On a microphallid metacercaria occurring in the ovaries of the sand crabs *Emerita asiatica* and *Albunea symnista* on the Madras Coast

SITA ANANTARAMAN AND T. SUBRAMONIAM

*University Zoology Laboratory, Madras 600005*

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**ABSTRACT**

A microphallid metacercaria resembling that of *Microphallus nicolli* (*Speiotrema nicoli*) Baer 1943, is reported from the sand crabs, *Emerita asiatica* and *Albunea symnista*, on the Madras Coast and tentatively identified as belonging to the genus *Microphallus*.

The larva is enclosed in a 3-layered spherical cyst, clothed with minute spines in the anterior two-thirds of the body, and shows short, blunt, postero-laterally directed intestinal crura, oval testes overlapped by large lobulated masses of vitellaria, a male genital papilla with no cuticular pockets, and a Y-shaped excretory vesicle.

High infection rates in the hosts were observed, all of 144 *E. asiatica* (16-31 mm long) and 22 of 23 *A. symnista* (13-27 mm long) examined between July and August 1974 having been infected in varying intensities. Strangely, only the female crabs harboured the metacercariae, in the connective tissue of the ovaries, the males being free. The ovary appeared to be the primary seat of infection, though parts of the liver may be involved. Neither the first intermediate host (gastropod), nor the definitive host (shore-birds?) is yet known.

1. **INTRODUCTION**

Studies on the life-histories of microphallid trematodes of shore-birds have demonstrated the involvement of various crustacean and xiphosuran second intermediate hosts, such as crabs, sand crabs, barnacles, and the king crab.1–11 Of these the sand crab, *Emerita analoga*, had been described as the host of the metacercaria of *Levisensiella cruzi* (?) by Young1 in the La Jolla region, California, USA. The metacercariae, occurring in dozens, were present in the connective tissue, chiefly of the liver lobules, of every crab over 6 mm in size examined.
Young obtained the cercarial stage of this parasite from the gastropod *Olivella biplicata* and identified it as a xiphidiocercaria of the Ubiquita type of Lebour. The metacercariae were tentatively assigned to the species *Levinseniella cruzi* of shore-birds *Limoso fedosa* and *Cataptrphorus semipalmatus ornatus*, as experimental transfer of the worm from the sand crab to the bird host was not attempted. The development of the cercaria into the metacercarial stage was followed in three sets of experiments in the crabs. All crabs over 6 mm long had natural infections with metacercariae, while those of 5 mm length or less were less frequently infected and revealed the presence of recently entered cercariae. Within two or three days of entry, the cercaria secreted a cyst within which it lay curled up as a metacercaria. Collectively, in the three sets of experiments, 31 of 62 tests showed successful infection, as against 6 of 46 in the controls. He also observed that larvae similar to those in *Emerita analoga* occurred in fiddler crabs, *Uca crenulata*, of the Mission Bay, near La Jolla, and in many other crabs in the vicinity.

Later, while describing a new species of *Levinseniella, L. charadriformis*, from California shore-birds, Young reviewed his earlier work and revised the tentative identification of *L. cruzi* as of *Spelotrema nicolli*, though it differed “in certain minor details”. He noted that the presence of all the four species of microphallids, one of *Maritrema*, two of *Spelotrema*, and one of *Levinseniella*, in shore-birds which had “recently fed on *Emerita* and of metacercariae excysting in the stomachs of the birds, is suggestive of the source of infection, but does not show which of the parasites is derived from this source, or whether more than one is so derived”. A captive specimen of the stone curlew (*Burhinus*), a charadriform bird, fed with sand crabs on several occasions between July 25 and August 15, 1940, was found on August 21, to harbour many adult worms, and numerous lately excysted juveniles of *Spelotrema*, apparently *S. nicolli*.

Ching referred to the studies of Young under *Microphallus nicolli* Baer 1943, as *Spelotrema* had been synonymized with *Microphallus*. She had also obtained metacercariae from about a hundred mole crabs, *Emerita analoga*, from Santa Barbara, California, identical with *M. nicolli*, adults of which were recoverable from a mouse 33 hr after feeding them. Specimens of *Microphallus* from *Larus glaucescens* in her collection resembled those from *E. analoga*, viz., *M. nicolli*.

The present account, apparently the next one relating to microphallid metacercariae from sand crabs, deals with those encountered in natural infections in the connective tissue of the ovaries of *Emerita asiatica*, as well as *Albunea symnista*, occurring on the shore of the Bay of Bengal in Madras, India.
2. Materials and Methods

While one of us (T.S.) was examining crustaceans for his studies on oogenesis, numerous circular bodies were consistently encountered in the connective tissue of the ovaries of the sand crabs Emerita asiatica and Albunea symnista, and were identified as metacercariae of microphaloid trematodes.

The encysted metacercariae were carefully liberated from the adherent tissues using a thin capillary tube, and examined in a fresh condition with intra-vitam staining (neutral red), or after preservation in 5% formalin or 70% alcohol followed by staining with borax carmine, acetic alum carmine, or Delafield’s haematoxylin, and permanent mounting in Canada balsem.

Metacercariae were observed to be continually moving inside the cysts, with their prominent vitellarian masses shifting their positions, and were liberated by rupturing the cyst wall with a fine needle, for examination and permanent mounting. Interestingly enough, when cysts were left in tap-water for some time, the outer wall disappeared in about 30 minutes, and the inner envelopes after prolonged immersion in it, as a result of which the larvae escaped and moved actively. Such larvae were also stained and mounted as above, for study and camera lucida drawings or photomicrographs.

3. Results and Discussion

(a) Description

The encysted metacercaria separated from the tissue shows a spherical shape, with a thick, tough, transparent outer cyst wall with two envelopes inside it (figures 1, 3, 4). The inner most one appeared to be thin, soft, resilient and refractile, changing its contour as the larva moved and when divested of the outer ones. A great deal of uniformity in shape and size (0.250–0.318 mm) of the metacercariae was evident.

The liberated larva (figure 2, 5, 6) is thin, pyriform, 0.4125–0.4375 mm long and 0.2125 mm broad. Fine and closely set body spines extend up to the acetabular region. Oral sucker circular, subterminal, and with a diameter of 0.0425 mm, is larger than the acetabulum, 0.0266–0.03 mm in diameter. The acetabulum, circular and muscular, is situated on the median ventral line, about a third of the length from the posterior end. The mouth, sub-terminal in position, is followed by a short pre-pharynx, 0.0133–0.0166 mm long, a thick muscular pharynx 0.01 × 0.0166–0.02 × 0.0266 mm, and a narrow, long oesophagus 0.09–0.1 mm, which bifurcates into short, blunt intestinal crura, disposed postero-laterally, each with a relatively clear lumen and an inner lining of one row of cells.
Figure 1. Camera lucida drawing of the encysted metacercaria to show the three envelopes and the larva inside.

The testes, one on each side, located almost behind the posterior ends of the intestinal crura and partly in the acetabular zone, are oval in shape, 0.043 × 0.066 mm, and transversely disposed. They are overlapped by the vitellarian mass on each side and are detected only with difficulty. The oval seminal vesicle lies below and partly anterior to the acetabulum, with a slightly convoluted ejaculatory duct leading into a penis and male papilla opening ventrally to the left of the acetabulum. No cuticular pockets were evident.

The ovary, oval in shape, 0.028 × 0.038 mm, is lodged on the right side of the larva, bounded by the right caecum above, right testis below, and the acetabulum towards the median line. The vitellaria are very prominent, large and lobed, the lobes fused in the middle and covering the testes, and almost filling the entire postero-lateral regions of the metacercarial body. They are the most conspicuous organs of the larva, and the first to be recognized even in the encysted metacercaria.

The excretory system appears as a pair of excretory canals proceeding anteriorwards from a Y-shaped vesicle with a short median stem opening terminally at the posterior end. The flame-cell pattern was not studied.

(b) TAXONOMIC RELATIONSHIPS

With the foregoing description, it is possible to conclude that the present metacercariae from *E. asiatica* and *A. symnista* resemble those described by Young\(^1,2\) most, but whether the two are identical is difficult to decide. The
Figure 2. Camera lucida drawing of the excysted metacercaria to show its organization. ac. — acetabulum; exc.v. — excretory vesicle; int. — intestine; m.p. — male papilla; o.s. — oral sucker; ov. — ovary; ph. — pharynx; s.v.— seminal vesicle; t. testis; v. vitellaria.
Figure 3. Photomicrograph of a number of metacercariae in situ in the ovarian tissue—in various stages of development.

Figure 4. Photomicrograph of a number of metacercariae in situ in the ovarian tissue—showing the formation of the envelopes.

Figure 5. Photomicrograph of a metacercaria in the process of escape from the cyst. The three membranes of the cyst wall are distinctly recognizable.

Figure 6. Photomicrograph of a liberated larva, in which the lobulated vitellaria are conspicuously seen.

(facing page 196)
identity of the latter had been subject to considerable uncertainty and discussion. Originally, it was supposed to belong to the species Levinseniella cruzi, but later confirmed as Spelotrema nicolli Cable and Hunnin 1940. Ching however included it under Microphallus nicolli Baer 1943, for the reason that the genus Spelotrema had been synonymized with Microphallus. If this synonymy is accepted, then Microphallus (Spelotrema) could be distinguished from Levinseniella and Maritrema, at least on the basis of the presence of variable numbers of cuticular pockets attached to the penis in Levinseniella5, 13 and the presence of a ring-shaped vitelline gland enclosing the testes in Maritrema.4 Cable and Hunnin 3 emphasized that “the terminal portions of the reproductive system seem to be subject to extreme modifications as indicated by the differences observed in the microphallid genera, Spelotrema, Maritrema, Levinseniella and Microphallus”. In Microphallus, no cuticular pockets have been described, and the vitellaria are highly lobulated, independent structures.

Based on these characters and its similarity with the metacercaria of Microphallus nicolli, the present form may be tentatively assigned to the genus Microphallus. Species of Microphallus reported from India include M. dicaecus and M. indicus,16 of which M. indicus has been synonymized with Bengaliniella dicaecus18 but the determination of the precise identity of the present form will have to wait till adults are raised from it in experimental infections in suspected hosts, presumably one of the shore-birds.

It is of interest to note here that Young had recovered excysted individuals from surf perches (Embiotoca and Abeona) fed on sand crabs, but did not attempt infection of birds. Cable and Hunnin,3 on the other hand, successfully infected young herring gulls, Larus argentatus, with S. nicolli, by feeding them with metacercariae from the blue crab, Callinectes sapidus. While Hadley and Castle4 failed to secure excysted individuals from shore-birds, rodents, kitten and the fowl fed on metacercariae (believed to be of Maritrema arenaria) from Balanus, Ching6 raised sexually mature Microphallus nicolli in a mouse, only 33 hr after feeding it with metacercariae from Emerita analoga. Stunkard7 had been successful in growing Levinseniella minuta to maturity in mice and hamsters, and Microphallus limuli in mice.17 Infection experiments in mice with the metacercaria under report are therefore being designed.

(c) Ecological Observations

In 28 collections made between 10th July and 16th August 1974, all of 144 (100%) E. asiatica ranging in size 16–31 mm, and 22 of 23 (96%) of A. symnista 13–27 mm in size, were found to be infected in various intensities. All of them were females. None of the 150 male specimens of E. asiatica
and none of the 30 males of *A. symnista* in the same collections harboured the metacercaria. Such heavy rates of infection in the crustacean second intermediate host have also been reported by earlier authors. Young\(^1\) had noted that “practically all crabs over 6 to 7 mm in length are infected”, Cable and Hunningen\(^3\) noted: “it has been known for several years that practically every blue crab in the Woods Hole area is infected with a microphallid cercaria”, and Hadley and Castle\(^4\) noted that “these cysts are found in practically all barnacles on rocky shores visited by ruddy turnstones.”

Seasonal fluctuations in the prevalence should also be expected, as it is improbable that high levels of infection would be attained uniformly throughout the year. Though he had not made a study of this aspect in *L. cruzi*, Young\(^1\) suggested that as the shedding of the cercariae was restricted to winter, as earlier observed by Lebour,\(^12\) a seasonal cycle in the life-history may be evident, and Hadley and Castle\(^4\) that those of *Maritrema arenaria* would not be present in the Woods Hole region in the summer months.

Perhaps the most interesting observation made in this study relates to the site of infection in and the sex of the intermediate hosts, namely, the connective tissue around the ovary, and inevitably therefore only in the females of *E. asiatica* and *A. symnista*.

The metacercariae of *L. cruzi* described by Young\(^1\) were found in the connective tissue of *E. analoga*, chiefly among the liver lobules, those of *Spelotrema nicollii* in certain slender fibres (connective tissue) which extend from the viscera to the bases of the legs of the blue crab, *Callinectes sapidus*\(^3\) and those of (?) *Maritrema arenaria* around the gut (in light infections), and every part of the body except the appendages and interior of the gut (in heavy infections) of the common barnacle, *Balanus balanoides*.

In the present study, the metacercariae were confined, except in the heaviest infections, to the connective tissue of the ovarian region, in both species of the sand crabs. When the males of one of the species, *A. symnista*, were examined, no metacercaria was present in the testis or associated organs, liver, or any other tissue. The ovary, when fully developed, extends so much as to overlap the entire area of the liver mass, and it is therefore possible, as might have been the case in the studies of Young,\(^1\) to mistake the host tissue of infection as the liver instead of the ovary. Indeed, this was our experience in the initial microscopical examinations. But, a critical study involving the careful dissection and extrication of the liver tubules from the ovarian fragments revealed that they were free, the larvae being borne only on the ovary; in the heaviest infections, however, a few metacercariae were seen in the portions of the liver which were underlying the ovary and intimately
involved with it. This observation was confirmed by an examination of the liver tubules and the ensheathing connective tissue in all the males of *A. symnista* (in which a contamination with the ovary or its connective tissue is out of question), and the absence of any larvae in them. The ovary therefore seems to be the preferred tissue for the metacercariae, and the ecological implications or significance of infection in crabs of only the female sex are yet to be understood.

In an earlier study, an adult trematodes belonging to several families, from shore-birds, *Larus, Sterna, Tringa* and *Gallinago*, have been reported in this area, but none representative of the Microphallidae. The molluscan intermediate host, and the fish or avian definitive host of the present metacercaria remain to be identified.

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