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Development of Oral Structure in *Salmonema ephemeridarum* (Nematoda: Spirurida: Cystidicolidae)

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The morphology of the oral region is important in the identification of adult and larval parasitic nematodes. This is nowhere more true than in the assignment of cystidicolid nematodes of fishes to one of approximately 23 genera. However, the small size of the anterior end of these worms has complicated identification of worms (Moravec 2007) and resulted in a poor understanding of the morphology of oral structures, including the oral structure of infective/third stage larvae/juveniles, which are found in crustacean and insects. The purpose of this study is to utilize Scanning Electron Microscopy (SEM) to document the morphogenesis of oral structures of the cystidicolid nematode *Salmonema ephemeridarum* (Linstow 1872) and to provide information on the certainty of the identification of third stage larva to genus and species.

Adult female worms were collected from brook trout, *Salvelinus fontinalis* (Mitchell 1814), captured in Rocky Saugeen River, Ontario, Canada in June 1987. Larvated eggs were fed to larval mayflies (Ephemeroptera) collected from the Eramosa River where it passes under Stone Road, Guelph Ontario (43.547363, -80.1997499). Mayflies successfully infected included *Stenonema ithaca*, *Stenonema* sp. and *Isonychia* sp. No nematode larvae were found in 60 mayflies collected at this locality, and no salmonids are present in this portion of the Eramosa River (Osmond 1971). Mayflies were held in an aquarium at 21°C and examined for parasite larvae at intervals for 62 days. Adult worms used for SEM were from natural infection of the brook trout. Worms processed for SEM were cleaned in saline preserved in 1.25% glutaraldehyde, post-fixed in 1% osmium tetroxide, dehydrated in an ethanol series, critical point dried using CO₂, mounted on stubs, coated with gold palladium alloy and viewed with a Jeol (Model JSM 35-C) scanning electron microscope. General terminology follows Anderson et al. (2009) and terminology of the oral morphology of third-stage and adult worms follows Appy (1981).

First stage larvae, present one to five days post infection (dpi), possessed an oral opening, a pore, and a hooked tooth (Fig. 1A). Second stage larvae (11 to 17 dpi) possessed a smooth circular oral opening, with presumptive lateral amphids (Fig. 1B). Oral structures of the third stage larva (>17 dpi) are visible inside the oral opening of some molting second stage larvae. The oral structure of third stage larvae consisted of a dorsoventrally elongate oral opening with broad, relatively flat pseudolabia emanating from the cuticular lining of the buccal cavity (stoma) (Fig. 1C). The sublabia appear contiguous ventrally and dorsally and their apical surface appears smooth. Four cephalic papillae and amphidial openings are visible. The oral morphology of adult worms is similar to third stage larvae except that in adults the sublabia appear disconnected ventrally and dorsally and have an indentation forming two lobes (Fig. 1D).

While SEM has become more common in the descriptions of adult cystidicolids (Appy 1981; Ko 1986; Moravec 2007) there are only a few cases where SEM has been used to describe third stage larva (Appy and Dadswell 1983; Moravec et al. 2003) and no studies have depicted first- and second-stage larvae. The boring tooth in first-stage larva was previously identified in light microscopic studies as a refractile body (Moravec 1967; Appy and Dadswell 1983) and is presumably used to penetrate the gut wall and gain access to the body cavity and migrate into the

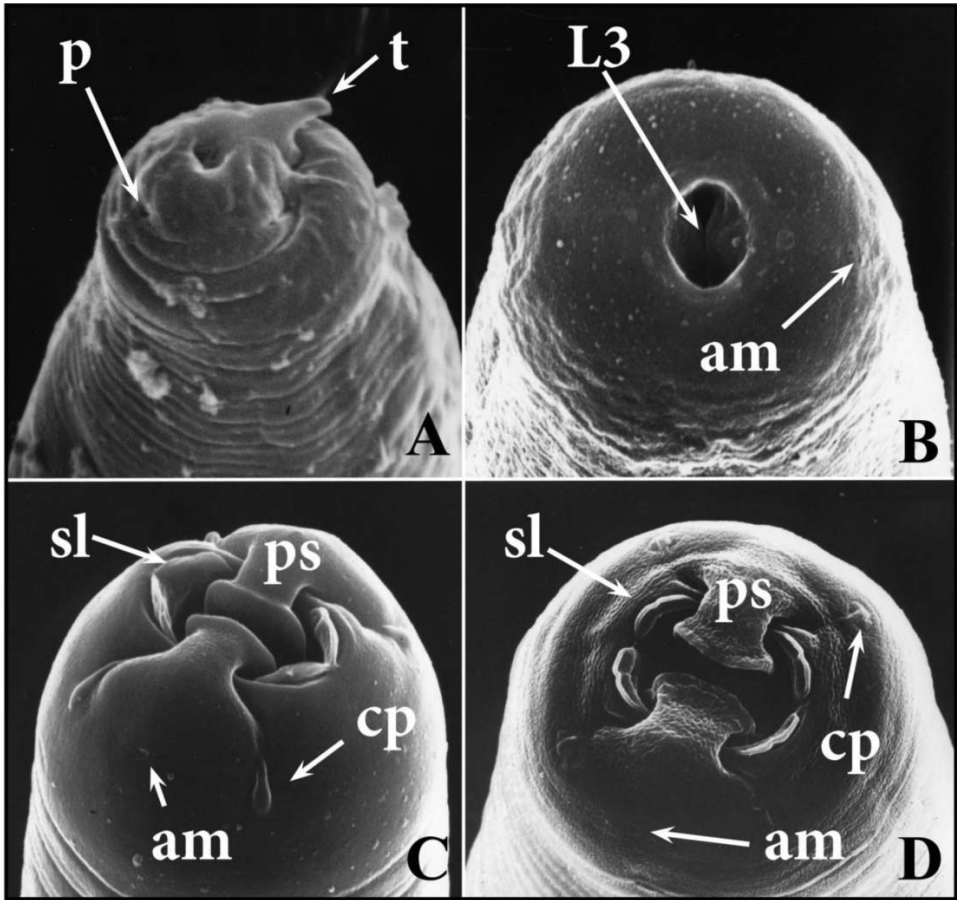


Figure 1. Scanning electron micrographs of the oral end of *Salmonema ephemeridiarum*: first stage larva (A), second stage larva (B), third stage larva (C) and adult (D). am = amphid, cp = cephalic papilla, L3 = third-stage larva, p = pore, ps = pseudolabium, sl = sublabia, t = tooth.

muscle tissue of the mayfly. The pore adjacent to the oral opening most likely provides a means for the larvae to release chemicals from glandular cells located adjacent to the oral opening; such cells have been previously identified in spirurid nematodes (Quentin and Poinar 1973). The second stage larvae, which are short-lived in the intermediate host, have simpler oral regions.

The oral morphology of third stage larvae are very similar to adult worms although the sublabia of adult worms appear to be more clearly separated and have a more defined notch in the anterior margin. SEM studies on larval stages of the allied genera *Capillospirura* and *Ascarophis* (Appy and Dadswell 1987; Appy and Butterworth 2011, respectively) also show the oral morphology of third stage larvae to be similar if not identical to the adult stage. As a result it is anticipated that third stage larvae of other cystidicolids found in invertebrates can be assigned to a genus and possibly to species in localities where the morphology of adult worms is well understood.

Cystidicolid nematodes are frequently associated with precocious development, including continued growth of infective larvae to a relatively large size and advanced development of reproductive structures in the intermediate host (Smith and Lankester 1979; Anderson and

Bartlett 1993; Anderson 2000). At least one species of *Ascarophis* has been experimentally shown to develop to the adult stage in the invertebrate host (Fagerholm and Butterworth 1988; Appy and Butterworth 2011). It is apparent that this precocious development of reproductive structures, which is thought to enhance transmission (Anderson and Bartlett 1993), also applies to somatic development of the worm including feeding structures, which are complete or nearly complete at the third stage in the intermediate host.

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