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OBSERVATIONS ON THE EARLY DEVELOPMENT OF *TRUTTAEDACNITIS CLITELLARIUS* (NEMATODA: CUCULLANIDAE)

Anindo Choudhury and **Terry A. Dick**

Department of Zoology, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2

There is little information on the life cycles of *Truttaedacnitis* spp. parasitizing sturgeons. Khromova (1975: Zool. Zh. 54: 449–452, (In Russian)) and Ivashkin and Khromova (1976: Cucullanata and Gnathostomatata of Animals and Man and the Diseases Caused by Them. Osnovy Nematodology, 27, Publ. House Nauka, Moscow, 436 pp. (In Russian)) suggested polychaetes as intermediate hosts of *T. sphaerocephala* (Rudolphi, 1809) based on cucullanid-like larvae recovered in natural infections of *Nereis diversicolor* (Müller) in the Caspian Sea and comparisons with larval worms recovered from the intestines of naturally infected sturgeons. In contrast, there is no information on the early development or transmission of *T. clitellarius* (Skryabina, 1966), a freshwater cucullanid of North American, Siberian and north-east Asian (Amur River) sturgeons. During work on the biology of sturgeon from the Saskatchewan River delta (T.A. Dick unpubl.), fresh sturgeon viscera were purchased from commercial fishermen and the spiral-valved intestines were found infected with *T. clitellarius*.

Live female *T. clitellarius*, recovered from the spiral-valved intestines of two sturgeon, were washed in 0.6% saline and placed in fresh saline or well water for 6 hours at 12–15°C, following which they released eggs. Released eggs were uncleaved, two-celled or in the morula stages of development. Eggs were incubated in well water at 12–15° C and at room temperature (21–23°C) and those that were in the two celled and morula stages developed into larvated eggs five days after incubation at room temperature (21–23°C) and 10 days after incubation at 12–15°C. No moulting or sheaths were associated with the larvae within the eggs. Larvae became transparent and active within the eggs as they developed and only one hatched and dead larva was found after 14 days of incubation at 12–15°C.

Four individuals of *Helisoma trivolvis* (Say) and two of *Physa jenessi* (Taylor) (both Mollusca: Gastropoda) were obtained from established (three-year) laboratory cultures of these snails and exposed to approximately 50 larvated eggs of *T. clitellarius* (incubated for two weeks), in a petri dish, at room temperature (21–23°C). Snails were observed to feed by scraping off the bottom where the nematode eggs were located. After 10 minutes of exposure, snails were transferred to clean culture dishes and maintained at room temperature on laboratory cultured algal fronds and *Artemia* nauplii. Ten days post exposure, one specimen of *Physa* was dissected and the viscera squashed under a cover slip on a slide and examined under a compound microscope at 400 × magnification. Nine larvae were found associated with the viscera and one nematode began coiling and uncoiling vigorously when freed from the surrounding visceral tissue. No haemocytic host response to the larvae or encapsulation was evident. Six larvae were recovered from the hepatopancreas of one dissected and squashed specimen of *Helisoma* 12 days after exposure. One specimen of *Helisoma* was dissected and squashed on a slide 20 days after exposure and examined under a compound microscope. Five larvae were observed moving slowly in the visceral tissue and had undergone little or no development since previous examination at 12 days post exposure. Controls of *Physa* (two individuals) and of *Helisoma* (two individuals) from the same cultures as the experimental snails were dissected out from their shells and the viscera squashed and examined under a compound microscope. No nematodes were observed in these snails.

Larvae recovered from infected snails were heat killed or fixed in hot 5% buffered neutral formalin (approximately 2% formaldehyde solution) and examined in saline (0.6%) or water. Larvae that could be extracted from the visceral tissue were also fixed with hot neutral buffered 5% formalin, washed in water, cleared in a solution of 5% glycerine (= glycerol) in 70% alcohol by allowing the alcohol to evaporate and, mounted and examined in glycerine. The following description of the larvae recovered from infected snails is based on six worms cleared and mounted in glycerine and measurements are reported in μm as mean ± S.D., followed by range in parentheses: Body 587 ± 38 (520–620) long, anterior end blunt (Figs. 1, 2); tail 67 ± 6 (55–70) long and tapering with characteristic posterior end (Fig. 3); prominent alae from cervical to anal region; deirids posterior to nerve ring (Fig. 2); ventral excretory pore well anterior to nerve ring (Figs. 1, 2), excretory duct long and conspicuous (particularly in freshly killed worms) (Fig. 1); nerve ring inconspicuous in cleared specimens; prominent dorsal oesophageal gland nucleus at base of oesophagus (Fig. 2), granular dorsal portion of distal oesophagus possibly duct of dorsal oesophageal gland (Fig. 2); oesophagus 193 ± 10 (185–210) long without anterior buccal expansion but slightly enlarged posteriorly (Fig. 2); cuticular valve at oesophagus-intestine junction inconspicuous; structure resembling the hemizonid visible in two specimens.

A direct comparison of the morphology of the larvae of *T. clitellarius*, recovered from infected snails, with hatched L2 larvae of *T. pybusae* Anderson, 1992 (Pybus et al. 1978: Can. J. Zool. 56: 1420–1429) suggests that the larvae of *T.*

Address for correspondence: T.A. Dick, Department of Zoology, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2; Phone: (204) 474 9245; Fax: (204) 275 6352; E-mail: Dick@bldgduff.lan1.umanitoba.ca

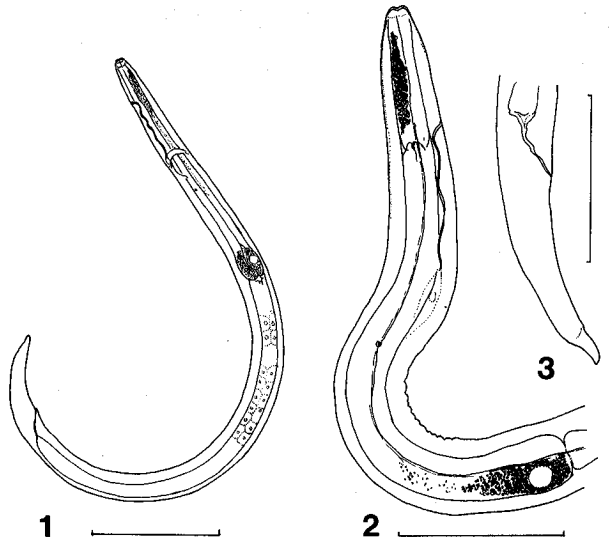

Fig. 1. Whole larva recovered from the visceral tissue of *Physa*. Only some of the intestinal cells are illustrated. Scale bar = 100 μm. **Fig. 2.** Anterior end of an unfixed freshly killed (heat killed) larva mounted in saline showing the granular anterior dorsal region of the oesophagus, dorsal oesophageal nucleus, excretory pore, deirids and lateral alae. (Oil immersion). Scale bar = 50 μm. **Fig. 3.** Posterior end of the worm in Fig. 2 showing peculiar terminal portion of the tail region and distinct rectum. Scale bar = 50 μm.

clitellarius are in the L2 stage and indicates that no development occurred within the snail host. The larvae of *T. clitellarius* described in this study are clearly distinguished from all described cucullanid larvae in possessing an excretory pore that is well anterior to the nerve ring (Figs. 1, 2). Furthermore, these larvae of *T. clitellarius* provide evidence that the anterior position of the excretory pore, which has been described in adult *T. clitellarius* (Choudhury A. and Dick T.A. 1996: Syst. Parasitol. 33: 89–99) is established early in development.

In other aspects of general morphology, the larvae of *T. clitellarius* are similar to other cucullanid larvae. For example, a granular dorsal portion of the anterior oesophagus (Figs. 1, 2) has been described from L2 larvae of *T. pybusae* (Pybus et al.; *op. cit.*), from L3 larvae of *T. truttae* (Moravec F. 1979: Folia Parasitol. 26: 295–307) and in unidentified *Cucullanus* larvae from the pericardium of the cyprinid, *Garra rufa* (Moravec F. and Rahemo Z.I.F. 1993: Folia Parasitol. 40: 145–146). The characteristic terminal region of the tail (Fig. 3) is similar to that shown in L2 larvae of *T. pybusae* (Pybus et al.; *op. cit.*). Several features present in this early larval stage of *T. clitellarius* such as the lateral alae, deirids and a prominent dorsal oesophageal nucleus (Fig. 2), also persist to the adult stage (Choudhury and Dick; *op. cit.*).

Eighteen days post-exposure of snails, two cultured lake sturgeon juveniles were each fed the viscera of one infected snail (one sturgeon was fed *Physa* and the other, *Helisoma*), by stomach intubation and subsequently maintained on a diet of ocean plancton. The infections in the snails were confirmed by gently flattening and examining their viscera. The two sturgeon juveniles were necropsied 45 and 62 days post intubation. No larvae were recovered from the gut lumen, the body cavity or the liver of the sturgeons, and there was no evidence for encapsulation in any of the organs (a histotropic phase is known for some cucullanids).

Little is known about the life cycle or larval development of most species of cucullanids (Anderson R.C. 1992: Nematode Parasites of Vertebrates. Their Development and Transmission. Commonwealth Agricultural Bureau International, Wallingford, 578 pp.). Life cycle studies on two other species of *Truttaedacnitis* do not allow any generalizations since *T. truttae* is transmitted to salmonids in Eurasia by lampreys harbouring L3 larvae (Moravec, 1979, *op. cit.*; Butorina T.E. 1988: Biol. Morya 4: 66–67 (In Russian)) while *T. pybusae* has a monoxenous life cycle in North American lampreys with a histotropic phase of development. However, Pybus et al. (*op. cit.*) suggested that the life cycle of *T. pybusae* was essentially heteroxenous because the same individual definitive host also acts as the intermediate host. Studies on other species of cucullanids indicate that the life cycle may involve intermediate fish hosts (in *Dichelyne cotylophora* (Ward et Magath, 1917), *Cucullanus* sp. – Baker M.R. 1984: Can. J. Zool. 62: 2062–2073; Moravec and Rahemo; *op. cit.*) or may be direct with a histotropic phase (in *Cucullanus cirratus* Müller, 1777, *C. chabaudi* Le-Van-Hoa et Pham-Ngoc-Khue, 1967 – Gibson D.I. 1972: Bull. Brit. Mus. Nat. Hist. Zool. 22: 153–170; Le-Van-Hoa and Pham-Ngoc-Khue 1967 – Bull. Soc. Pathol. Exot. 60: 315–318 (In French); Valovaya M. 1979: Parazitologiya 13: 540–544 (In Russian)).

Gibson (*op. cit.*) suggested a number of possibilities in the life cycles of cucullanids including 1) a free-living phase of growth of the hatched larvae, 2) earlier site of infection in the definitive host and 3) presence of an intermediate host. Of these, a free-living growth phase can be ruled out in *T. clitellarius* since hatching was rare and a hatched larva was found dead soon after hatching. An earlier site of infection of the definitive host was considered and the livers and gut of the sturgeon fed snails were examined by squashing but no larvae were observed. However, it is possible that the larvae of *T. clitellarius* in snails are not infective to the definitive host (sturgeons) since they are L2 larvae and that gastropods act as paratenic hosts in transmitting these larvae to other intermediate hosts, e.g., fish, where development to the infective stage occurs. Fish form an important part of the diet of some acipenserids (*Huso* spp., *A. stellatus*, *A. gueldenstaedti*, *A. transmontanus*) and a small but constant part of the diet of most acipenserids (Holčík J. 1989: The Freshwater Fishes of Europe. Vol. 1, Pt. II. General Introduction to Fishes. *Acipenser*. AULA-Verlag, Wiesbaden; Scott W.B. and Crossman E.J. 1973: Bull. Fish. Res. Bd. Can. 184, 966 pp.). Potential intermediate fish hosts in our study area (central Canada) include sculpins (Cottidae) since they were found in the diet of lake sturgeon surveyed from this area (Choudhury A. and Dick T.A. 1993: J. Fish Biol. 42: 571–584).

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