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Present occurrence of *Anguillicola novaezelandiae* (Nematoda: Dracunculoidea) in Europe and its development in the intermediate host

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Abstract. An examination of a sample of European eels, *Anguilla anguilla* (L.), collected from Lake Bracciano near Rome in 1993, the only known European locality with the occurrence of the introduced swimbladder nematode *Anguillicola novaezelandiae* Moravec et Taraschewski, 1988, revealed for the first time the presence of two *Anguillicola* species, *A. novaezelandiae* and *A. crassus*. In view of the investigations carried out by current authors in Bracciano Lake in the years 1982–1992, it is apparent that the latter species has been introduced into the lake quite recently, where it quickly became a dominant species. The development of *A. novaezelandiae* was experimentally studied in the copepod intermediate host, *Cyclops strenuus*, for the first time. The copepods were infected with nematode second-stage larvae at 21–22°C; fully developed infective third-stage larvae were obtained 13 days p.i. The general morphology of individual larval stages of *A. novaezelandiae* was similar to that of larvae of the related species *A. crassus*.

Of the two species of *Anguillicola* that were introduced into Europe at the beginning of the 1980s, *A. crassus* Kuwahara, Niimi et Itagaki, 1974 originating from East Asia quickly spread in eel populations in most European countries, whereas *A. novaezelandiae* Moravec et Taraschewski, 1988 originating from New Zealand has been known to occur only in Bracciano Lake in central Italy (Moravec and Taraschewski 1988, Moravec 1992). Although the first species has been intensively studied, including its biology and pathogenicity, both in East Asia and Europe (see e.g. De Charleroy et al. 1990, Bonneau et al. 1991, Molnár et al. 1993, Moravec et al. 1993, Nagasawa et al. 1994), the latter is little-known and its life cycle remains completely unknown.

Anguillicola novaezelandiae was described from short-finned eels, *Anguilla australis* Richardson, from New Zealand and later it was recorded from the same host species from Australia (Moravec and Taraschewski 1988, Moravec and Rohde 1992). In Europe, this species was first reported as *Anguillicola australiensis* Johnston et Mawson, 1940 by Paggi et al. (1982) from *Anguilla anguilla* (L.) from Bracciano Lake in Italy, where it had apparently been brought in along with a stock of *Anguilla australis* introduced into the lake in 1975 (see Paggi et al. 1982, Moravec and Taraschewski 1988). It has not been reported from Europe since.

The purpose of this paper is to describe a situation in the occurrence of *Anguillicola* nematodes in eels in Bracciano Lake in recent years and to present the results of experimental study of the development of *A. novaezelandiae* in the copepod intermediate host.

MATERIALS AND METHODS

The following samples of eels, *Anguilla anguilla* (L.), were examined for the presence of helminth parasites from Bracciano Lake near Rome, Italy, in the period between 1988–1993: 10 eels (body length 33–39 cm) collected in May 1988, 2 eels (37 and 43 cm) in January 1992, 11 eels (29–41 cm) in March 1992, 10 eels (44–74 cm) in October 1992, and 40 eels (31–41 cm) in October 1993. The eels were trapped by fishermen and then they were transported alive to the laboratory in Rome where they were immediately dissected. The eels were measured and individually identified to species according to the features given by Tesch (1983) in order to exclude a possible presence of the introduced species *Anguilla australis*. The *Anguillicola* specimens from the eel swimbladders were washed in physiological saline and then fixed with 4% formaldehyde in saline solution for subsequent species identification. Live gravid females of *Anguillicola* collected in October 1993 were placed in physiological saline in plastic vials cooled with ice and were transported to the laboratory in České Budějovice, Czech Republic, where they were used for

an experimental study. The nematodes for experiments were first identified to species, using the size of the buccal capsule (examined under the light microscope) and the shape of the cephalic end as the principal differentiating features (see Moravec and Taraschewski 1988). From this material, only two gravid females of *A. novaezelandiae* (size of buccal capsule 30–33 x 12 μm) were obtained, each containing only a small quantity of fully developed eggs with motile larvae inside. These females were placed in a petri dish (diameter 15 cm) filled with water and their bodies were torn up with preparation needles and eggs released from the uteri. After removing the remnants of the nematode bodies, about 300 copepods (*Cyclops strenuus* Fischer), originating from a small pond near České Budějovice, were added. These were kept at laboratory temperature (21–22°C). The copepods were fed with *Paramecium* and examined at intervals of several days. Nematode larvae were immobilized and fixed by heating in a drop of water on a glass slide over a flame for several seconds and examined with a light microscope. Drawings were made with the aid of a Zeiss microscope drawing attachment.

RESULTS

A. Infection of eels with *Anguillicola* nematodes in Bracciano Lake in 1988–1993

All samples of eels taken until 1992 showed the presence of only one *Anguillicola* species, *A. novaezelandiae*. The examination of eels carried out in May 1988 revealed a prevalence of 80% and an intensity of infection 1–27 (mean 11) nematodes per eel. Also the examination of two eels in January 1992 indicated a high degree of infection: both eels proved to be infected with *A. novaezelandiae* with the intensity 5 and 8 nematodes in a fish. On the other hand, the eels examined in March 1992 harboured only dead nematodes and remnants of their bodies, with prevalence 36% and intensity 1–3 (mean 2) nematodes per eel. The sample taken in October of the same year (1992) consisted mostly of large eels and it was probably the reason why the prevalence of *A. novaezelandiae* was only 20%, with an intensity 2–7 (mean 5) nematodes per fish (only two smallest eels were infected). With the exception of the eel sample from March 1992, gravid females of *A. novaezelandiae* were found in the swimbladders in addition to conspecific males, young females and fourth-stage larvae.

A very different situation was found in October 1993 when, besides *Anguillicola novaezelandiae*, *A. crassus* was also recorded for the first time in this locality. While the prevalence of *A. novaezelandiae* was only 21% and the intensity 1–3 (mean 2) nematodes per eel, the prevalence of *A. crassus* was considerably higher (47%), with an intensity 1–14 (mean 4) nematodes per fish. No mixed infections were recorded. Gravid females with larvated eggs in uteri were present in both *A. novaezelandiae* and *A. crassus*. In addition to live *Anguillicola*, the swimbladders of most eels contained either the remnants of nematode bodies from previous infection or the swimbladder walls were markedly thickened, which was also a sign of a previous infection with *Anguillicola* (see Molnár et al. 1993).

B. Development of *A. novaezelandiae* in the intermediate host

Experimental infection of copepods

Like other congeneric species, *Anguillicola novaezelandiae* is ovoviviparous and its mature eggs *in utero* contain fully formed, motile second-stage larvae. Unfortunately, the two nematode females used in this experiment contained mostly eggs without fully developed larvae; only very few eggs contained motile larvae. As a consequence, only few experimental copepods (about 5%) became infected, with an intensity of 1, exceptionally 2, larvae per copepod.

When the eggs were released into the water, the second-stage larvae hatched from the egg shell, remaining, however, inside the loosened cuticular sheath from the first moult. These larvae actively moved in the water, attracting copepods which swallowed them readily. During the penetration of larvae through the copepod gut to its haemocoel, the second-stage larvae lost their cuticular sheath from the first moult. While developing in the haemocoel of this intermediate host the larvae increased in size and underwent one (second) moult. At the temperature of 21–22°C, the fully formed third-stage larvae, already liberated from the cuticular sheath of this moult, were found in copepods on the 13th day p.i. During the development in the copepod intermediate host, the larvae of *A. novaezelandiae* grew considerably in length, the body of third-stage larvae being approximately three times longer than that of free second-stage larvae. The third-stage larvae remained unencapsulated in the haemocoel of the intermediate host, but they were less active than second-stage larvae, with a tendency to stay spirally coiled.

The third larval stage is already infective for the definitive host. The rest of about 30 copepods surviving in the experiment (it was not determined whether some of them were infected) were added to a small aquarium with 5 small eels (body length 12–13 cm). The eels were examined after 7, 9, 12, 20 and 33 days, respectively, but none were found to be infected.

Morphology and larval development

a) Eggs Fig. 1A

The mature eggs are irregularly oval or nearly spherical, with a thin membraneous shell with almost smooth surface; their size is 105–114 x 63–90 μm. When laid,

the eggs contain a fully formed, motile second-stage larva; the larva retains the first-stage cuticle forming a loose sheath around the body. In water the second-stage larvae hatch from the egg shell.

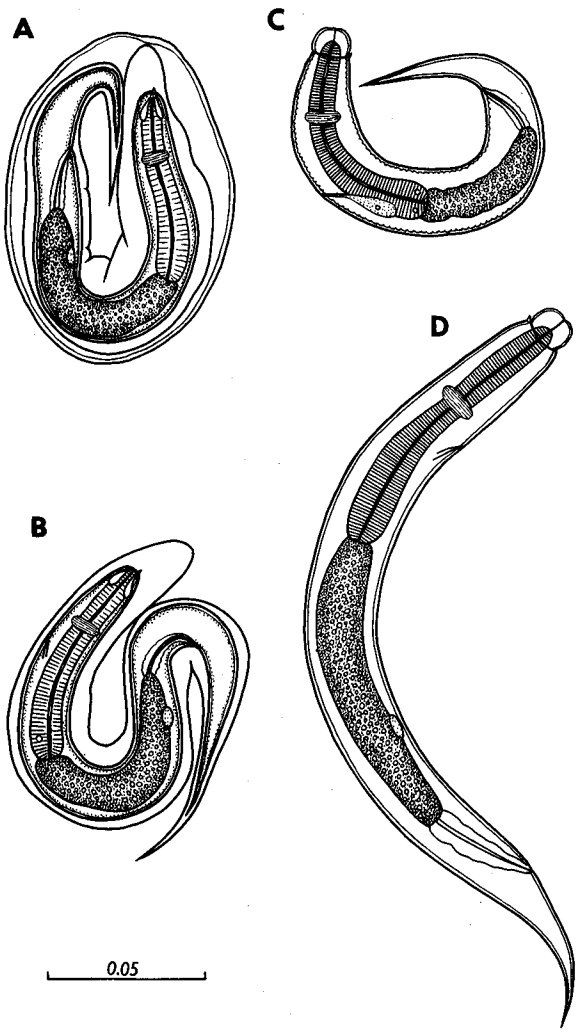

Fig. 1. *Anguillicola novaezelandiae*, second-stage larva. **A** – larva inside the egg shell; **B** – hatched larva still inside a loosened cuticular sheath from the first moult; **C** – larva from the copepod 2 days p.i.; **D** – larva from the copepod 6 days p.i. (Scale bar in mm.)

b) Free second-stage larvae Fig. 1B

Hatched second-stage larvae remain inside the cuticular sheath from the first moult. Their body is slender, whitish to translucent, 207–216 μm long and 15 μm wide; the maximum width of cuticular sheath is 30–36 μm. The cephalic end is armed with a dorsal conical cuticular tooth. The cuticle is very thin and smooth. The anterior end of the larva is provided with two distinct, drop-like refractive formations (probably proteolytic glands). The internal organization of the body is not readily visible. The oesophagus is 72–78 μm long, its posterior part is somewhat expanded. The nerve ring and excretory pore are situated 27–30 μm and 36–45 μm, respectively, from the anterior end. The intestine is relatively wide, sparsely granulated; the rectum is a thin-walled, colourless tube. The tail is conical, sharply pointed, 48–60 μm long. A small, barely visible genital primordium is situated ventrally in posterior half of body.

c) Development of second-stage larvae in the intermediate host Figs. 1C, D

Having penetrated into the haemocoel of the copepod, the larvae loose their cuticular sheath from the first moult, but their morphology is still very similar to that of free second-stage larvae. However, the body of the larva obtained 2 days p.i. (Fig. 1C) was markedly shorter and somewhat plump (178 μm long and 21 μm wide). Its cephalic end was broad, with its anteriormost portion globular, separated from the body by a cuticular constriction; this part of the cephalic end, measuring 9 x 15 μm, can be retracted into the body. The larval tooth was situated dorsally at the base of the globular cephalic part. The inner layer of the cuticle of this larva appeared to be densely transversally wrinkled. The length of the oesophagus was 60 μm, the nerve ring and the excretory pore were 24 μm and 33 μm, respectively, from the anterior extremity. The tail was 42 μm long.

In the following days, the larvae again continue to grow, but their morphology remains practically the same. The larva obtained 6 days p.i. (Fig. 1D) measured 320 μm in length and 25 μm in width. Its cuticle was smooth. The oesophagus measured 120 μm, the nerve ring and the excretory pore were situated 69 μm and 78 μm, respectively, from the anterior extremity. The length of the sharply pointed tail was 81 μm. The larvae showed no signs of the next moulting.

Due to a very small number of infected copepods remaining in the experiment, the next copepods were examined only 13 days p.i. when already fully formed third-stage larvae were recorded in them. Therefore, the second moult of larvae was not observed.

d) Third-stage larvae (infective larvae) Fig. 2

Two larvae of this stage were obtained from the copepods 13 days p.i. These were slender, their anterior (oesophageal) part was whitish, whereas the posterior part contained a light brown intestine. Their body was 810–816 μm long and 39 μm wide. Their cuticle appeared to be almost smooth, with two very narrow lateral alae extending along the whole body length. Deirids were not observed. The cephalic end of the larva was rounded, the mouth was provided with two small lateral, anteriorly directed sclerotized teeth; each tooth was followed posteriorly by a sclerotized apparatus situated at level of the anterior end of oesophagus, which appeared

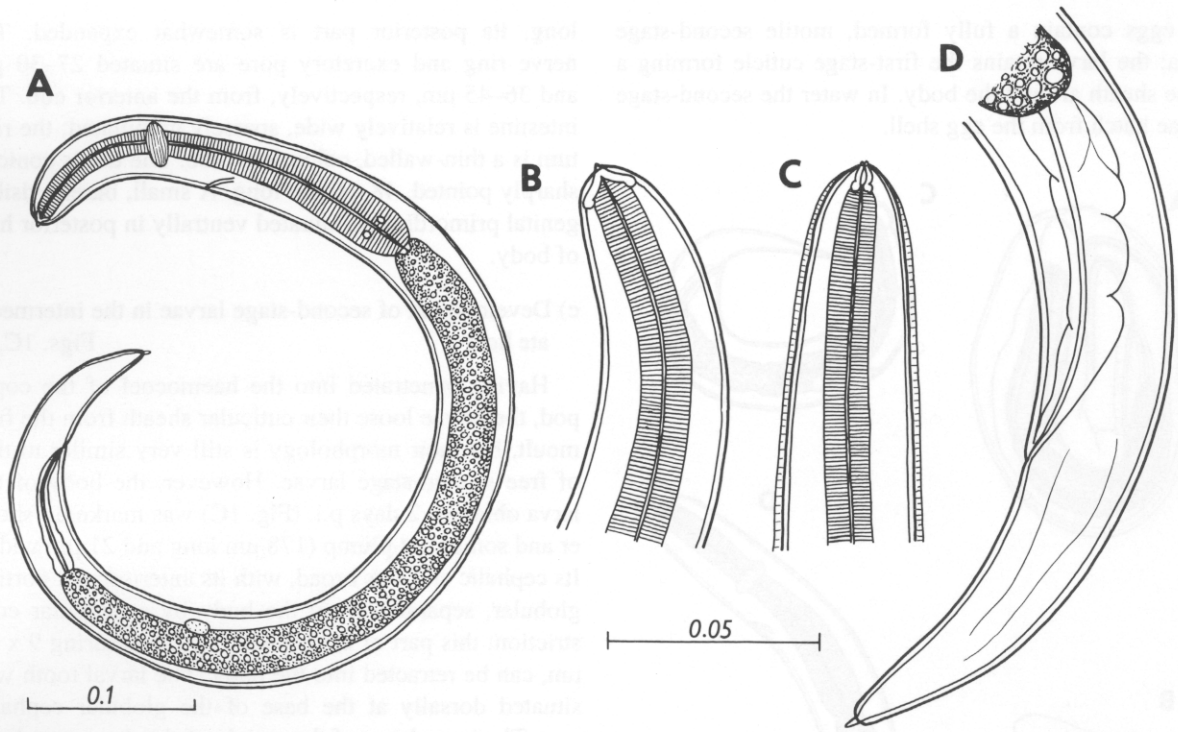

Fig. 2. *Anguillicola novaezelandiae*, third-stage (infective) larva from the copepod intermediate host (13 days p.i.). **A** – general view; **B, C** – cephalic end, lateral and dorsoventral views; **D** – tail. Scale bars in mm.

as bifurcate in lateral view; this apparatus was 9 µm long and 15 µm wide. Cephalic papillae were indistinct. The oesophagus was long, slender, distinctly broader at its posterior part; it was 249–252 µm long (31% of the whole body length) and 15 µm and 27 µm wide at its anterior and posterior parts, respectively. The oesophagus opened into the intestine through a valve. The nerve ring encircled the narrow anterior part of oesophagus 102–105 µm from the anterior extremity; the excretory pore was located somewhat behind the nerve ring level, 135–138 µm from the cephalic end. The intestine was straight, containing numerous brownish granulae. The rectum was a hyaline tube 75 µm long; rectal glands were not well visible. The tail was conical, 66 µm long, bearing a distinct small cuticular spike (3 µm long) at its tip; the length of the tail represented 8% of the whole body length. A small oval genital primordium was located ventrally, approximately at the border of the second and the third thirds of the body length, i.e. 609–618 µm from the anterior extremity.

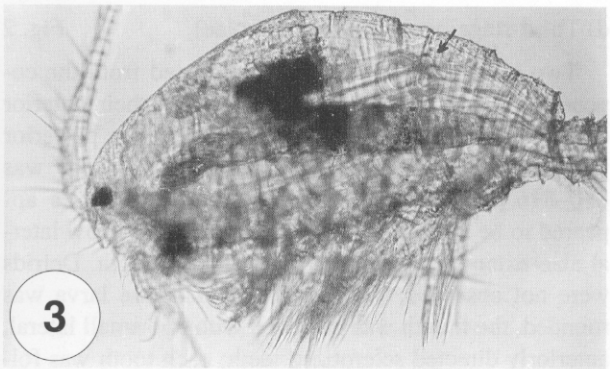

Fig. 3. Larva of *Anguillicola novaezelandiae* (arrow) in the haemocoel of the copepod (x 95).

DISCUSSION

It has already been stated in this paper that Bracciano Lake in Italy has been the only known locality in Europe where *Anguillicola novaezelandiae* occurs. The first record of this swimbladder parasite of eels (reported as *A. australiensis*) is that by Paggi et al. (1982) who found it in about 40% of eels, *Anguilla anguilla* (L.), examined from this locality in February 1982. The results of the present paper show that, until 1992, *A. novaezelandiae* was the only species of this genus occurring in the lake with the prevalence of up to about 80% in medium-sized eels in some seasons. It has already been indicated by Paggi et al. (1982) that this parasite was brought into Bracciano Lake along with a stock of *Anguilla australis* introduced into the lake in 1975.

Bracciano Lake is the second largest lake in central Italy, being situated at 164 m above sea level. Its shape is almost circular with a circumference of 31 km and a surface of more than 5 000 hectares. The lake is of a tectonic origin and is completely isolated from the sea,

which may be the reason why *A. novaezelandiae* remained restricted in its distribution to this lake and, on the other hand, *A. crassus* did not invade the lake for many years. The European eel, *Anguilla anguilla*, is not an autochtonous fish species in this lake and the local eel populations originated from different sources, both from the sea (rivers) and eel farms. According to information provided by local fishermen, the short-finned eels, *Anguilla australis*, both small and large eels, originated from New Zealand, were introduced several times into Bracciano Lake, for example in 1982 and 1988. Within the last five years, *Anguilla australis* have no longer been introduced into Bracciano Lake and only the European eels, *Anguilla anguilla*, of Italian origin (both from the rivers and eel farms) have been used for a re-stocking of the lake twice a year. Apparently, due to this re-stocking, another *Anguillicola* species, *A. crassus*, has been introduced into Bracciano Lake quite recently, as is indicated by our findings. It is not surprising, because *A. crassus* is widespread in both wild and farmed eels in Italy.

As far as the authors know, the situation where one eel species is infected by two different species of *Anguillicola* in the same locality is quite unique. Moreover, none of these two *Anguillicola* species is indigenous to Europe, but originated from two different zoogeographical regions (New Zealand and East Asia), and the European eel is not their original host. The results of our observations from 1993 show that *A. crassus* became quickly a dominant species in Bracciano Lake. However, it will be interesting to follow the interactions of both these nematode species and the further development of the situation in the lake over the next years.

At present the genus *Anguillicola* is represented by five species, all parasitic in the swimbladder of eels. Of them, the life cycles have hitherto been studied only in *A. globiceps* (Wang and Zhao 1980) and *A. crassus* (e.g., Hirose et al. 1976, Kim et al. 1989, Petter et al. 1989, 1990, De Charleroy et al. 1990, Fioravanti and Restani 1992, Moravec et al. 1993, 1994, Moravec and Konecny 1994). In both these species, various cyclopoid copepods served as the natural and experimental intermediate hosts, in *A. crassus* also the ostracods *Cypria ophthalmica* and *Notodromas monacha* (Petter et al. 1990, Bonneau et al. 1991, Moravec and Konecny 1994). The results of the present study show that also the life cycle of *Anguillicola novaezelandiae* involves a copepod intermediate host.

The morphology of adult *Anguillicola novaezelandiae* and *A. crassus* indicates that both species are closely related which reflects in the similarity of their larval stages. The results of the present study show that the second- and third-stage larvae of *A. novaezelandiae* are morphologically indistinguishable from the same larval stages of *A. crassus* (see Moravec et al. 1993) and they undergo similar morphological and biometrical changes while developing in the intermediate host. In contrast to *A. crassus*, the rate of the development of larval *Anguillicola novaezelandiae* seems to be shorter. While Moravec et al. (1993) observed the fully formed third-stage larvae of *A. crassus* already liberated from the cuticular sheath only 20 days p.i. at 20–22°C, the same larvae of *A. novaezelandiae* were found in copepods as early as 13 days p.i. at approximately the same temperature (21–22°C).

Since there are known many fish species serving as paratenic hosts of *Anguillicola crassus* (Petter et al. 1989, De Charleroy et al. 1990, Haenen and van Banning 1990, Thomas and Ollevier 1992, Moravec and Konecny 1994), it is highly probable that paratenic parasitism is common in *A. novaezelandiae* too.

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