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Studies on cercariae from the Kuwait Bay. VI. Description and surface topography of *Cercaria kuwaitae* VI sp. n. (Trematoda: Haplosplanchnidae)

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Abstract: A new haplosplanchnid cercaria, *Cercaria kuwaitae* VI sp. n., was found in the prosobranch snail *Cerithidea cingulata* in the Kuwait Bay. Details are presented on the morphology and behavior of the cercaria and the encystment process. The new cercaria is a biocellate, distome, with a prominent single sac-like intestinal cecum extending well posterior to the ventral sucker and develops in simple sporocysts. It differs from known haplosplanchnid cercariae in the absence of finger-like processes on the tail, and the presence of V-shaped excretory vesicle extending beyond ventral sucker and the presence of cervical glands. The surface topography of the cercaria and its sporocyst is examined by scanning electron microscopy. This is the first haplosplanchnid cercaria to be described from a *Cerithidea* species.

Key words: Trematoda, Haplosplanchnida, *Cercaria kuwaitae* VI sp. n., cercaria, sporocyst, metacercaria, *Cerithidae cingulata*, surface topography, Kuwait

INTRODUCTION

The prosobranch gastropod *Cerithidea cingulata* (Gmelin 1790) is common in intertidal mudflats of the Kuwait Bay. Species of *Cerithidea* from the California coast (Martin, 1955; Yoshino, 1975; Sousa, 1983), Gulf of Mexico (Holliman, 1961), Madras coast (Gnana Mani and Hanumanthra Rao, 1993), the Caribbean (Cable, 1956) and Tokyo Bay (Ito, 1956; Harada and Suguri, 1989) were found to harbor a variety of larval digenetic trematodes represent the families Cyathocotylidae, Echinostomatidae, Heterophyidae, Microphallidae, Notocotylidae, Philophthalmidae, Renicolidae and Schistosomatidae. In the Kuwait Bay, Abdul-Salam and Sreelatha described two cyathocotylids (1993a & b), two heterophyids (1993c & d) and one philophthalmid (1994) cercaria from *C. cingulata*. This study describes a new haplosplanchnid cercaria from *C. cingulata*. The surface topography of the cercaria and the sporocyst was examined by scanning electron microscopy (SEM).

MATERIALS AND METHODS

Specimens of *Cerithidea cingulata* infected with haplosplanchnid cercariae were collected from the mudflats along the southern shores of Kuwait Bay, approximately 10 km west of Kuwait City. Larval stages were studied alive and fixed in AFA and stained in acetic alum carmine. Vital staining with neutral red aided examination of cercarial features. For measurement, larval stages stained with neutral red and immobilized in the refrigerator were drawn with the aid of a camera lucida. All

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measurements are given in micrometers with averages in parentheses. For SEM, living cercariae and daughter sporocysts were fixed in 2.0% 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 5 mins at 4°C, dehydrated in a series of anhydrous acetone and critical point dried. The specimens were coated with gold-palladium and then examined in a Jeol JSM-840 scanning electron microscope.

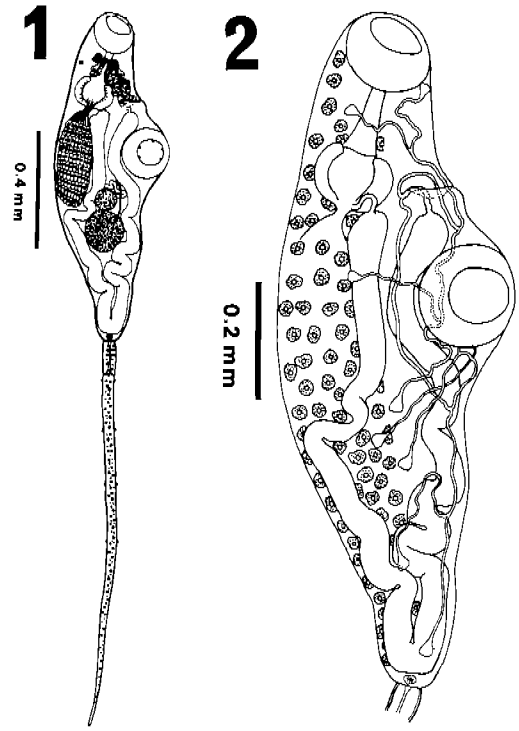
The cercaria described in this paper is designated by locality and number following the system adopted by Cable (1956) from Sewell (1922).

RESULTS

Haplosplanchnidae Poche, 1926
Cercaria kuwaitae VI sp. n.

Description

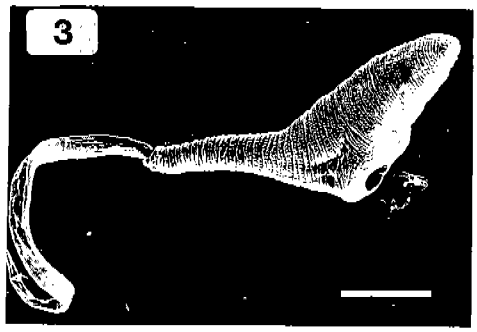
Cercaria (Figs. 1,2): Biocellate distome cercaria, body elongate, thick at ventral sucker level (Fig. 3). Body tegument aspinose, folded into concentrically arranged ridges, domed papillae run in bilaterally symmetrical longitudinal lines (Fig. 4). Oral sucker subterminal, with ridges radiating outward from oral opening, bears small papillae with openings on outer margin, 14 domed papillae on lip, arranged in 2 circles, 10 external, 4 internal. (Figs. 4,5). Ventral sucker within anterior body half, surrounded by 4 types of domed papillae arranged in concentric circles, 6 flat on outer margin, 8 small, 8 paired on lip, 6 knob-like inside (Fig. 6). A pair of eyespots, variable in shape, at level of prepharynx. Immature cercariae covered with transparent envelope. Cystogenous glands prominent in immature (intrasporocyst) specimens only, spherical to oval, numerous, occupying entire body. Cervical glands (salivary glands) numerous, extend to prepharynx level, gland ducts extend anteriorly, open posterior to oral sucker. Mouth sub-terminal; prepharynx short, muscular; pharynx massive, about midway between suckers; esophagus muscular; intestinal cecum single, sac-like,

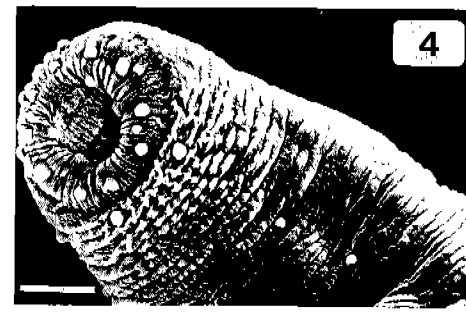


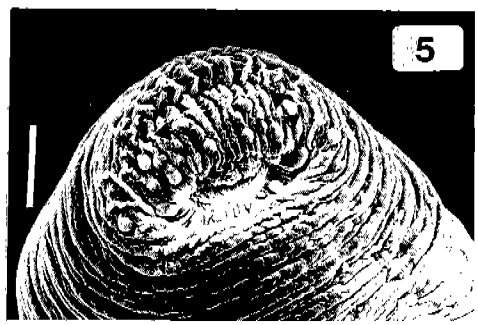
Figs. 1-2. Drawings of *Cercaria kuwaitae* VI, cercaria. **1.** Ventral view of entire cercaria. **2.** Excretory system and cystogenous glands.

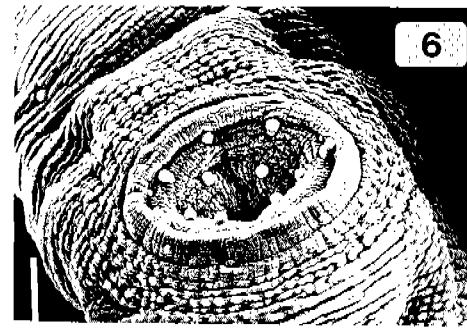
extending well posterior to ventral sucker. Reproductive primordia differentiated, single testis, ovary, at about middle of hindbody, send ducts toward genital pore. Genital pore median, just anterior to ventral sucker, with sucker-like structure prominent in immature specimens (Fig. 7). Excretory vesicle V-shaped, voluminous, extending beyond ventral sucker, terminating in a narrow main excretory tubule, receiving anterior and posterior collecting tubules, each receiving tubules from 2 groups of flame cells, flame cell pattern 2 [(2+2) + (2+2)] = 16. Primary pores at sides of tail, near base. Tail long, slender, unadorned, finely annulate, bears two lateral rows of 4 domed papillae; parenchyma staining reddish with neutral red. Cercaria body dimensions are shown in Table 1.

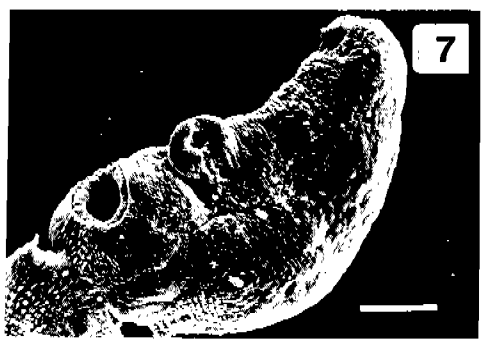
The cercaria is strongly photopositive, swims actively by wriggling the tail in a figure 8 movement, while the suckers are contracted. Within an hour of emergence the cercaria







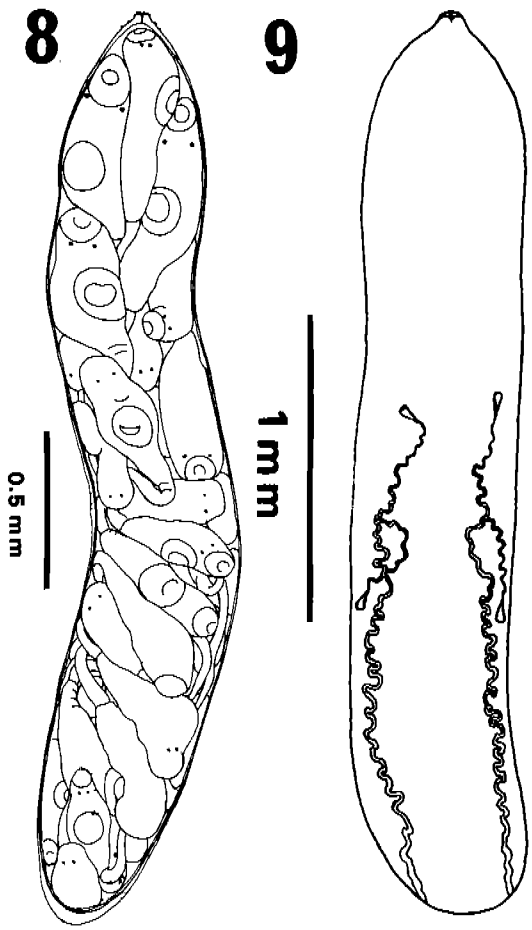




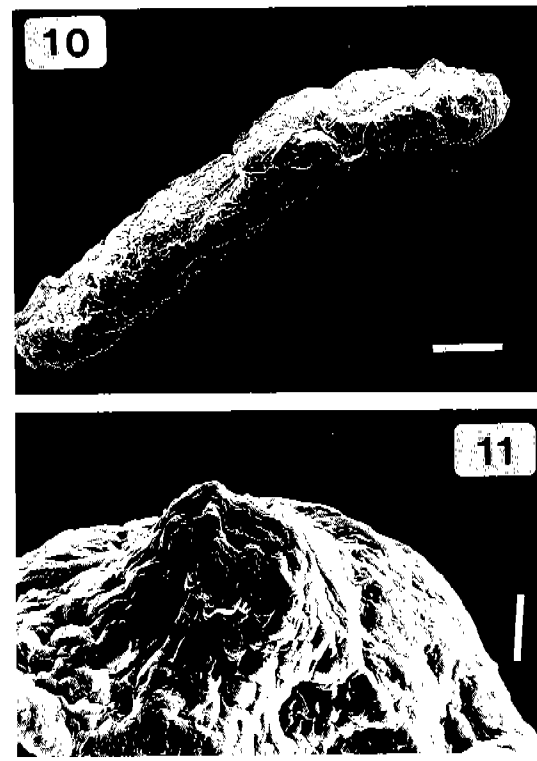
Figs. 3-7. Scanning electron micrographs of *Cercaria kuwaitae* VI, cercaria. **3.** Lateral view of an entire cercaria showing tegumental surface folded into concentrically arranged ridges, ventral and oral suckers, and finely annulate slender tail. Scale bar = 100 μm. **4.** Sub-terminal region showing averted oral sucker with ridges radiating from the oral cavity and domed papillae concentrically arranged around the oral lip. The body papillae disposed in bilaterally symmetrical longitudinal lines. Scale bar = 20 μm. **5.** Sub-terminal region showing inverted oral sucker exposing two types of domed papillae, large and small with openings (arrowheads). Scale bar = 20 μm. **6.** Mid-ventral region showing the ventral sucker with centrally radiating ridges and surrounded by domed papillae of various types. Scale bar = 20 μm. **7.** Immature cercaria showing genital pore surrounded by a sucker-like structure, located anterior to the ventral sucker. Scale bar = 40 μm.

Table 1. Body dimensions (μm) of the cercariae of *Cercaria kuwaitae* VI sp. n.

Organ		Range (mean)
Body	length	910-1144 (1011.4)
	width	338-468 (377)
Tail	length	989.4-1326 (1110.8)
	width	37.5-50 (42)
Distance of ventral sucker from anterior end		370-440 (404)
Ventral sucker		142.5-202.5 (160.3) × 132.5-185 (161.8)
Oral sucker		112.5-150 (133.3) × 125-162.5 (144.3)
Prepharynx		90-110 (96)
Pharynx		75-125 (102) × 100-160 (129)
Cecum		234.6-459 (335.6) × 102-204 (140.8)
Eyespots		12.5-17.5 (15.5)



Figs. 8-9. Drawings of *Cercaria kuwaitae* VI, sporocyst. **8.** Entire mature sporocyst containing cercariae. **9.** Excretory system.



Figs. 10-11. Scanning electron micrographs of *Cercaria kuwaitae* VI, sporocyst. **10.** Mature sporocyst showing elongate body bearing impressions of intrasporocyst cercariae. Scale bar = 100 μm. **11.** The anterior end showing terminal birth pore. Scale bar = 10 μm.

settles on the bottom and crawls by lashing the tail before start of the encystment process.

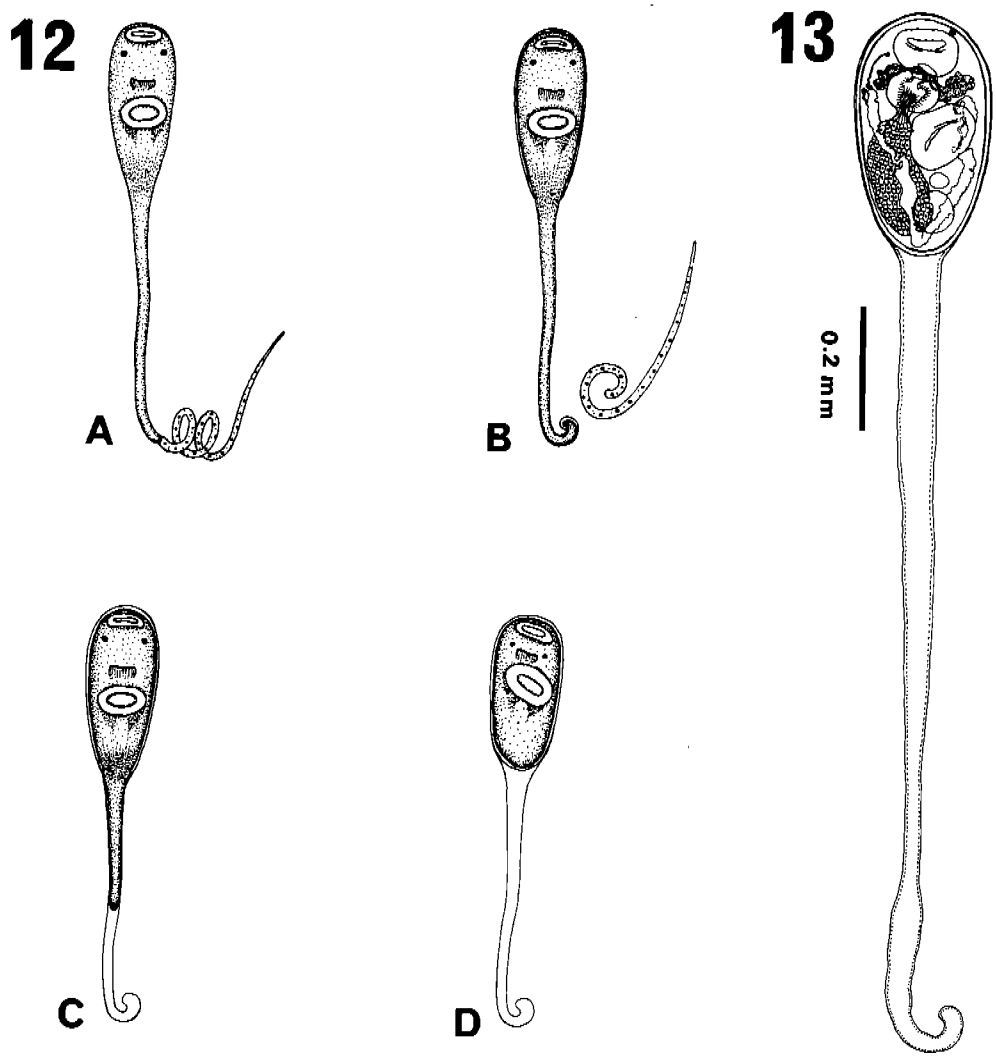
Sporocyst (Figs. 8,9): The cercaria develops in elongate sporocysts, 1625-3125 (2655) long, 475-625 (552.5) wide (Fig. 10). The body is covered with a thin, elastic transparent envelope. The anterior end is slightly pointed, bearing a redia-like birth pore (Fig. 11). The excretory formula is 2[(1) + (1)] and excretory pores are located toward the posterior end of the body. The mature sporocyst contains 5-15 cercariae.

Metacercaria (Figs. 12A-D,13): The cercaria starts encystment process by rounding up the anterior end and extensive elongation of the posterior half of the body (Fig. 12A). The elongated portion lashes vigorously forcing the tail to detach (Fig. 12B). The detached tail swims very actively for a few minutes. The curved distal end of the extended portion anchors the metacercaria to floating objects or solid surfaces. Simultaneously, as the cyst wall is forming, the extended portion contracts back leaving behind the thin hyaline outer layer as a flexible empty cord, 910-1450 (1153) long, 65-90 (74.5) wide (Fig. 12C). Once the encystment process is completed, a symmetrically ovoid cyst is formed, 380-440-(404) long, 220-270 (248) wide, surrounded by the inner layer, 5-7.5 (5.3) thick (Figs. 12D,13). The encystment process lasts about 1 min.

Species description

Type host: *Cerithidea cingulata* (Gmelin 1790).

Type locality: Shuwaikh, Kuwait Bay, Kuwait.



Figs. 12-13. Drawings of *Cercaria kuwaitae* VI, metacercaria. **12A-D**. Process of cercaria encystment (freehand). **13.** Ventral view of an entire metacercaria.

Date of collection: October-February, 1994.
Infection site: Gonads and hepatopancreas.
Prevalence: 2.8% of 640 snails.
Specimens deposited: Helminth collection, Department of Zoology, University of Kuwait (Accession No. KUHC-C6).

DISCUSSION

By its single sac-like intestinal cecum, pair of eyespots, well developed suckers, thick aspinose tegument, flame cell pattern in groups of two and development in simple sporocysts *Cercaria kuwaitae* VI belongs to the family Haplosplanchnidae. Cable (1954) and Fares and Maillard (1975), investigated experimentally the life cycle of *Haplosplanchnus acutum* and *H. pachysomus*, respectively. Haplosplanchnid cercariae develop in marine prosobranch snail and shortly after emergence they encyst on vegetation. Fishes, especially mullet become infected by ingesting vegetation infested with the metacercariae. *Cercaria kuwaitae* VI shares basic haplosplanchnid characteristics with the cercariae: *C. haplocoecum* from *Assiminea*

japonica in Japan (Ogata, 1943), *C. caribbea* XXXVIII from *Cerithium variabile* in Puerto Rico (Cable, 1954 & 1956) and *Haplosplanchnus pachysomus* from *Hydrobia ventrosa* in the Mediterranean (Fares and Maillard, 1975), but differs in the absence of finger-like projections on the tail, the presence of cephalic glands, and the V-shape rather than spherical excretory bladder.

Surface detail of the cercaria is obscured by a thick tegument folded into a series of concentrically arranged ridges. The pattern of the tegumental folds may, among other functions, protect the cercaria during the free-living stage in the sea without hindering flexibility needed for swimming and encystment process. The body surface of the cercaria is devoid of spines and attachment of the adult stage to the host tissue is provided by the well developed suckers. Distinct types of domed papillae are observed on the tegument of *C. kuwaitae* VI, mostly on and around the suckers, and ciliated papillae such as those found on cercariae actively seek out the second intermediate host are absent. Domed papillae have been found on the tegument of a variety of trematodes and TEM studies of the tegumental syncytium revealed sensory bulbs associated with these papillae (Smyth and Halton, 1983). The morphological features and pattern of distribution of papillae on the body and suckers of the cercaria indicate that they may act as contact or stretch receptors during the search for a suitable substratum for encystment. The number, structure and arrangement of the papillae on suckers of the cercaria appear to be constant and may provide basis for correlating it to the adult. Sakamoto and Ishii (1978) showed that while that cercariae of *Schistosoma mansoni* and *S. japonicum* are quite similar in their tegumentary structures, both the number and distribution of their tegumental papillae are distinctly different.

Cercaria kuwaitae VI is the first haplosplanchnid to be described from a *Cerithidea* species. Although several adult haplosplanchnid species have been reported from fishes in the Arabian Gulf and Indian Ocean region (Gupta and Ahmad, 1979; Madhavi, 1979; Ahmad, 1985; Abdul-Salam and Khalil, 1986), determination of the identity of the new cercaria awaits experimental tracing of the life cycle.

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