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NOTE

Development of *Anguillicola crassus* (Dracunculoidea, Anguillicolidae) in experimentally infected Balearic congers *Ariosoma balearicum* (Anguilloidea, Congridae)

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ABSTRACT: The development of *Anguillicola crassus* in experimentally infected *Ariosoma balearicum* (Anguilloidea, Congridae) kept in seawater was studied in the laboratory. In parallel trials the effect of water salinity on the development of larval *A. crassus* in European eels *Anguilla anguilla* was also investigated using eels kept in seawater of a salinity of 34‰. Both eel species were orally inoculated with L_3 larvae of *A. crassus* and then maintained for up to 3 mo at 18°C in seawater. 110 d post infection, no adult but larval (L_3 and L_4) stages of *A. crassus* were detected in the swimbladder wall of Balearic congers, although this period of time was sufficient for the parasites to develop to the adult stage in European eel kept in seawater. The results presented suggest that the definitive host specificity of *A. crassus* comprises species of the family Anguillidae (i.e. the genus *Anguilla*), but not members of the Congridae. Theoretically however, *A. balearicum* might serve as a metaparatenic host. Factors determining the definitive host range of *A. crassus* remain to be elucidated. Water salinity does not seem to act as a factor affecting definitive host specificity once the parasite has become ingested by the eel.

KEY WORDS: *Anguillicola crassus* · *Anguilla anguilla* · *Ariosoma balearicum* Development · Salinity · Metaparatenic host

Nematodes of the genus *Anguillicola* are widely distributed swimbladder parasites of eels. *Anguillicola* species have been recorded from various eel species of the monotypic family Anguillidae as definitive hosts (Moravec & Taraschewski 1988). *Anguillicola crassus* has been very successful in colonising eel species and their populations by transcontinental displacement and then by a quick spread within the continent. Originating from East Asia where it naturally parasitized *Anguilla japonica* and later on also cultivated European eels *Anguilla anguilla* (see Nagasawa et al. 1994), *A. crassus* became a very abundant parasite in *A. anguilla* throughout Europe (Würtz et al. 1998, Sures et al. 1999) and North Africa (El Hilali et al. 1996, Maamouri et al. 1999) in the 1980s and 1990s. From 1995 the parasite also occurred and reproduced in the American eel *Anguilla rostrata* along the east coast of the United States (Johnson et al. 1995, Fries & Williams 1996, Barse & Secor 1999). At present, 3 members of the genus *Anguilla* are known to serve as final hosts of *A. crassus*, while various other fish species as well as amphibians and even invertebrates may serve as paratenic hosts (Moravec & Konecny 1994, Moravec 1996, Székely 1996, Moravec & Škoriková 1998).

The successful spread of *Anguillicola crassus* is also due to the ability of the nematode to survive and reproduce in eels under many conditions that the host can withstand. In European eels caught in freshwater, adult *A. crassus* did not become negatively affected when the hosts were maintained in water with salinities ranging from freshwater to 100% seawater over a period of 4 wk (Kennedy & Fitch 1990). In nature, transmission of *A. crassus* occurs in brackish water lagoons with salinities ranging between 10 and 40‰ (Kennedy et al. 1996) as well as in the Baltic Sea (Höglund et al. 1992, Reimer et al. 1994). Accordingly, marine eel species belonging to the family Congridae may ingest L_3 larvae and become infected with *A. crassus*.

It was the aim of the present investigation to check whether the final host range of *Anguillicola crassus* includes Anguilliformes of the family Congridae or whether it is limited to the family Anguillidae, i.e. the genus *Anguilla*. As the water salinity obviously did not affect the development and viability of *A. crassus* in *Anguilla anguilla*, the Balearic conger *Ariosoma bale-*

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aricum was used to investigate the final host specificity of the nematode under marine conditions.

Material and methods. European eels *Anguilla anguilla* L., 1758 (Anguilloidea, Anguillidae) with a length ranging between 35 and 45 cm were obtained from a commercial fish farm (Limnotherm, Bergheim, Germany) known to be free of *Anguillicola crassus*. 3 eels were placed in a 100 l tank and maintained in aerated water with a salinity of 34‰ at a water temperature of 18°C. The eels were allowed to adapt to the salinity step by step by adding marine salt to tap water each week until a final salinity of 34‰ was reached after 6 wk.

3 Balearic conger *Ariosoma balearicum* Delaroche, 1809 (Anguilloidea, Congridae) with a length ranging between 28 and 34 cm caught in the Mediterranean Sea near the Island of Giglio (Italy) were transported alive to the laboratory, placed in a 100 l tank and maintained in aerated seawater (34‰) at a water temperature of 18°C.

Anguilla anguilla as well as *Ariosoma balearicum* were infected with third-stage larvae (L_3) of *Anguillicola crassus* perorally with a stomach tube. Freshwater eels were inoculated with 7 larvae while the marine eels were infected with 40 L_3. The third-stage larvae were obtained by feeding second-stage larvae (L_2) from the swimbladder lumen of naturally infected eels to planktonic copepods, mainly comprising *Thermocyclops* cf. *crassus* and *Mesocyclops leuckarti* (for details see Knopf et al. 1998). Immediately after infection the fish were placed back in seawater and maintained unfed for up to 110 d under marine conditions. At the end of the experiment the fish were killed and the swimbladder was examined for living and dead larvae and adults of *A. crassus*. As L_3 and L_4 cannot be distinguished from each other perfectly by means of light microscopy (Blanc et al. 1992), all larvae with a body length exceeding 1.5 mm were counted as L_4 according to the suggestion of Knopf et al. (1998).

Results and discussion. Only 1 out of 3 *Anguilla anguilla* which were adapted to seawater and then infected with L_3 larvae survived over several months. This individual was killed at 110 d post infection (p.i.). Its swimbladder contained 1 male adult *Anguillicola crassus*. As neither L_3 nor L_4 were found, the recovery rate could be given as 14%. This result confirms the finding of Kennedy & Fitch (1990) that *A. crassus* can establish and survive in *A. anguilla* under marine conditions, although infection levels decrease with an increase in salinity (Kirk et al. in press).

In contrast, only 1 *Ariosoma balearicum* died after 34 d p.i. In the swimbladder of this eel 67% L_3 and 33% L_4 were recorded (Fig. 1). This ratio had changed to values of 33% L_3 and 67% L_4 in the second eel maintained for 110 d p.i. The third *A. balearicum* was found

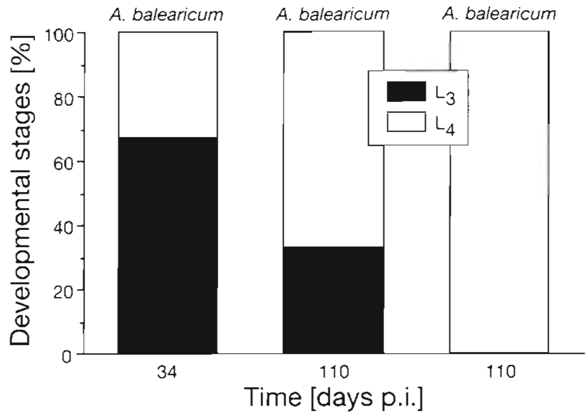

Fig. 1. Relative percentages (%) of different developmental stages of *Anguillicola crassus* out of the total number of specimens recovered from the swimbladder of 3 specimens of *Ariosoma balearicum* kept in seawater

to contain only L_4 at 110 d p.i. The recovery rates could be given as 15% for the first 2 *A. balearicum* and 10% for the third eel. Thus, it is obvious that L_3 larvae of *Anguillicola crassus* are able to moult to the L_4 stage in the swimbladder wall of *A. balearicum*. However, although the period of time was long enough for a complete development into the adult stage, no adult worms were recorded in the lumen of swimbladders of *A. balearicum*.

In a recent study Knopf et al. (1998) elucidated the time required for complete development of L_3 larvae in European eels kept in freshwater at a temperature of 18°C. First adult worms (12%) were recorded 50 d p.i., reaching values of approximately 85% of the total number of recovered worms on Day 80 p.i. From this day onwards the ratio of larval stages (L_3 and L_4) changed only little and remained below 15%. A developmental period of approximately 50 d at 18°C (Knopf et al. 1998) is consistent with earlier results of Haenen et al. (1996), who first observed *Anguillicola crassus* in the swimbladder lumen of eels 48 d p.i. at 18 to 20°C. Following Day 80 p.i., more than 80% of the worms became adult and this ratio remained nearly unchanged until Day 110 p.i. (Knopf et al. 1998). Thus, a period of 110 d should also be sufficient for *A. crassus* to develop in *Ariosoma balearicum* to the adult stage. The fact that in Balearic congers *A. crassus* did not reach the adult or at least the preadult stage but just moulted from the third-stage larvae into the fourth-stage larvae is most likely due to host-specific factors and not due to the water salinity, as can be concluded from the parallel experiment with European eels. Obviously, the host range of *A. crassus* does not include true seawater eels (congers) like *A. balearicum*, although the salinity itself does not act as the limiting factor. More likely, the final host range only comprises

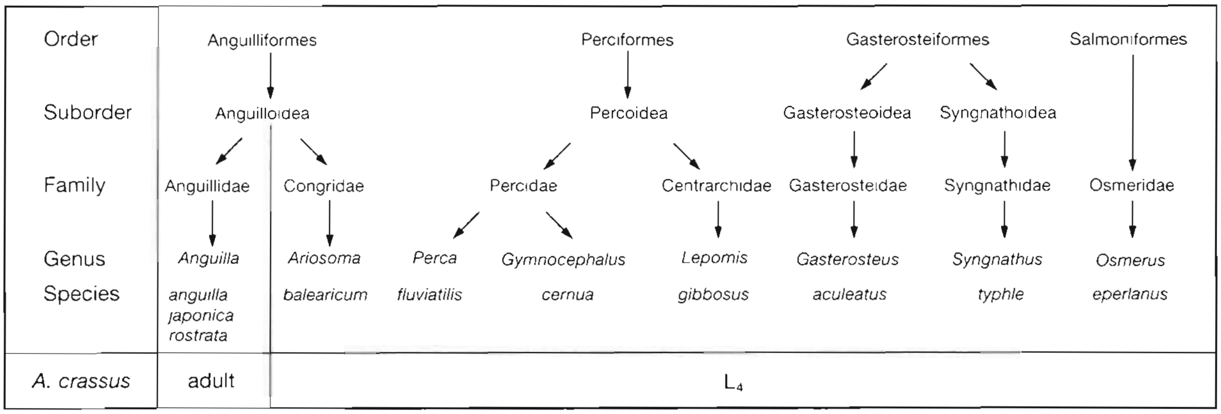

Fig. 2. Taxonomic position of fish species (Deckert et al. 1991) found to act as definitive or metaparatenic hosts for *Anguillicola crassus*. Data are from this paper and from the literature (De Charleroy 1990, Haenen & Van Banning 1990, Thomas & Ollevier 1992, Reimer et al. 1994, Moravec 1996)

members of the monotypic family Anguillidae (Tesch 1999) but not marine eels of the family Congridae.

In a field study Reimer et al. (1994) described the deep-snouted pipefish *Syngnathus typhle* as a potential paratenic host of *Anguillicola crassus* naturally containing fourth larval stages of the parasite. Additionally, different freshwater fish species are known to act as such metaparatenic (stadiogenous) hosts or even paradefinitive hosts (see Odening 1976, Moravec & Konecny 1994, Moravec 1996), enabling the parasite to develop into the L_4 larval stage (De Charleroy et al. 1990, Haenen & Van Banning 1990, Thomas & Ollevier 1992, Moravec 1996). The systematic positions (according to Deckert et al. 1991) of the definitive and metaparatenic hosts of *A. crassus* so far described are summarised in Fig. 2. In addition to the 3-spined stickleback *Gasterosteus aculeatus* and *S. typhle*, both belonging to the order Gasterosteiformes, 3 species known to serve as metaparatenic hosts are the perciform fish species *Gymnocephalus cernua*, *Perca fluviatilis* and *Lepomis gibbosus*. It seems most likely that other perciform fish could also be found infected with L_4 larvae of *A. crassus*, as the suborder Percoidea contains 26 families. As for salmoniformes, only *Osmerus eperlanus* was found to be infected with L_4 larvae (Haenen & Van Banning 1990), whereas no larval stages of *A. crassus* could be detected in *Salmo trutta fario* and *Salmo salar* in a field study by Thomas & Ollevier (1992). Following the conception and terminology of Odening (1976), cyprinids, one of the biggest groups of freshwater fish, seem to belong to the euparatenic (astadiogenous) hosts, as only L_3 larvae were recorded in the species investigated (Haenen & Van Banning 1990, Moravec & Konecny 1994, Székely 1995). In all, more than 30 fish species are known to act as such euparatenic hosts (Székely 1995, Moravec 1996).

Until now, 7 different fish species, including the Balearic conger, are known to act as potential metaparatenic hosts. Among them, 4 fish species are either truly marine fish or at least able to live in brackish water (Balearic conger, deep-snouted pipefish, smelt, 3-spined stickleback) and 3 are freshwater fish (river perch, ruffe and pumpkinseed). Thus, a relatively broad range of possible metaparatenic hosts for *Anguillicola crassus* seems to exist in every habitat which will be invaded by eels in the run of their life cycle. However, one still does not know to what extent intermediate hosts and the different categories of paratenic hosts contribute to the maintenance of the parasite's life cycle.

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