outcome may ultimately be fatal. More work is needed to understand the mechanisms that initiate these cuticular lesions of nematode parasites and the role they might play, if any, in causing the expulsion of these parasites from their respective hosts.

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Research Note

Eustrongylides sp. (Nematoda: Dioctophymatoidea): First Report of an Invertebrate Host (Oligochaeta: Tubificidae) in North America

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As adults, nematodes of the genus *Eustrongylides* parasitize piscivorous birds. Infection results in mortality of the avian host (Locke, 1961, Avian Dis. 5:135–138; Locke et al., 1964, Avian Dis. 8:420–427). Third- and especially fourth-stage larvae of *Eustrongylides* spp. are known to parasitize a wide variety of vertebrate intermediate hosts. Freshwater fish are the most common vertebrate intermediate hosts, but fourth-stage larvae from fish will invade the tissues of various reptilian and mammalian hosts (including man) if infected fish are ingested uncooked. An invertebrate as the first intermediate host has been suspected, but has only been confirmed for *Eustrongylides excisus* Jägerskiöld, 1909 in freshwater oligochaetes in the delta of the Volga River (Karmanova, 1965, Trudy Gel'mint. Lab. Akad. Nauk SSSR 15:86–87).

In the Chesapeake Bay area of the United States a frequent vertebrate intermediate host for *Eustrongylides* is the benthic mummichog, *Fundulus heteroclitus* (L.). A recent study of the prevalence of larval *Eustrongylides* in the mummichog (Hirshfield et al., 1983, J. Fish Biol. 23: 135–142) led those authors to hypothesize that an increased prevalence of the nematode in mummichogs in the warmer waters of a power plant might be related to an increased abundance of oligochaetes, the suspected first intermediate hosts. The present report is part of a study of oligochaetes undertaken in areas where mummichogs were infected to determine whether the oligochaetes were infected with larval *Eustrongylides*. This report describes the single third-

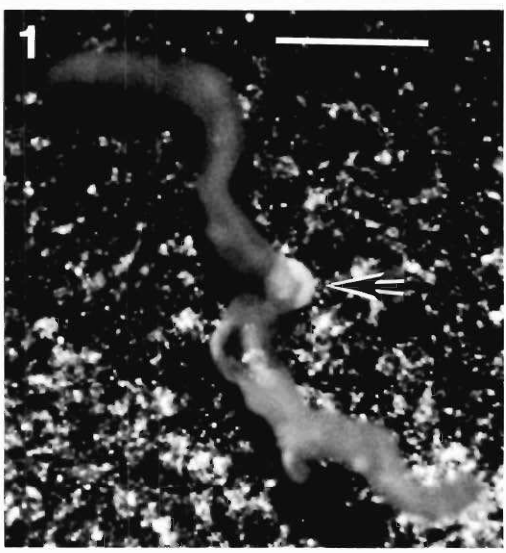
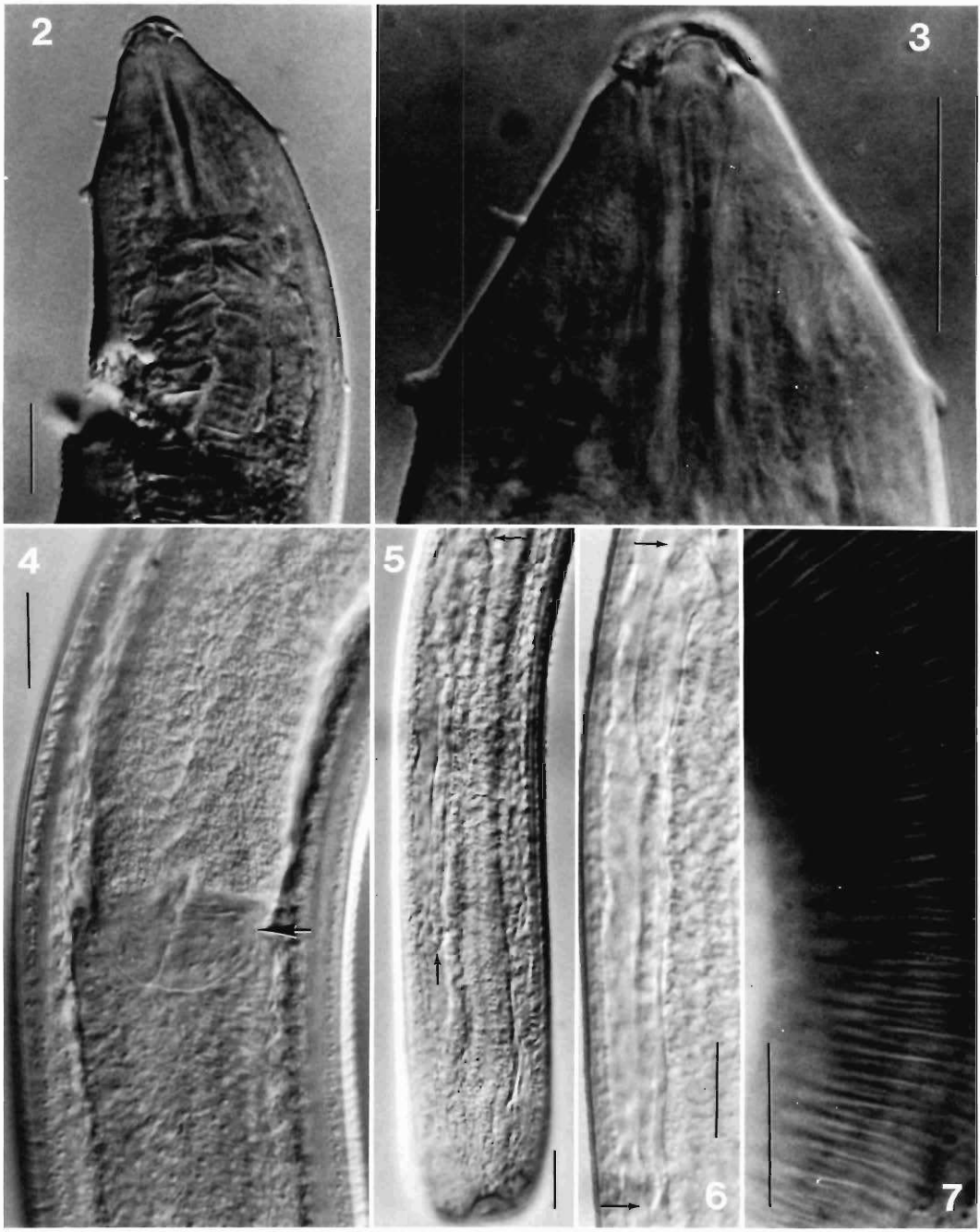


Figure 1. Oligochaete (Tubificidae) ruptured by parasitic nematode, *Eustrogylides* sp. (third-stage larva) (arrow); scale bar, 1 mm.



Figures 2–7. ***Eustrongylides*** **sp. third-stage larva from tubificid oligochaete. Scale bars, 25 μm. 2. Anterior end showing conical head and tubular buccal capsule. 3. Anterior extremity showing spine-like papilla of internal circle and lobe-like papilla of external circle. 4. Esophageal-intestinal valve (arrow) separating esophagus (with granules) from intestine. 5, 6. Posterior end; arrows at anterior flexure and blind terminus of immature reproductive tract (6, higher magnification). 7. Cuticle showing irregular annulation.**

stage larval *Eustrongylides* sp. recovered in the study. This is the first report of a natural infection of an oligochaete by *Eustrongylides* in North America.

The oligochaete host was collected August 16, 1983 from a subtidal fringe marsh in Quantico Creek, near the Possum Point Power Station. Quantico Creek is a small shallow tributary of the Potomac River between Occaquan Bay and Aquia Creek on the Virginia shore. This area is transitional between fresh and oligohaline water (salinity varies annually between 0.0 and 0.5 ppt). The sediment was 95% sand, 5% silt/clay and contained a large amount of organic detritus. A total of 1,767 oligochaetes was collected from seven locations throughout the Maryland portion of the Chesapeake Bay of which 422 were collected at Quantico Creek. Only one of 1,767 oligochaetes examined was infected, and it contained a single *Eustrongylides* third-stage larva (Fig. 1). Because the infected oligochaete was immature and damaged by the nematode, it could not be identified past the family level. The oligochaete was classified as a Tubificidae without capilliform chaetae. All of the mature tubificids collected in the sample were *Limnodrilus* spp., with approximately half identified as *Limnodrilus hoffmeisteri* Claparede and half as *Limnodrilus cervix* Brinkhurst (both typical and variant forms). Therefore, the infected oligochaete was probably a *Limnodrilus*. The remains of the oligochaete and the nematode have been deposited in the U.S. National Parasite Collection, USDA, Beltsville, Maryland as USNM Helm. Coll. No. 78225.

The identification of *Eustrongylides* to species is based on characteristics of adults so we must refer to our larval specimens as *Eustrongylides* sp. However, von Brand and Simpson (1944, J. Parasitol. 30:121–129) obtained an adult male *Eustrongylides ignotus* Jägerskiöld, 1909 from an in vitro culture of larval specimens from *Fundulus heteroclitus* collected in the Chesapeake Bay area. This appears to be the only species of *Eustrongylides* that has been identified from this area.

Fundulus heteroclitus is a non-selective benthic feeder, gaining its energy from animal matter (Prinslow et al., 1974, J. Exp. Mar. Biol. Ecol. 16:1–10), and it is likely that *F. heteroclitus* consumes oligochaetes proportional to their abundance in the study area. However, the feeding habits of *F. heteroclitus* have not been studied in areas of low salinity such as the study area. The low incidence of infection in oligochaetes in the area sampled should not rule them out as normal intermediate hosts for *Eustrongylides*. If large numbers of the oligochaetes are eaten by the fish, a low incidence of infection in the oligochaetes may still be effective in establishing a high incidence of infection in the fish. Such a relationship was demonstrated for a tapeworm, *Echinococcus multilocularis* Leuckart, 1863, with a low incidence of infection in rodents and a high incidence in foxes (Rausch and Schiller, 1951, Science 113:57–58).

The third-stage nematode recovered from the oligochaete is 3.89 mm long with a conical anterior extremity and a bluntly rounded posterior extremity. The mouth is dorsoventrally elongated with one large, broad, lateral lip on each side, and with single, shorter and narrower dorsal and ventral lips. The buccal capsule is thin and 65 μm long (Fig. 2). The conical anterior extremity bears two circles of cephalic papillae. The lateral papillae of both internal and external circles are located more anteriorly than the subventrals and subdorsals. The subdorsal and subventral pairs of the internal circle of six spine-like papillae are located slightly more than half way from the dorsoventrally elongated mouth to the external circle of papillae, which is located 47 μm posterior to the anterior extremity (Figs. 2, 3). The six papillae of the external circle are lobe-like, broader, and protrude less than those of the internal circle (Figs. 2, 3). The esophagus is 1.17 mm long, uniformly thick, occupies two-thirds of the body diameter, and has a prominent cuticular lining and an esophageal–intestinal valve (Fig. 4). The posterior half of the esophagus is full of granules (Fig. 4). The nerve ring is near the anterior end of the esophagus, 100 μm from the anterior extremity. The lumen of the intestine is open and the anus is terminal and recessed (Fig. 5). A reproductive system extends anteriorly 300 μm before reflexing to a narrow blind terminus (Figs. 5, 6). The cuticle is annulated irregularly (Fig. 7). Somatic papillae are not visible on the surface of the cuticle with oil immersion, perhaps due to the annulation, but their presence is indicated by lateral rows of subsurface nuclei in optical section.

Descriptions of third-stage larvae of *Eustrongylides* spp. are extremely rare. To our knowledge

the only previous descriptions are: 1) third-stage *E. excisus* by Karmanova (1965, loc. cit.); 2) third-stage *E. tubifex* by Sprinkle (1973, Thesis, Ohio State Univ. 60 pp.) and by Crites (1982, Clear Technical Report No. 258, Ohio State Univ., Ctr. for Lake Erie Area Res., Columbus, Ohio, July 1982, 83 pp.); and 3) third-stage *E. mergorum* (Rudolphi, 1809) by Fagerholm (1982, Acta Acad. Aboensis, Ser. B 40:11–19). Recently, two third-stage larval *Eustrongylides* sp. from *Fundulus* sp. collected in the Chesapeake Bay area became available for study. They will be described elsewhere (Lichtenfels and Pilitt, 1986, Proc. Helminthol. Soc. Wash. 53. In press) along with a discussion of characters useful for separating third- and fourth-stage *Eustrongylides* sp.

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Research Note

Methods for Long-Term Collection of Fish Feces

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The collection of helminth eggs and protozoan spores from the feces of fish over an extended period is often required in parasite life history studies. For fish several centimeters in length, a separation funnel apparatus can be modified to accommodate a fish (Fig. 1). If a benthic or benthopelagic host is used, a plastic screen can be placed on the bottom of the funnel. The grid must be large enough to allow fecal material to pass through to the collection tube. This method, however, may not be suitable for larger fish or fish that would not readily adapt to the above apparatus. For benthic and benthopelagic hosts, a rubber sheath can be used to collect fecal material. Fish approximately 15 cm in length can be anesthetized and placed in a nonlubricated condom (Fig. 2). Smaller fish can be placed in a physician's finger cot. A small split-shot fishing weight should be placed in the end of the sheath to prevent the tip from floating. The method of securing the sheath depends upon the species of host. Usually the sheath's collar can be secured between the pectoral fin and the opercular cover by a rubber band. Care should be taken that the band not be so tight as to injure the host. The pectoral fin can be placed through a small cut in the sheath. If necessary, the sheath can be further attached by spot glueing (Histoacryl, Tri-Hawk, Los Angeles, California) the collar to the fish. To guard against possible bacterial infection or accelerated fecal decomposition, antibiotics can be added to the sheath without affecting the viability of helminth eggs or protozoan spores. The caudal fin should be checked for pathology due to compression. In a helminth life cycle experiment using *Gillichthys mirabilis* the sheaths were removed and the contents collected weekly for 3 wk with no apparent pathology to the host. The helminth eggs were separated from the feces and mucus using standard concentration techniques.

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