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Studies on Cercariae from Kuwait Bay
III. Description and Surface Topography of *Cercaria Kuwaitae* III sp. n. (Digenea: Opisthorchioidea)

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Abstract

The pleurolophocercous cercaria, *Cercaria kuwaitae* III sp. n. was found in the mud snail *Cerithidea cingulata* in Kuwait Bay. It resembles closely larvae in the superfamily Opisthorchioidea. Details are presented on the morphology and behaviour of the cercaria. The surface topography of the cercaria and the redia was examined by scanning electron microscopy.

Key words: *Cercaria kuwaitae* III sp. n., *Cerithidea cingulata,* Kuwait, Digenea, Ultrastructure

Introduction

Kuwait Bay is located in the northwestern corner of the Arabian Gulf. The intertidal region of the Bay is dominated by large areas of mudflats formed by settlement of suspended material originating from the Tigris and Euphrates. The mudflats support an extremely varied animal and plant life (Clayton, 1986). The molluscan fauna along the coast of Kuwait was studied by Glayzer *et al.* (1984), a total in excess of 435 molluscs was recorded. However, the parasitic fauna of the Bay and the role of molluscs in the transmission of trematode infections have not been investigated yet.

The present study describes a new pleurolophocercous cercaria, *Cercaria kuwaitae* III sp. n., from *Cerithidea cingulata* in Kuwait Bay. The surface topography of the cercaria and the redia was examined by scanning electron microscopy (SEM).

Materials and Methods

The snail host, *Cerithidea cingulata,* was collected from the mudflats along the southern shores of Kuwait Bay, about 10 km west of Kuwait City. Larval stages were studied alive and as fixed in AFA solution (Ethylalcohol-formalin-acetic acid) and stained in acetocarmine or Ehrlich's hematoxylin. Vital staining with neutral red aided examination of cercarial features. Larval morphometrics were taken from at least 10 specimens fixed in hot AFA. Drawings were made to scale from fixed specimens with the aid of a camera lucida. All measurements are given in microns with averages in parentheses. The cercaria described in this paper was given numerical system of designation adopted by Cable (1956).

For SEM, living cercariae and rediae were fixed in 2% glutaraldehyde in 0.1 M sodium cacodylate buffer pH 7.2 for 1 hr at 4°C. Following the appropriate buffer wash, the specimens were post fixed in 1% osmium tetroxide in the same buffer for 10 min at 4°C. Dehydration was in a graded series of anhydrous acetone. Specimens suspended in acetone were dried in a Technics critical point drying apparatus, coated with gold-palladium and examined in a Jeol JSM-840 scanning electron microscope.

Results

Description

Opisthorchioidea Looss, 1899

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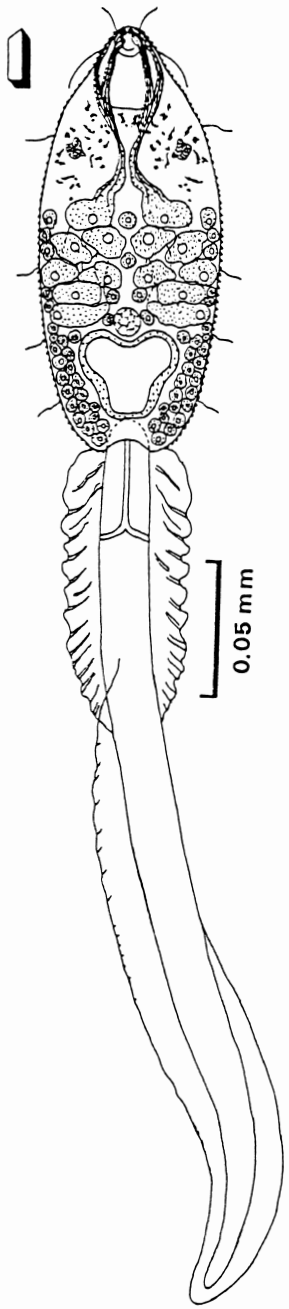
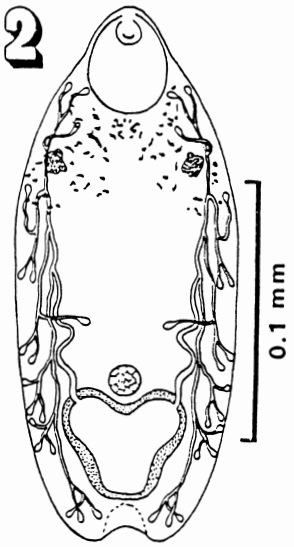
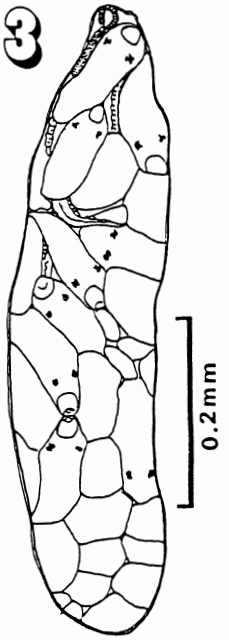






Figs. 1–3 *Cercaria kuwaitae* III sp. n.
1. Entire cercaria, ventral view.
2. Cercaria, excretory system.
3. Mature redia.

Cercaria kuwaitae III sp. n.

Type host: *Cerithidea cingulata* (Gmelin, 1791).

Site of infection: Hepatopancreas.

Infection rate: 0.4% of 1258 snails examined.

Type locality: Shuwaikh, Kuwait Bay, Kuwait.

Date of collection: April 1989.

Specimens: deposited in the helminth collection of the Department of Zoology, University of Kuwait.

Species diagnosis (Figs. 1–3)

Body 122.5–237.5 (177.5) long, 50.0–105.0 (69.8) wide at midlevel. Tail attached subterminally; excluding finfolds, 316.2–397.8 (365.2) long, 15.3–25.5 (20.4) wide at base. Plicated lateral finfolds, extend along the anterior one-third of tail, 95.0–100.0 (97.0) long, 10.0–13.8 (12.7) wide. Plicated dorso-ventral finfold begins dorsally a short distance anterior to posterior margins of lateral finfolds, continues around tip of tail anteriorly for about one-third tail length, 202.5–242.5 (213.6) long, 8.8–12.5 (10.4) wide. Two irregular eyespots of brown granules, 6.3–10.0 (8.6) long, 5.0–10.0 (7.9) wide, located about midway between oral sucker and penetration glands. The distance between both eyespots 25.0–32.5 (28.8). Body filled with finely granular cystogenous cells more numerous in posterior half. Brownish pigments scattered all over the body more distinct in anterior region. Entire body surface with minute spines forming parallel transverse rows, decreasing in size and number posteriorly; tail stem smooth. Three pairs of lateral bristles in oral sucker region, 3 posterior pairs of lateral bristles. Oral sucker 28.8–37.5 (33.4) long, 20.0–30.0 (27.3) wide. Oral opening transversely oval, small, subterminal; oral cavity flask-shaped; prepharynx, pharynx not observed. Dorsal surface of oral opening lined with two alternating transverse rows of 10 upper and 9 lower acicular spines. Acetabulum undeveloped. Seven large penetration glands on each side, 5 lateral, 2 medial, between eyespots level and excretory bladder. Gland ducts in 2 bundles, 3 outer and 4 inner, on each side close to midline, passing dorsally over oral sucker to open in a crypt above oral opening. Excretory bladder transversely oval to V-shaped, with thick, granular wall. Caudal excretory tubule short, bifurcating to open at lateral pores on tail. Main excretory tubules joining antero-lateral margins of bladder, extending anteriorly to mid body, where it receives anterior and posterior collecting tubules, each with 3 groups of 3 flame cells. Excretory formula $2[(3+3+3)+(3+3+3)]=36$. Genital primordium, oval mass of nuclei just anterior to excretory bladder.

Development in whitish, slender, elongate redia, 255.0–663.0 (397.8) long, 102.0–173.4 (134.6) wide, containing germ balls at various developmental stages and 1–5 mature cercariae. Birth pore 20.0–30.0 (24.4) from anterior end. Pharynx 25.0–30.0 (27.8) long, 19.0–24.0 (21.2) wide, cecum short. Tegument thin, with minute bristles at cephalic end.

Behaviour

The cercaria swims in a jerky manner without showing pronounced phototactic tendencies. It swims, head first, to the surface of the water and then drifts slowly to the bottom with the body downward. The oral sucker is constantly in searching action. The cercaria rests in aggregates in shaded regions.

Surface topography

From SEM images, the body of the cercaria comprises projected anterior organ (cephalic organ), flat ventral surface forming a shoulder with the anterior organ, convex dorsal surface and cylindricl tail with conspicuous lateral and dorso-ventral plicated finfolds (Figs. 4–6). The body surface is provided with simple pointed, backwardly directed spines, decreasing in length posteriorly (Figs. 5, 7). Modified spines, which differ from the more posteriorly located spines in being longer, more curved surround the anterior organ (Figs. 7, 9). The tegument surface bears conspicuous transverse folds interconnected to form an anastomosing reticulum, uniformly marked by numerous pits (Figs. 7, 10), which may represent openings of cystogenous glands. The anterior organ comprises a protrusible rasping oral sucker equipped with two rows of acicular spines on the dorsal lip, whose points

project into the oral opening (Figs. 8, 9). Oral opening located on the ventral lip of the oral sucker (Fig. 8). Ventral border of the oral opening appears to be stiff lined with minute pores and flanked by three pairs of small pointed spines (Fig. 8). Oral sucker is surrounded by prominent pores (Fig. 8), probably penetration glands openings. Numerous presumed ciliated sensory structures are symmetrically arranged ventrally on both sides of median line (Fig. 5) and on region of the anterior organ (Figs. 8, 9). The sensory structures generally consist of a tegumentary collar from which cilia of variable size and shape may project. The upper lip of the oral sucker is surrounded by four long cilia, about 5 μm in length, and numerous short cilia (Fig. 8). Two modified cilia, over 10 μm in length with bell-shaped tips are located on the ventral side of anterior organ (Fig. 9). Tail stem is smooth, spineless and with few ciliated sensory structures towards the posterior region (Fig. 11).

SEM observations of the redia shows a cylindrical body with parallel transverse ridges and an oral opening at anterior tip (Fig. 12). The tegument is covered with closely packed short microvilli-like structures (Fig. 15). Ciliated sensory structures, of various length, surround the oral opening region (Figs. 13, 14). By contrast, many domed papillae occurs on the remainder of the body (Fig. 15).

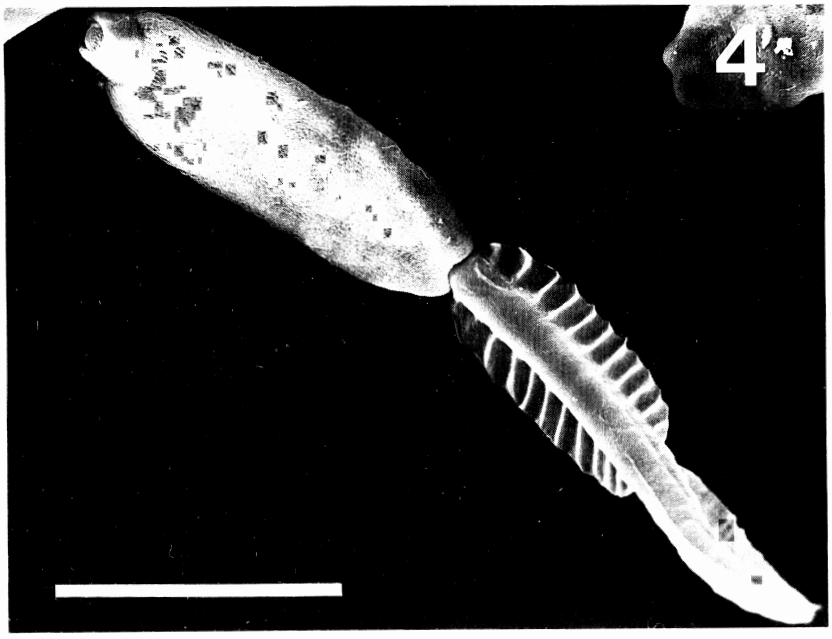
Discussion

The term pleurolophocercous was coined by Sewell (1922) to describe biocellate monostome cercariae having well developed caudal finfolds. Cable (1956) designated the opisthorchioid group to include pleurolophocercous cercariae sharing the following general characteristics: two eyespots, oral sucker a protrusible penetration organ armed with preoral spines, tail long with finfolds and penetration glands 7 pairs with ducts opening dorsal to oral opening. Development in rediae in the digestive gland of prosobranch gastropods, encystment in fish and adults found in piscivorous vertebrates. In view of the morphological characteristics outlined by Cable, it is evident that *Cercaria kuwaitae* III belongs to the opisthorchioid group of pleurolophocercous cercariae. Life history studies have related cercariae of the opisthorchioid group to the families Cryptogonimidae (Cable and Hunninen, 1942; Cable, 1956; Cribb, 1986), Heterophyidae (Ochi, 1931; Martin, 1950a; 1950b; 1958; 1959; Cable, 1956) and Opisthorchiidae (Martin, 1950c; Cable, 1956).

Cercaria kuwaitae III is similar to the marine pleurolophocercous cercariae; *Cercaria caribbea* X and *C. caribbea* XI from *Cerithidea costata* in Sucia Bay, Puerto Rico (Cable, 1956), *Heterophyes cercaria* I, *H. cercaria* II, *H. cercaria* III, *Stictodora cercaria* I and *S. cercaria* II from *Pirenella conica* in Lake Burullus near the Mediterranean Sea (Martin, 1959) and *C. coruscantis, C. cursitans* and *C. vivata* from *C. scalariformis* in Apalachee Bay, Gulf of Mexico (Holliman, 1961). Comparison of certain morphological characteristics between *C. kuwaitae* III

Figs. 4–11 Scanning electron micrographs of *Cercaria kuwaitae* III sp. n.

4. Ventral view showing body and caudal finfolds. Bar = 100 μm.
5. Ventral view of body showing anterior organ and longitudinal rows of ciliated sensory structures. Bar = 10 μm.
6. Lateral view of body showing anterior organ, shoulder-like expansion of flat ventral surface and convex dorsal surface. Bar = 10 μm.
7. Lateral view of anterior end showing tegumental surface, spines on anterior organ and oral sucker. Bar = 10 μm.
8. Anterior organ with protruding oral sucker showing pores possibly openings of penetration glands, two alternating rows of cephalic spines, two pairs of long cilia and numerous short cilia on the dorsal lip of the sucker. Oral opening, located on ventral lip of oral sucker, bordered ventrally by minute pores and flanked by three pairs of pointed spines. Bar = 1 μm.
9. Ventral view of anterior organ with partially protruding oral sucker showing a pair of modified cilia with bell-shaped tips and numerous small cilia. Bar = 10 μm.
10. Tegumental surface showing reticulate pattern of surface folds, small pointed spines, ciliated sensory structures and numerous small pores possibly openings of cystogenous glands. Bar = 10 μm.
11. Tail stem showing part of a lateral finfold and a ciliated sensory structure. Bar = 10 μm.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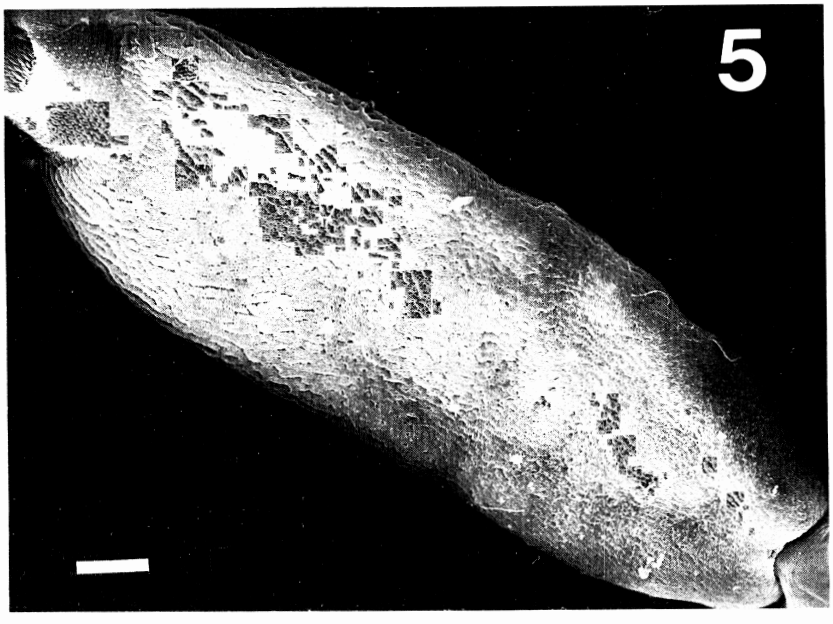
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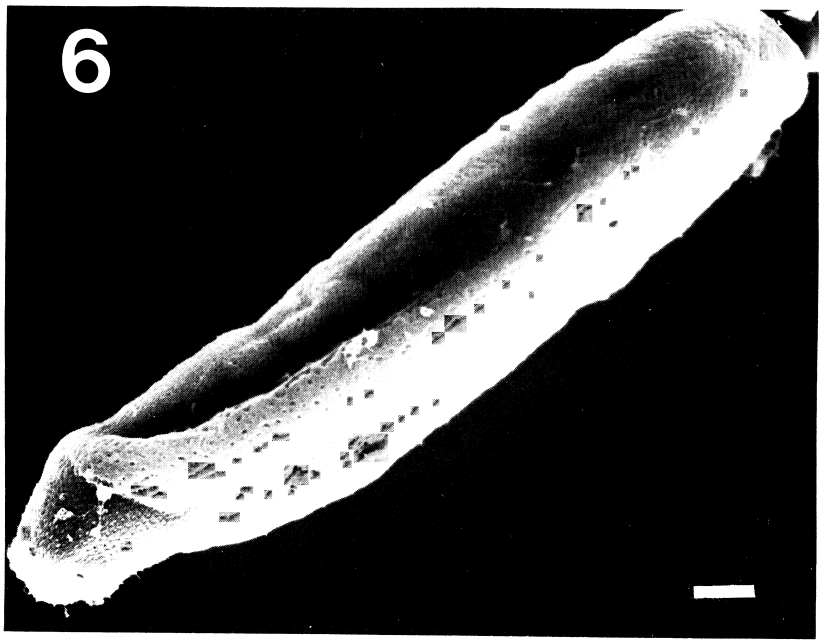
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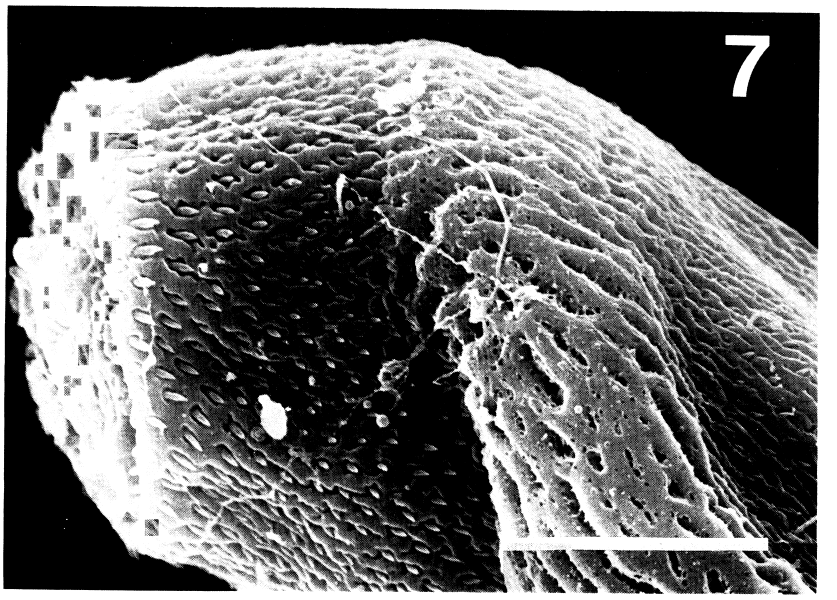
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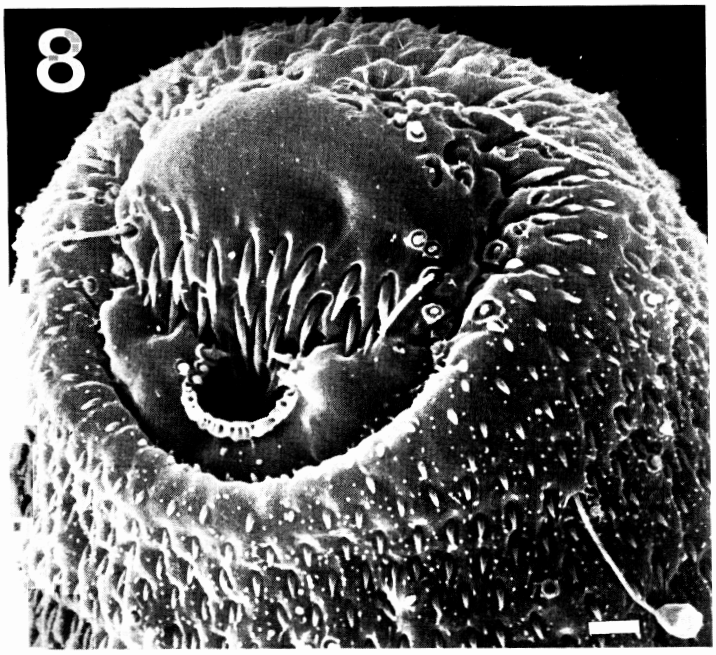
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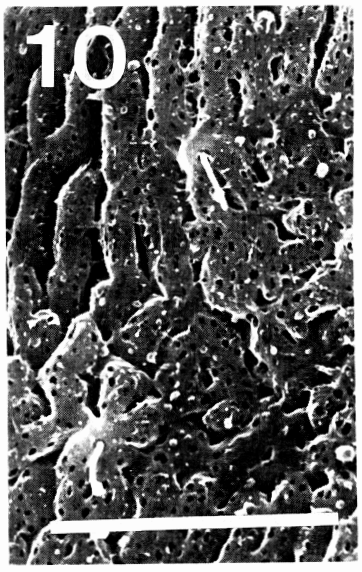
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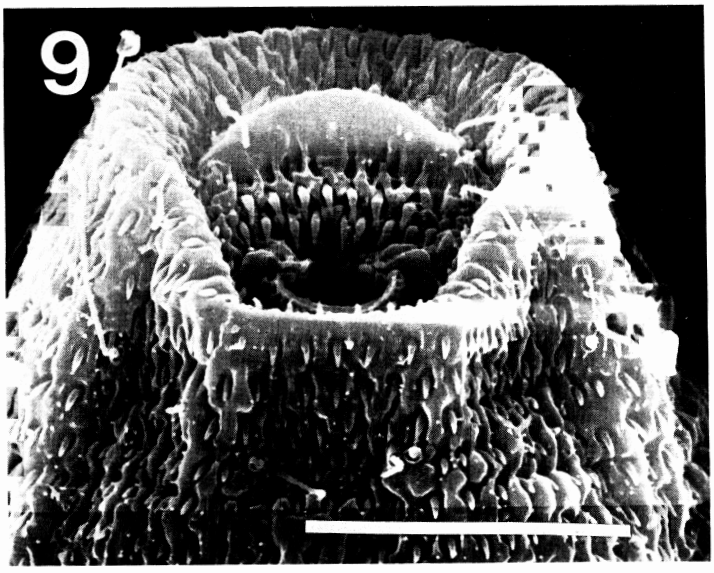
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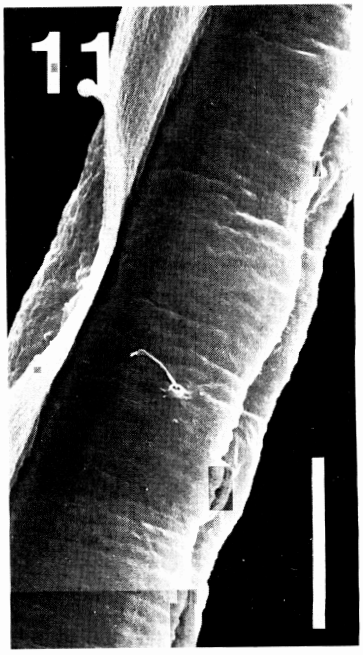
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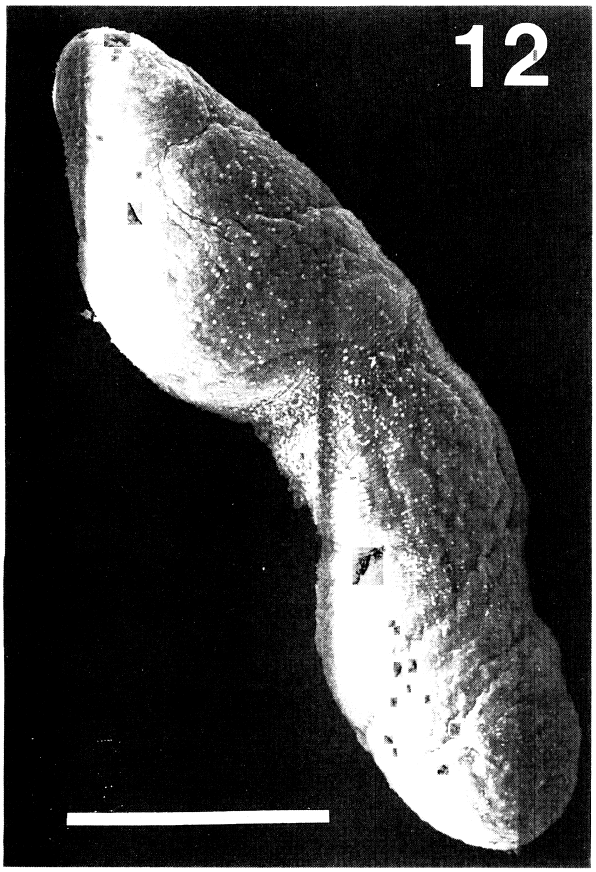
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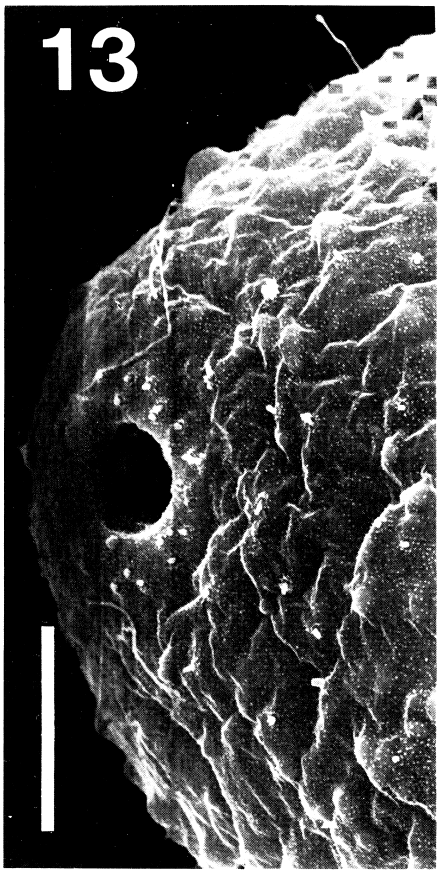
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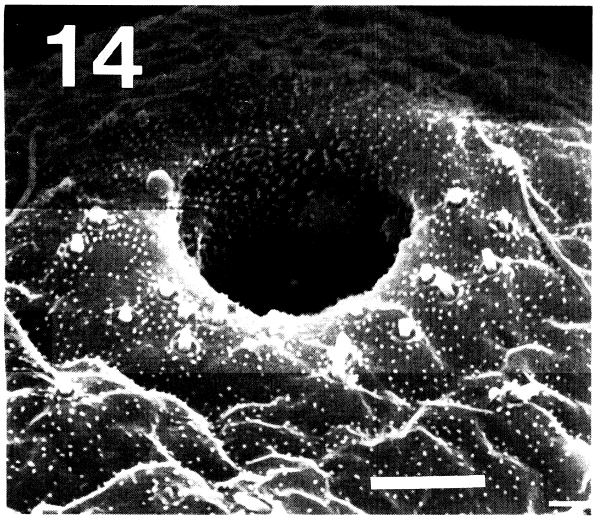
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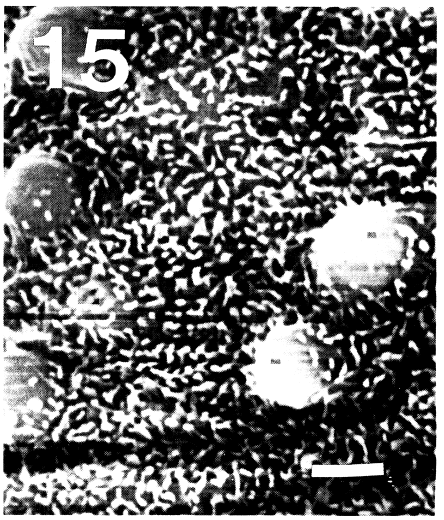
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and the similar cercariae were summarized in Table 1. In addition to the differences shown in Table 1, the present species differs from *H. cercaria* I, *H. cercaria* II and *H. cercaria* III by possessing lateral caudal finfolds, and from *S. cercaria* I and *S. cercaria* II by the disposition of the penetration glands in 5 lateral and 2 medial rows on each side of the body. Variations in the

Table 1 Comparison of certain characteristics between *Cercaria Kuwaitae* III and similar species of pleurolophocercous cercariae

Species of Cercaria	Flame Cells No.	Eyespots Shape	Preoral* Spines	Body-Tail Length Ratio	Reference
Cercaria caribbea X	N.A.	Cuboid	6	1:1.9	Cable (1956)
C. caribbea XI	20–24	Irregular	4;8	1:1.9	Cable (1956)
C. kuwaitae III	36	Irregular	9;10	1:2.1	Present study
C. coruscantis	16	Cuboid	Lacking	1:2.5	Holliman (1961)
C. cursitans	24	Cuboid	7;6	1:1.9	Holliman (1961)
C. vivata	24	Cuboid	Lacking	1:2.6	Holliman (1961)
Heterophyes cercaria I	36	Irregular	4–6;7–10;7–10	1:2.1	Martin (1959)
H. cercaria II	36	Irregular	N.A.	1:2.8	Martin (1959)
H. cercaria III	36	Irregular	N.A.	1:3.3	Martin (1959)
Stictodora cercaria I	36	Irregular	5–6;7–9;7–9	1:3.1	Martin (1959)
S. cercaria II	36	Irregular	5–6;7–9;7–9	1:2.6	Martin (1959)

N.A.: Not available.
*: Number of spines in each row starting from the row nearest mouth.

Figs. 12–15 Scanning electron micrographs of redia of *Cercaria kuwaitae* III sp. n.
12. Entire view showing cylindrical body, oral opening at anterior tip and tegumental annulations on the middle region of body. Bar = 100 μm.
13. Anterior end showing oral opening and ciliated and domed sensory structures. Bar = 10 μm.
14. Anterior end showing long and stout ciliated sensory structures surrounding oral opening. Bar = 1 μm.
15. Tegumental surface showing microvilli-like projections and domed papillae. Bar = 1 μm.

number and arrangement of preoral spines, as shown in this study and in a study by Dhanumkumari *et al.* (1991) on pleurolophocercous cercariae from freshwater snails, appears to be species specific. The use of this feature in the taxonomy of the opisthorchiod cercariae may prove to be helpful in solving current taxonomic confusion due to striking morphological similarities.

The general surface topography of *C. kuwaitae* III, as revealed by SEM, resembles that reported for the heterophyid cercaria, *Cryptocotyle lingua* (Rees, 1974; Koie, 1977). However, it differs from the above mentioned species in size, shape and arrangement of tegumentary spines and cilia. The basic morphological features of cilia in *C. kuwaitae* III are similar to that described for cercariae of other digeneans (Smyth and Halton, 1983; Koie, 1985).

The exact function of cilia remains unknown, however, based on ultrastructural features and patterns of distribution chemo- and mechanoreceptive functions have been ascribed to them (Nuttman, 1971; Page *et al.*, 1980). It is conceivable that cilia on *C. kuwaitae* III are involved in locating the second intermediate host and in selecting point of penetration into the host skin. Initial exploratory contact with the host surface is probably made by the pair of modified cilia on the anterior organ, with its unique bell-shaped tip acting as mechanoreceptors. Subsequent mechanisms of attachment and penetration into the host skin, is probably facilitated by rasping oral sucker, preoral spines and chemicals released from pores surrounding oral sucker. The basic ultrastructural organization of the tegument of rediae of *C. kuwaitae* III resembles that described for rediae of other digeneans (Hoskin, 1975; Rees, 1980; Fried and Awatramani, 1992). The pattern of distribution of ciliated and domed sensory structures on the tegument indicates that they may function as mechanoreceptors during movement and feeding on the molluscan host tissue. On the other hand, densely arranged microvilli-like structures on the tegumental surface is consistent with an absorptive function.

Cercaria kuwaitae III is the first opisthorchioid cercaria to be described from a mollusc in the Arabian Gulf. The definitive host in the present is not known, however, it is possible that the adult develops in one of the sea birds which are abundant in the locality where the infected snails were collected.

References

1) Cable, R. M. (1956): Scientific survey of Puerto Rico and the Virgin Islands. marine cercariae of Puerto Rico. N. Y. Acad. Sci., 14, 491–577.
2) Cable, R. M. and Hunninen, A. V. (1942): Studies on the life history of *Siphodera vinaledwardsii* (Linton) (Trematoda: Cryptogonimidae). J. Parasitol., 28, 407–422.
3) Clayton, D. A. (1986): Ecology of mudflats with particular reference to those of the northern Arabian Gulf. Proceedings of the First Arabian Gulf Conference on Environment and Pollution. University of Kuwait, 83–96.
4) Cribb, T. H. (1986): The life cycle and morphology of *Stemmatostoma pearsoni,* gen. et sp. nov., with notes on the morphology of *Telogaster opisthorchis* Macfarlane (Digenea: Cryptogonimidae). Aust. J. Zool., 34, 279–304.
5) Dhanumkumari, C., Hanumantha, R. K. and Shyamasundari, K. (1991): Two species of heterophyid larval digeneans from a thiarid gastropod *Thiara tuberculata* (Muller) from India. Bol. Chil. Parasitol., 46, 14–18.
6) Fried, B. and Awatramani, R. (1992): Light and scanning electron microscopical observations of the daughter rediae of *Echinostoma trivolvis* (Trematoda). Parasitol. Res., 78, 257–259.
7) Glayzer, B. A., Glayzer, D. T. and Smythe, R. (1984): The mollusca of Kuwait, Arabian Gulf. J. Conch., 1, 311–330.
8) Holliman, R. B. (1961): Larval trematodes from the Apalachee Bay Area, Florida, with a checklist of known marine cercariae arranged in a key to their superfamilies. Tulane Stud. Zool., 9, 2–74.
9) Hoskin, G. P. (1975): Light and electron microscopy of the host-parasite interface and histopathology of *Nassarius obsoletus* infected with rediae of *Himasthla quissetensis.* Ann. N. Y. Acad. Sci., 266. 497–512.
10) Koie, M. (1977): Stereoscan studies of cercariae, metacercariae and adults of *Cryptocotyle lingua* (Creplin 1825) Fischoeder 1903 (Trematoda: Heterophyidae). J. Parasitol., 63, 835–839.
11) Koie, M. (1985): The surface topography and life-cycles of digenetic trematodes in *Limanda limanda* (L.) and *Gadus morhua* (L.) (Summary). D. Sc. thesis, Univ. Copenhagen, Denmark.
12) Martin, W. E. (1950a): *Euhaplorchis californicusis* n. gen., n. sp., Heterophyidae, Trematoda, with

notes on its life-cycle. Trans. Amer. Microsc. Soc., 69, 194–209.

13) Martin, W. E. (1950b): *Parastictodora hancocki* n. gen., n. sp. (Trematoda: Heterophyidae), with observations on its life cycle. J. Parasitol., 36, 360–370.

14) Martin, W. E. (1950c): *Phocitremoides ovale* n. gen., n. sp. (Trematoda: Opisthorchiidae), with observations on its life cycle. J. Parasitol., 36, 552–558.

15) Martin, W. E. (1958): The life histories of some Hawaiian heterophyid trematodes. J. Parasitol., 44, 305–323.

16) Martin, W. E. (1959): Egyptian heterophyid trematodes. Trans. Amer. Microsc. Soc., 79, 172–181.

17) Nuttman, C. J. (1971): The fine structure of ciliated nerve endings in the cercaria of *Schistosoma mansoni*. J. Parasitol., 57, 855–859.

18) Ochi, S. (1931):Studies on brackish water fish trematoda. On the life history of *Pygidiopsis summus*. Tokyo Iji Shinshi, 2712, 346–353.

19) Page, M. R., Nadakavukaren, M. J. and Huizinga, H. W. (1980): *Ribeiroia marini*: Surface ultrastructure of redia, cercaria, and adult. Int. J. Parasitol., 10, 5–12.

20) Rees, G. (1974): The ultrastructure of the body wall and associated structures of the cercaria of *Cryptocotyle lingua* (Creplin) (Digenea: Heterophyidae) from *Littorina littorina* (L). Z. Parasitenkd., 44, 239–265.

21) Rees, G. (1980): Surface ultrastructure of the redia of *Parorchis acanthus* Nicoll (Digenea: Philophthalmidae). Z. Parasitenkd., 63, 33–46.

22) Sewell, R. B. S. (1922): Cercariae Indicae. Indian J. Med. Res., 10, 1–370.

23) Smyth, J. D. and Halton, D. W. (1983): The Physiology of Trematodes. Cambridge University Press, Cambridge.