Surface morphology of *Probolocoryphe uca* (Sarkisian, 1957) (Digenea: Microphallidae) from Kuwait Bay

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**Abstract**

The surface ultrastructure of *Probolocoryphe uca* (Digenea: Microphallidae), recovered from a rat experimentally fed on crabs, *Nanosesarma minutum* (Brachyura: Grapsidae), naturally infected with the metacercariae, was studied by scanning electron microscopy. The flukes were leaf-like, ventrally concave and pyriform or ovoid in outline. The anterior end was modified into a sucker-like organ, comprising a protrusible disc-shaped structure surrounded by single-pointed spines. This organ is probably involved in the attachment and feeding process in a manner similar to the action of the oral suckers. Apart from the sucker-like organ, the entire tegumental surface was covered with triangular spines with multi-pointed tips. Ciliated, dome-shaped papillae were observed, singly or in groups, arranged symmetrically on the sucker-like organ and around the oral and ventral suckers. Kuwait Bay constitutes a new geographical record and the crab *N. minutum* is a new second intermediate host record for *P. uca*.

**Introduction**

Members of the family Microphallidae Travassos, 1920 are minute flukes parasitic in the intestine of vertebrates. Studies on the life-histories of microphallids in aquatic environments have demonstrated a three-host life-cycle, involving a mollusc, a crustacean and a vertebrate. Otagaki (1958) erected the genus *Probolocoryphe*, with *P. asadai* (Otagaki, 1958) Otagaki, 1958 as the type-species, for an adult worm recovered from experimental animals fed on crabs naturally infected with the metacercariae. The other two species in the genus are *P. glandulosa* (Coil, 1955), described from adults from shore birds, and *P. uca* (Sarkisian, 1957), described from metacercariae from experimentally infected crabs, *Uca crenulata*. *Probolocoryphe* spp. are morphologically characterised by their peculiar anterior end, which appears under light microscopy (LM) as an angular basal bulb surmounted by a conical projection. In the present study, the surface morphology of *P. uca*, which had been recovered from a rat experimentally fed on crabs, *Nanosesarma minutum* (Brachyura: Grapsidae), naturally infected with the metacercariae, was studied using scanning electron microscopy (SEM).

**Materials and methods**

Crabs, *Nanosesarma minutum* (De Mann), harbouring the metacercariae of *Probolocoryphe uca* were collected on the eulittoral zone of Kuwait Bay, in the vicinity of Kuwait City, during May, 1998. Adult *P. uca* were recovered from the small intestine of an albino rat 72 hr after being fed with infected crabs. For LM, live metacercarial cysts and adult specimens of *P. uca* were stained with 0.5% neutral red. Photographs were taken using an Olympus AHBS3 research photomicrographic microscope system. For SEM, live adult specimens of *P. uca* were washed with cold physiological saline and fixed in a solution containing 4% formaldehyde and 1% glutaraldehyde in a 0.1m phosphate buffer (pH 7.2) at 4°C. Following the appropriate buffer wash, the specimens were post-fixed in 1% osmium tetroxide in the same buffer for 5 min at 4°C. Dehydrated in a series of anhydrous acetone and then examined in the Jeol JSM-6300 scanning electron microscope. Voucher specimens are deposited in The Natural History Museum, London, BM(NH) 1998.4.9.6.
Results

Figure 1A,B shows the general features of the cyst and adult stages of Probolocoryphe uca as seen under LM (for a detailed LM study, see Sarkisian, 1957).

SEM observation

The body of P. uca was oval and ventrally concave (Figures 2,3). The tegumental surface was covered with multi-pointed spines arranged in transverse rows (Figure 4). The anterior end was modified into a sucker-like organ, comprising a protrusible disc-shaped structure, 30μm in diameter. The surface of the disc was highly wrinkled and devoid of spines. Its rim was covered with peg-like spines bearing longitudinal grooves (Figures 5,6) and was fused with the upper lip of the oral sucker. Spines on the rim were approximately 4 times the size of those protruding from the tegument. The oral sucker was thin-lipped and bore prominent papilla on the lower lip (Figure 6). Only one type of tegumentary sensory papilla was observed throughout the body, appearing as a dome-shaped tegumental swelling with a short cilium protruding from an apical collar (Figures 4–6). These papillae were disposed singly or in groups and arranged in a bilaterally symmetrical pattern (Figure 2). They were particularly abundant on the centre of the sucker-like organ (Figure 5) and around the oral sucker (Figure 6). The ventral sucker was located in the middle of the body; its lip was not well defined and encircled by papillae (Figure 7). The genital pore was apparent as a circular depression of the tegument lateral to the ventral sucker (Figure 7). The inner lining of the genital pore opening was provided with minute tegumental protuberances. Laurer’s canal opened medially and postero-dorsally on the body (Figure 3). Spermatozoa were often observed protruding from the apertures of genital atrium and Laurer’s canal. The excretory pore opened at the posterior end of the body (Figure 2).

Discussion

The surface topography of Probolocoryphe uca was generally similar to that of other digeneans which have been previously studied (Lee et al., 1985; Chai et al., 1992; Koie, 1992; Srisawangwong et al., 1997; Uga et al., 1998). The multi-pointed (serrate) tegumental spines and the ciliated dome-shaped papillae char-