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Third-stage larvae of *Daniconema anguillae* (Nematoda: Dracunculoidea) in the subcutaneous tissue of eel *Anguilla anguilla*

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Abstract. *Daniconema anguillae* Moravec et Køie, 1987 larvae measuring 1.64–1.76 mm were occasionally found in considerable numbers in the fins and subcutaneous connective tissue of approximately 50% of eel *Anguilla anguilla* (L.) sampled from Lake Balaton, Hungary. The larvae were noted for their slender body, very long tail with a rounded tip, a densely transversely striated cuticle, and the presence of boring tooth and large kidney-shaped amphids on the cephalic end. The larvae could easily be recovered from the above mentioned organs by placing them into isotonic saline solution. No disease signs or pathological changes attributable to the larval infection could be observed. The only histological indication of host reaction was the appearance of macrophages adhering to the body surface of larvae and of cells with spherical nucleus in areas around the larvae. A possible life cycle pattern of *D. anguillae* is discussed.

The examination of a histological section made from an eel collected from Lake Balaton in 1991 revealed the cross-sections of nematode larvae (Fig. 2) in the fins. Subsequently, in some cases larvae were demonstrated also in fresh preparations made from the connective tissue of the fins. Further dissections of fish showed that the parasites occurred in the subcutaneous connective tissue much more frequently than in the fins.

Of the nematodes parasiting eels, besides the extremely common infection *Anguillicola crassus* Kuwahara, Niimi et Itagaki, 1974 (Molnár et al. 1991, Székely et al. 1991), only *Daniconema anguillae* Moravec et Køie, 1987 shows a considerable occurrence in Lake Balaton eels (Molnár unpublished). Therefore, it was natural that these larvae, morphologically different from those of *Anguillicola,* were considered to belong to *D. anguillae*, and further studies to verify their species appurtenance were performed.

In this work, morphological evidence is presented to support that the larvae belong to the genus *Daniconema* Moravec et Køie, 1987, and a probable developmental cycle is proposed on the basis of the nature, location and frequency of larval occurrence.

MATERIALS AND METHODS

A total of 142 eels, *Anguilla anguilla* (L.), with the body length ranging between 22 and 76 cm, were examined. The fish were collected by electrofishing from three different regions (western, middle and eastern parts) of Lake Balaton from March to November, 1993.

After the first histological observation of nematode larvae, we tried to detect further specimens by examining the eels' fins under stereomicroscope. It was later found that the larvae could be isolated by placing the skin of flayed eels into physiological saline, as the larvae actively migrated from the skin into the solution. After leaving the skin, the larvae showed vigorous motion in the saline solution and were collected from the Petri dish with the help of a pipette. The collected larvae were rinsed several times, selected and transferred into fresh saline, then fixed in 80% alcohol or 10% buffered formalin.

For examination by scanning electron microscopy (SEM), the specimens were postfixed in 1% OsO_4, dehydrated through an ethanol series and aceton and subjected to critical point drying. The specimens were coated with gold and examined with a Jeol JSM-6300 scanning electron microscope at an accelerating voltage of 15 kV.

For histological examination, samples were excised from the fins and skin of eels that had proved infected and were fixed in Bouin's solution. Paraffin-embedded sections 4 μm in thickness were stained with haematoxylin and eosin.

RESULTS

Larvae were successfully isolated from the skin of 74 out of the 142 eels examined. In addition, larvae regarded as *Daniconema* were isolated from the fins of 47 eels and from the abdominal cavity of one fish. About two-thirds of eels exceeding 60 cm in length proved to be infected; at the same time, the infection could not be demonstrated in fish shorter than 24 cm. The infection occurred in all three habitats examined. All the isolated

larvae were of nearly identical size. In physiological saline, the larvae maintained their viability for 48–96 hours at 24°C and after short periods of rest, showed vigorous vibrating motion. In tap-water the larvae survived for 12–24 hours. The larvae were quickly ingested by copepodes of undetermined species; however, they did not penetrate the haemocoel of the latter and could be collected from them after 4–6 hours in the digested state only.

Adult specimens of *D. anguillae* were frequently found in the swimbladder of eels but their prevalence was not examined.

lateral amphids present (Fig. 3), cephalic papillae indistinct; size of amphids in lateral view 2 x 9 μm. Deirids not observed. Under cuticle, along nearly entire body, numerous round cells with refractile nuclei visible. Mouth formed by very fine, short tube, attached to anterior portion of oesophagus. Oesophagus simple, not yet clearly divided into muscular and glandular parts, being 330–642 μm long and 9–10 and 18 μm wide at its anterior and posterior ends, respectively. Oesophagus not well visible due to presence of conspicuous hypodermal cells. Nerve ring and excretory pore 177–201 and 279–297 μm, respectively, from anterior extremity. In-

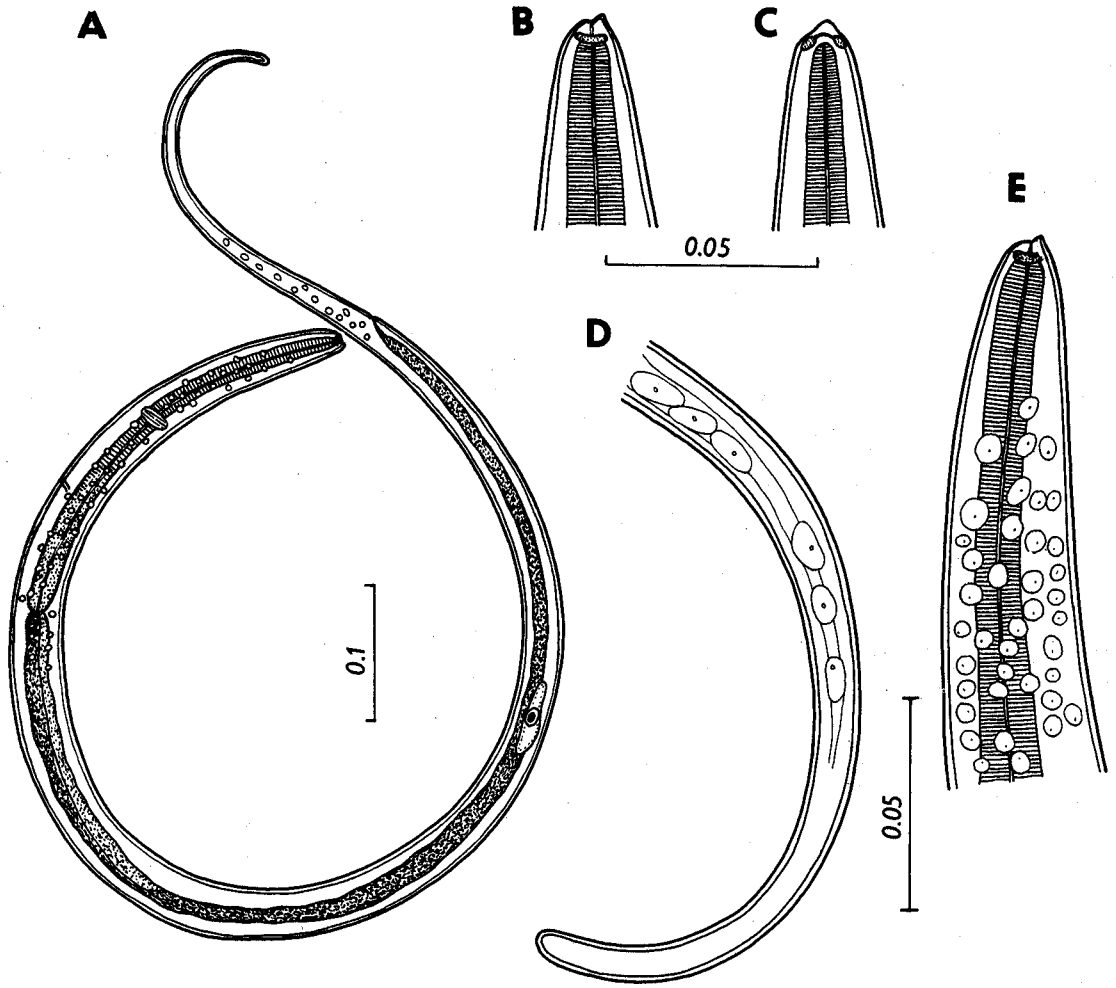
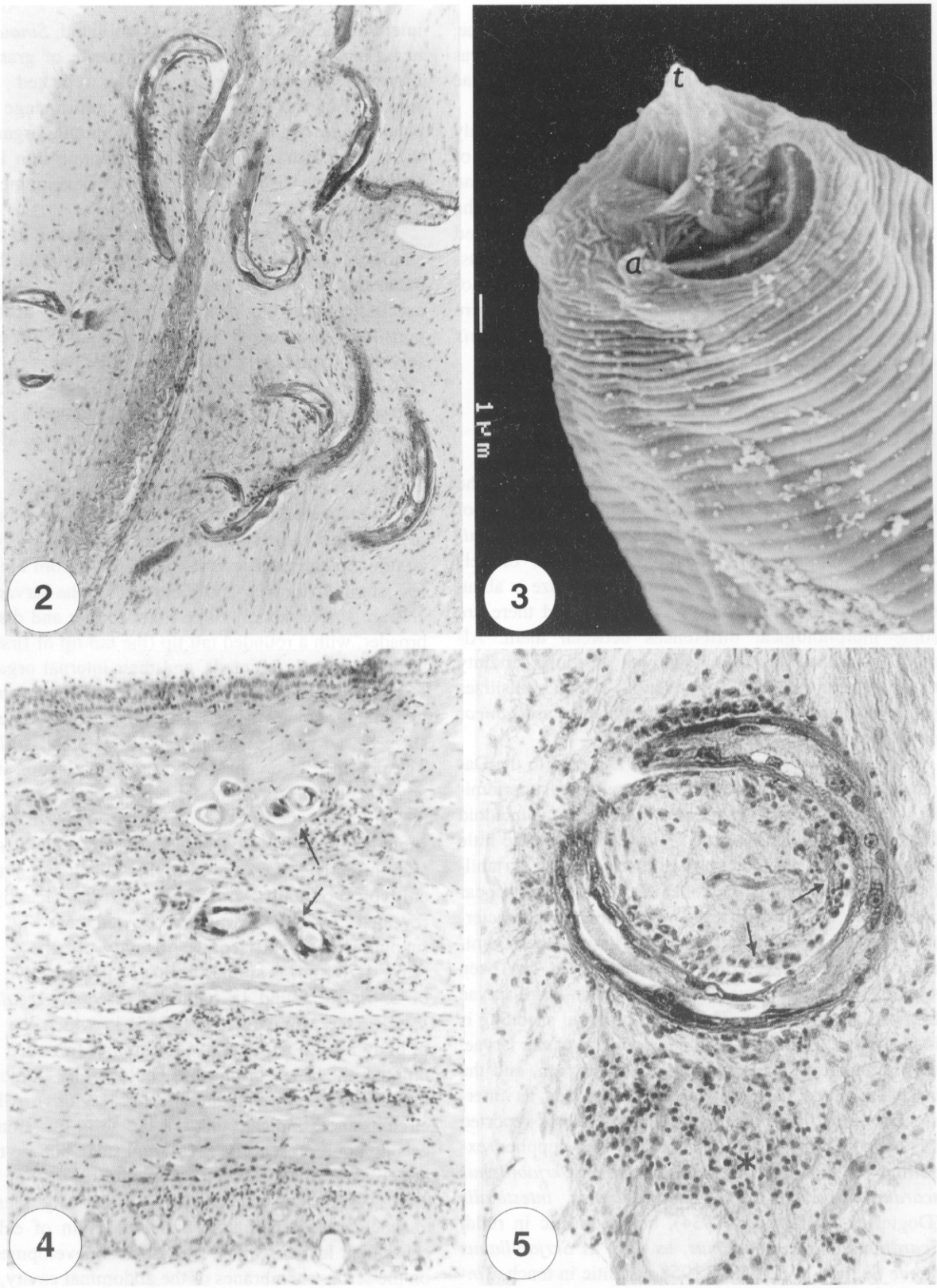


Fig. 1. Line drawings of *Daniconema anguillae* third-stage larvae. **A** – general view; **B, C** – cephalic end, lateral and ventral views; **D** – posterior part of tail; **E** – anterior end of body, lateral view.

Description of larvae (based on 10 specimens studied by light microscopy and a few specimens studied by SEM) (Fig. 1): Body whitish, thin, elongate, 1.64–1.76 mm long and 42–54 μm wide, with dense transverse striation of cuticle. Cephalic end somewhat narrowed. Mouth aperture almost circular, surrounded by two lip-like elevations. Dorsal elevation triangular in dorsoventral view, forming an anteriorly protruding boring tooth. Two conspicuously large, kidney shaped testine light-coloured, narrow, straight; rectum short hyaline tube. Genital primordium large, situated 0.96–1.03 mm from the anterior end of body. Tail narrow, markedly long, with rounded tip; its length 345–387 μm, forming 20–24% of whole body length.

In histological sections the larvae were found in the loose connective tissue of the skin and fins (Figs. 2 and 4) where they occurred in irregularly coiled forms, not causing substantial tissue reaction. In some cases



Figs. 2–5. Fig. 2. *Daniconema* larvae seen in cross section of the connective tissue of an eel's fin. Haematoxylin and eosin, x 110. **Fig. 3.** SEM micrograph of cephalic end of *Daniconema anguillae* third-stage larva. t = dorsal tooth; a = amphid. **Fig. 4.** *Daniconema anguillae* larvae in the fin of an eel. The transected larvae (arrow), wound up several times, are located in the loose connective tissue under the multilayered epithelium and compact corium layer covering the fins. Haematoxylin and eosin, x 110. **Fig. 5.** Longitudinal section of a *Daniconema anguillae* larva in the fin of an eel. Note macrophages (arrow) adhering to the cuticle of the larva and the appearance of cells with spherical nucleus (asterisk) in the loose connective tissue surrounding it. Haematoxylin and eosin, x 300.

macrophages were seen adhering to the body of larvae, and the appearance of cells with spherical nucleus was observed in the connective tissue surrounding the larvae (Fig. 5).

Changes in the skin and fins were observed in only one case, when the markedly reddened skin and fins of a 72 cm long eel showed the signs of haemorrhages and inflammation. Stereomicroscopic examination of the fins allowed for several hundred larvae to be counted (Fig. 4). That case however, could not be unambiguously connected with the larval infection, as no bacteriological examination was performed and the fins were covered with large masses of *Myxobolus portucalensis* Saraiva et Molnár, 1990 cysts.

DISCUSSION

Morphologically the nematode larvae found in the subcutaneous tissue of eels are strongly reminiscent of the first-stage larvae found in the uterus of female *Daniconema anguillae*, a specific tissue parasite of eels (Moravec and Køie 1987). However, their size is about three times larger than that of the latter and there are some morphological differences between them. Although it cannot be ruled out with absolute certainty that the larvae belong to a nematode which parasitizes some piscivorous birds, their identification as *Daniconema* seems to be well supported.

Daniconema anguillae, a species assigned to the Daniconematidae family of the Dracunculoidea superfamily is closely related to members of the dracunculoid families Skrjabillanidae, and Guyanemidae. While little is known about the life cycles of the species of Skrjabillanidae, knowledge of Guyanemidae life cycles appear completely unknown. These nematodes are characterized by their parasitic activity in the tissue and abdominal cavity of fish. Further characterization can been seen by the continuous shedding of first-stage larvae from the female uterus. This mode of larval shedding is indicative of a filarioid-type development of the larvae, their circulation in the lymph and blood path, and the involvement of haematophagous arthropods as intermediate hosts. This type of development was reported by Tikhomirova (1970, 1975, 1980) who supplied experimental evidence that the larvae of *Skrjabillanus scardinii* Molnár, 1966 and *Molnaria intestinalis* (Dogiel et Bykhowsky, 1934), both parasitic in rudd, *Scardinius erythrophthalmus*, as well as *Skrjabillanus tincae* Schigin et Schigina, 1958 parasitic in tench, *Tinca tinca*, are taken up from the host's tissue by carp lice, *Argulus* spp. (Branchiura), serving as the intermediate hosts in the body cavity of which the nematode larvae develop to the third stage. Carp lice (*Argulus*) were also found by Rudometova (1974, 1975) to serve as the intermediate hosts of another skrjabillanid, *Sinoichthyonema amuri* (Garkavi, 1972), a parasite of grass carp, *Ctenopharyngodon idella*. During repeated "blood sucking" of the fish, the infective third-stage larvae penetrate through the crustacean's mouth organs into the skin of fish; from there they migrate into internal organs of the fish host for further development and maturation.

Daniconema anguillae may be assumed to have a similar life cycle. The location of the larvae in the subcutaneous connective tissue, i.e. in a place most easily accessible to the blood-sucking intermediate hosts, supports this assumption. This is consistent with the observation made by Elkan and Reichenbach-Klinke (1974) who detected in the blood of eels the "microfilariae of a *Philometra* sp", which is presumably identical with the first-stage larvae of *D. anguillae* (see Moravec and Køie 1987).

The general morphology and measurements of the larvae recovered from the subcutaneous tissue and fins of eels show clearly that they are third-stage larvae. In contrast to conspecific first-stage larvae from the females' uterus (Moravec and Køie 1987), the larvae from eels are approximately three times longer and distinctly broader, with a rounded tail tip (the tail tip of first-stage larvae is sharply pointed), and their internal organs are well developed. The general structure of the mouth of these larvae somewhat resembles that found in the third-stage larvae of other dracunculoids (e.g. *Anguillicola*). The presence of a cephalic boring tooth at this stage is not exceptional in nematodes, and is present, for example, in third-stage larvae of some anisakids. In addition, some features of these larvae (a distinct transverse striation of cuticle, presence of highly developed amphids) are typical of adult *D. anguillae*, as it has recently been revealed by SEM studies carried out by Køie (unpublished). The length of body of these larvae (1.64–1.76 mm) is similar to that of the infective larvae of *S. tincae* found in naturally infected *Argulus foliaceus* (see Moravec 1978, 1985) in which the length of body ranged within 1.39–1.60 mm. Moreover, in our opinion, the larvae of this size could not be extracted by the presumable intermediate host (? *Argulus*). All these circumstances indicate that the larvae in question from eels do not represent the first larval stage but are typical infective third-stage larvae.

However, it is difficult to explain why these advanced larvae accumulate under the skin of eels, because their location in the sites of final development, i.e. on the serous membranes of the abdominal cavity, intestine or swimbladder would seem more logical. The only explanation would be that these third-stage larvae, after their penetration into the body of an eel from an intermediate host, are temporarily concentrated under the skin of the host fish from where they later migrate to

other tissues. It is noteworthy however, that all these larvae from subcutaneous tissue and fins were nearly morphologically and biometrically identical, showing no signs of further development. Furthemore, juvenile forms located in the abdominal cavity were not as large as those in the third stage larvae. This contradiction in size could most likely be answered by experimental studies. These studies might examine the presence of *Argulus* specimens occuring in eels. Interestingly enough, during this study no *Argulus* infestation was observed on eels from Lake Balaton.

A dense aggregation of small, unidentified dracunculoid (reported as philometrid) larvae has been reported several times from superficial body tissues and internal organs of sharks (e.g., Rosa-Molinar et al. 1983, Benz et al. 1987) which are known to be the definite hosts of dracunculoids of the genera *Phlyctainophora, Granulinema* and *Lockenloia.*

It seems that the larval parasitosis reported in this work does not result in the appearance of disease or pathological changes even if infection is intensive. The cellular response of the host is indicated only by the presence of macrophages adhering to the surface of larvae and by the proliferation of round cells around certain larvae.

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