

## RESEARCH NOTE

FIRST EXPERIMENTAL OBSERVATIONS ON THE DEVELOPMENT OF *RHABDOCHONA DENUDATA* (NEMATODA: RHABDOCHONIDAE) IN THE INTERMEDIATE HOST

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**Abstract.** Two species of mayfly nymphs, *Habroleptoides modesta* (Hagen) and *Habrophlebia lauta* (Eaton) (Ephemeroptera: Leptophlebiidae), were found as suitable experimental intermediate hosts of the fish nematode *Rhabdochona denudata* (Dujardin, 1845) (Rhabdochonidae), an intestinal parasite of Palaearctic cyprinids. The parasite's eggs were obtained from nematode gravid females parasitizing the European chub, *Leuciscus cephalus* (L.), of the Rokytná River, Czech Republic. At the water temperature of 9°C, the eggs of *R. denudata* were ingested by the intermediate host and the hatched, toothed (with a minute cephalic boring tooth) first-stage larvae about 200 µm long penetrated into the body cavity of the mayfly. There the larvae grew and moulted twice (on 16–18 and 30–35 days p.i.) before attaining the third stage, which is already infective for the fish definitive host. Infective larvae of this stage were about 1.5 mm long and their morphology resembled that of adults except for the vestibule structure; they became encapsulated as in those of other congeneric species.

Although species of *Rhabdochona* Railliet, 1916 belong to the most frequent and most widely distributed parasites of freshwater fishes, very little is so far known about their development and life cycles (Moravec and Huffman 2001). In addition to few records of *Rhabdochona* larvae from naturally infected intermediate hosts, complete or incomplete life cycles have been experimentally studied in the European species *R. ergensi* Moravec, 1968 and *R. phoxini* Moravec, 1968 and in the North American species *R. canadensis* Moravec et Arai, 1971 and *R. kidderi texensis* Moravec et Huffman, 1988 (Moravec 1972, 1976, Barger and Janovy 1994, Moravec and Huffman 2001). In the Helminthological Symposium in Slovakia, Moravec (1991) presented his preliminary experimental observations on the development of another European species of *Rhabdochona*, *R. denudata* (Dujardin, 1845), but these results have not yet been published; therefore, the purpose of the present paper is to put them on record.

*Rhabdochona denudata*, the type species of the genus, is a common parasite of many species of cyprinids and some other fishes, which is widely distributed in Europe and Palaearctic Asia (Moravec 1994). Its life cycle was not previously studied experimentally. Only Shtein (1959) reported the finding of *R. denudata* advanced larvae from the naturally infected mayfly nymphs *Heptagenia* sp. and *Ephemerella* sp. in Karelia, Russia and infective larvae of this species were also recorded by Moravec (1989, 2004) from the naturally infected mayfly nymphs *Ephemera danica* Müller, *Ecdyonurus aurantiacus* (Burmeister) and *Caenis macrura* Stephens in the Czech Republic. Vojtková (1971) reported an advanced larva of *R. de-*

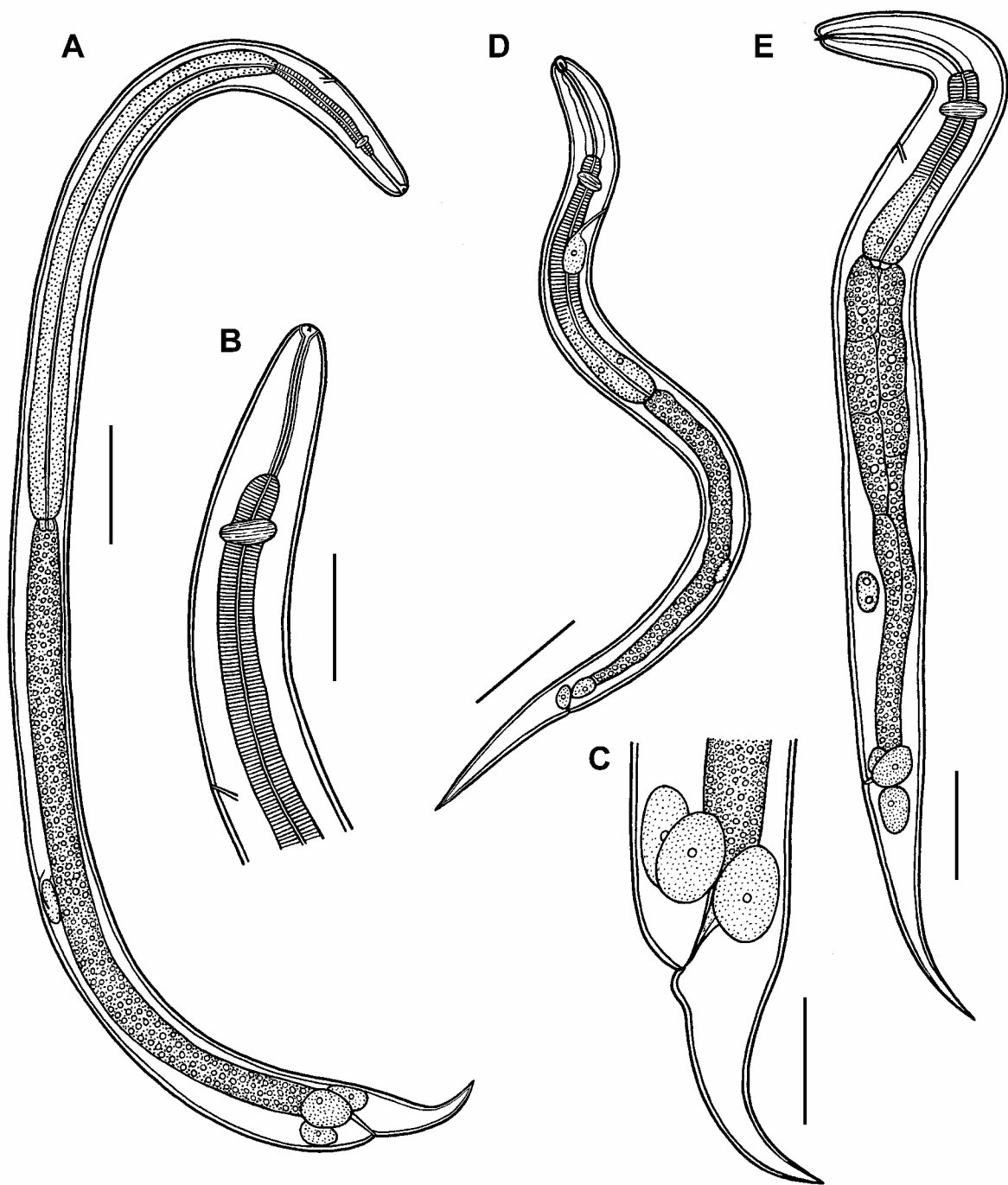
*nudata* from the caddis-fly larva *Hydropsyche* sp. from the Czech Republic, but in fact it was a misidentification of another congeneric species, *Rhabdochona hellichi* (Šrámek, 1901) (see Moravec 1995).

The methods of study of the *Rhabdochona denudata* development were the same as those described in the author's earlier papers dealing with other *Rhabdochona* spp. (Moravec 1972, 1976, Moravec and Huffman 2001). The mature eggs of the parasite were obtained from the uteri of female nematodes recovered from chub, *Leuciscus cephalus* (L.), from the Rokytná River in the Czech Republic; these already contained fully formed first-stage larvae. The parasite's eggs were placed in small Petri dishes (dia. 5 cm) with mayfly nymphs and a small amount of detritus and pieces of dry leaves. The dishes were covered with a fine nylon cloth fixed with a rubber ring and submerged into running water in large dishes. The experiments were carried out at the water temperature of 9°C. Although nymphs of several mayfly species were employed in the experiments, only two species of the Leptophlebiidae, *Habroleptoides modesta* (Hagen) and *Habrophlebia lauta* (Eaton), became infected.

After the eggs of *R. denudata* were ingested by these experimental intermediate hosts, the toothed (with a minute cephalic boring tooth) first-stage larvae of the parasite (Fig. 1D), about 200 (189–200) µm long and 9–12 µm wide, were released and penetrated through the intestinal wall into the body cavity of the mayfly. The larvae were localized primarily in the abdomen, but partly also in the thorax of the intermediate host, where they freely moved. The larvae grew (Fig. 1E) and moulted twice (on the 16–18th and 30–35th day p.i.) before they attained the third, infective stage. The third-stage larvae (Fig. 1A–C) were slender, about 1.5 mm long and 68 µm wide, and their general morphology already resembled that of adults. However, in contrast to adults, typical of them was the so-called *Cystidicola*-type of stoma with only two longitudinal lateral thickenings, projecting in form of two small conical teeth at the anterior end of prostom (Fig. 1B), and the character of the genital primordium (Fig. 1A). These larvae became encapsulated in small, transparent, thin-walled lens-shaped capsules located mainly in the abdomen and less frequently in the thorax of the mayfly.

The morphology and measurements of individual larval stages of *R. denudata* from intermediate hosts were very similar to those of *R. ergensi* and *R. phoxini* and it is practically impossible to distinguish these species on the basis of their larvae. However, as indicated by the findings of *R. denudata* larvae (reported as *Rhabdochona* sp.) from naturally infected mayflies (Moravec 1989), the third-stage larvae of this species can develop further in the intermediate host if they stay in it for a longer period and they may undergo at least one more moult. This phenomenon was called precocity by Anderson (1988) and it has been observed in several *Rhabdochona* spp. (see Gustafson 1939, 1942, Moravec 1976). Such cases were

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**Fig. 1.** *Rhabdochona denudata* (Dujardin, 1845), larvae from the body cavity of experimentally infected mayfly nymphs *Habrophlebia lauta*. **A–C** – infective third-stage larva 36 days p.i. (**A** – general view; **B** – anterior end; **C** – caudal end); **D** – first-stage larva 3 days p.i.; **E** – advanced first-stage larva 7 days p.i. Scale bars: **A** = 100  $\mu\text{m}$ ; **B**, **C** = 50  $\mu\text{m}$ ; **D**, **E** = 30  $\mu\text{m}$ .

recorded also by Shtein (1959) and Vojtková (1971) in naturally infected intermediate hosts harbouring subadult forms of *R. denudata* and *R. hellichi* (reported as *R. denudata*), respectively. In such cases, it is possible to identify the species.

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