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Larval Nematodes Parasitic in Shellfish

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There has been an increased interest in the parasitology of marine mollusks in recent years, especially pertaining to those species that are of economic importance. From the standpoint of shellfish biologists, this interest stems primarily from the possible effects of parasites on these mollusks; however, there is another reason why such parasites are important. Specifically, since several species of mollusks, such as certain oysters and clams, are commonly consumed by humans in the raw or barely cooked state, the potential of transmission of such parasites from mollusks to humans and their establishment in man must be considered. Thus, the pragmatic importance of parasites harbored by shellfish involves the focal interest of shellfish biologists as well as that of the public health-oriented parasitologist.

A third reason for the increased interest in parasites of mollusks rests with the fact that zooparasites are useful as antigens in studies designed to elucidate the immunologic responses of mollusks. This is especially true if the intent is to contribute to our understanding of the process of encapsulation, commonly known as granuloma formation. In such instances, the helminths are particularly useful.

As indicated by the title of this presentation, my remarks will be limited to larval nematodes, a group of parasites of marine mollusks that has been sadly neglected. I shall commence by stating that, in my opinion, the reasons why there is a dearth of published information pertaining to this category of parasites are because 1) larval nematodes are extremely difficult to identify, and 2) many are tissue, rather than luminal, parasites, and consequently require special procedures to isolate which are not routine, e.g., enzyme digestion and concentration.

Only a few species of nematodes have been reported from commercially important marine mollusks, although they have been recorded from a number of terrestrial, freshwater, and a few commercially unimportant marine mollusks (Gerichter, 1948; Pelseneer, 1928). Abbreviated accounts of the known species in marine shellfish are presented below. For those interested in identification, redescriptions of these species have been presented earlier (Cheng, 1967).

ECHINOCEPHALUS UNCINATUS MOLIN, 1858

Molin (1858) erected the genus *Echinocephalus* to include *E. uncinatus* found in the spiral valves of the elasmobranch *Trygon brucco* in the Adriatic Sea. The original description was based on two distinct types of specimens. Molin designated one specimen, 7.0 mm long with 6 rows of cephalic hooks, as the female, and two others, 24-35 mm long with 30 rows of hooks, as males. Later, Baylis and Lane (1920) considered Molin's males to be identical with *E. spinosissimus*, and the female as representative of *E. uncinatus*.

As a result of studies by Millemann (1963), it is now generally agreed that the specimens of *Echinocephalus* studied by Molin (1858) and Baylis and Lane (1920) actually represent the larval forms of species, the adults of which are yet unknown. Also, Millemann has stated that the early juvenile stages (L_1 and L_2) of *Echinocephalus* spp. have 8 rows of cephalic hooks, which can only be appreciated by examining en face preparations. The definitive number of rows of such hooks is not attained until the L_3 stage. Millemann's interpretation is that Molin's males represent the true *E. uncinatus* and considers *E. spinosissimus* as a synonym.

Larval *E. uncinatus* have been reported in several species of marine mollusks. Specifically, von Linstow (1904) found it encysted in the adductor muscle of the pearl oyster, *Margaritifera vulgaris*, in Ceylonese waters, and Baylis and Lane (1920) reported it in the pelecypod *Pinna* sp. In addition, Hopkins (1935) reported what he believed to be the larva of *E. uncinatus* collected by Van Cleave in the gonad of a sea urchin, *Arbacia punctulata*, at Woods Hole, Mass., and Johnston and Mawson (1945) reported the occurrence of this larva in the gastropods *Polinices conica* and *Katylesia scalarina* from Australia. It is noted that there is some doubt whether these larvae are all *E. uncinatus*. Nevertheless, these few reports do serve to point out that the larvae of *Echinocephalus* spp. occur in a variety of marine invertebrates, among which mollusks are apparently a common group. The definitive host of *Echinocephalus* spp. is usually an elasmobranch (Yamaguti, 1961).

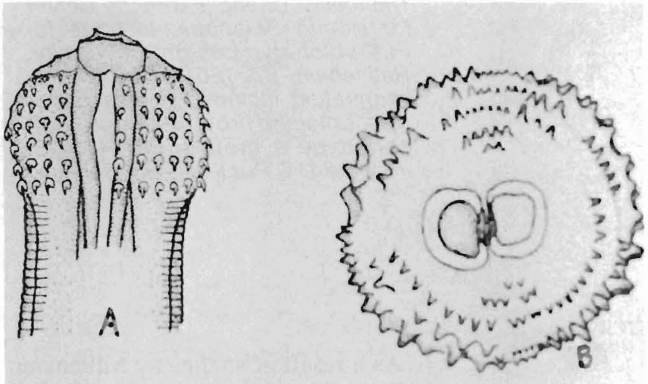

Figure 1.—*Echinocephalus pseudouncinatus*. (A) Anterior end of second-stage larva, lateral view; (B) en face view of head of second-stage larva. (Redrawn after Millemann, 1963).

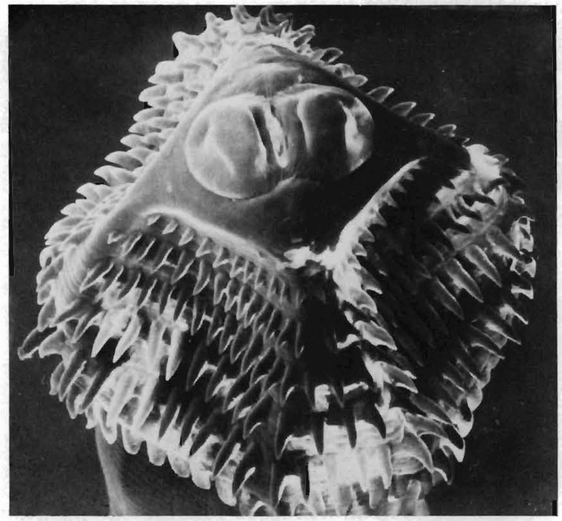

Figure 2.—Scanning electron micrograph of cephalic end of third-stage larva of *Echinocephalus crassostreai*. 400×.

ECHINOCEPHALUS PSEUDOUNCINATUS MILLEMANN, 1951

This species (Fig. 1) was described by Millemann (1951) based on larvae found encysted in the foot of the pink abalone, *Haliotis corrugata*, collected at San Clemente Island in southern California. In addition, it also occurs in the southern green abalone, *Haliotis fulgens*.

Echinocephalus pseudouncinatus larvae burrow into the foot of abalones where they encyst in the ventral portion. This results in a blisterlike protrusion on the foot. In addition, the burrowing motions of the larvae prior to encystment apparently weakens the foot musculature and decreases its efficacy as a hold-fast structure. As a result, parasitized abalones are readily detached from rocks.

It is noted that Millemann (1951) has reported that only old abalones are parasitized. It remains unknown whether this means that younger ones are refractile or if the parasite prefers older mollusks.

ECHINOCEPHALUS CRASSOSTREAI CHENG, 1975

The definition of this species (Fig. 2) by Cheng (1975a) was based on specimens of second- and third-stage larvae from the Japanese oyster, *Crassostrea gigas*, collected from oyster beds off the coasts of Hong Kong and The People's Republic of China.

The L_3, which was the most commonly encountered stage, occur primarily in the gonoducts of the molluscan host.

Although examination of routinely stained sections of parasitized oysters revealed only slight pathological changes, for example the development of a tunic of reaction elements surrounding the parasite-enclosing gonoduct (Cheng, 1975a), histochemical studies have revealed considerably more. It has been found that the reaction tunic is comprised of connective tissue fibers, which are comprised, at least in part, of complex carbohydrates that are sulfated and rich in acidic groups; tightly packed Leydig cells, which include glycogen and carboxylated polyanions; glycogen-containing hemocytes, which also enclose carboxylated polyanions; brown cells, and myofibers. Of these elements, only the myofibers are native to this region (Cheng, 1975b).

The presence of *E. crassostreai* larvae in the gonoductal vestibule results in hyperactivity and secretion of gland cells embedded in epithelial lining. Furthermore, if larvae should invade the gonad, some damage is inflicted on adjacent molluscan gametes.

Because of the structural separation of the intragonoductal parasites from the periductal reaction complex, Cheng (1975b) advanced the hypothesis that the reaction is stimulated by molecules secreted and/or excreted by the nematodes and which are able to permeate through the lining epithelium and subtending basal lamina. I wish to emphasize this interpretation since insufficient attention, in my opinion, has been placed on pathogenesis resulting from parasite-secreted molecules in invertebrate hosts. Of course, direct evidence for such deleterious molecules must rest with their isolation, characterization, and studying their effects experimentally.

Echinocephalus crassostreai serves as an example of a parasite which is of public health importance. Ko et al., (1974) demonstrated that L_3 from oysters, upon ingestion by a cat and a rhesus monkey, penetrated the stomach and intestines. This is not totally surprising since the genus *Echinocephalus* is a member of the family Gnathostomatidae, which also includes *Gnathostoma*, the larvae of which genus are well known to cause gastric and other types of granulomatous lesions in humans if accidentally ingested.

SULCASCARIS SULCATA (RUDOLPHI, 1819)

Cobb (1930) described *Paranisakis pectinis* based on a single immature specimen found in the visceral mass of a scallop, *Pecten,* collected at Beaufort, N.C. Gutsell (1930) found the same nematode in *Aequipecten maximus* collected from the same location. Years later, Hutton (1964), while examining the bay scallop, *Aequipecten gibbus,* collected off the east coast of Florida, found numerous specimens of a larval nematode which he identified as Cobb's worm. However, as the result of finding a short, anteriorly projecting caecum, Hutton transferred it to the genus *Porrocaecum.* It is noted, however, that this nematode has been tentatively designated as *Paranisakiopsis pectinis* by Perkins et al. (1975) who reported its occurrence in the foot and adductor muscles of the surf clam, *Spisula solidissima,* from Virginia and North Carolina. Since then, J. Ralph Lichtenfels has informed me that this nematode has been identified by J. F. A. Sprent as the larva of *Sulcascaris sulcata,* the adult of which is a parasite of loggerhead turtles, *Caretta caretta,* and the green turtle, *Chelonia mydas.* This has been confirmed by Lichtenfels. Also, Lichtenfels has informed me that the L_3 of the nematode also occurs in the whelk, *Busycon caniculata,* and the moon snail, *Lunatia heros.*

Earlier, Cheng (1967) reported larval *S. sulcata* (Fig. 3) in the adductor muscles of *A. gibbus* and *A. irradians* off the coasts of North Carolina, South Carolina, and Florida, and later (Cheng, 1973) reported this larval nematode in 2.3 percent of 400 specimens of *A. irradians* from off the coast of North Carolina. The larvae were commonly, although not always, found in the adductor muscle, causing it to take on a brownish color. Furthermore, the presence of more than one larva, commonly two to four, causes sufficient damage to the muscle that much of the tonicity was lost. It is now known the brownish color associated with this parasite is, at least in part, due to *Urosporidium spisuli,* a pigmented hyperparasite (Perkins et al., 1975).

The occurrence of larvae of *S. sulcata* in scallops and other species of marine mollusks is also of potential public health significance since the larvae of certain species of related genera belonging to the family Anisakidae are known to cause what has been designated as anisakiasis in humans if accidentally ingested (see Jackson, 1975 and Cheng, 1976 for reviews). It is noted that Norris and Overstreet (1976) have reported the occurrence of the related anisakid *Thynnascaris* in three species of marine mollusks, the gastropods *Cantharus cancellarius* and *Thais haemastoma floridana* and the cephalopod *Lolliguncula brevis.* In addition, Dolgikh (1966) has reported another related anisakid, *Thynnascaris adunca (=Contracaecum aduncum),* in the marine gastropods *Cyclonassa neritea* and *Nassa reticulata* in the Black Sea. Valter (1968) has reported success at experimentally infecting the gastropod *Margarites groenlandicus* with the same parasite, and Kikuchi et al. (1969, 1972) and Shiraki (1969) have reported *Contracaecum* larvae in the cephalopod *Todarodes pacificus* in Japanese waters.

The life cycles of anisakids are highly complex and still little understood (Cheng, 1976). The definitive hosts in the case of those larvae that have been found in marine mollusks, including commercially important shellfish, could be a fish, a bird, or a marine mammal. If it is a homeotherm, then the larvae in mollusks are potentially infective to humans (Cheng, in press).

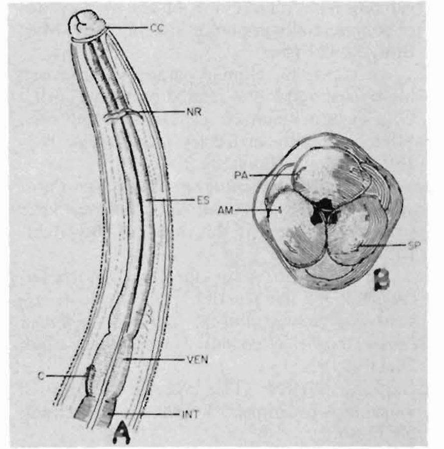

Figure 3.—*Sulcascaris sulcata.* (A) Lateral view of anterior portion of larva from *Aequipecten gibbus;* (B) en face view of anterior terminal of larva. (Redrawn after Cobb, 1930.) AM, amphid; C, caecum; CC, cephalic constriction; ES, esophagus; INT, intestine; NR, nerve ring; PA, papilla; SP, submedian papilla; VEN, ventriculus.

COMMENTS

As indicated by the brief review presented, only a few species of nematodes have been reported as naturally occurring parasites of commercially important marine shellfish, and all of these have been larvae, mostly L_3. This does not mean that nematode parasitism is rare in shellfish. Cheng (1976), for example, has noted the occurrence of an unidentified species embedded in the Leydig tissue of *Crassostrea virginica* collected in St. Mary's Creek, St. Mary's County, Md.

As stated, it is suspected that the reasons no more have been reported are 1) because larval nematodes are difficult to identify, and 2) the recovery of nematodes from mollusks require nonroutine procedures.

Except for *Echinocephalus crassostreai* in *Crassostrea gigas,* detailed pathological effects, both morphological and physiological, inflicted by these helminths on their molluscan hosts have not yet been studied. Nevertheless, the ability of *Echinocephalus pseudouncinatus* and *Porrocaecum pectinis* to weaken the adductor muscles of their pelecypod hosts renders them of importance to the shellfish industry.

Finally, since certain species of shellfish are consumed either raw or in the partially cooked state, the occurrence of larval nematodes in them represents potential public health hazards. The danger is real in view of what is known about the pathogenicity of *E. crassostreai* to mammals and human anisakiasis.

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