

Surface structures of *Brachylaimus fuscatus* (Digenea: Brachylaimidae) metacercaria

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Abstract. *Brachylaimus fuscatus* metacercaria develops unencysted in the terrestrial snail *Ponsadenia duplocincta*. For the first time in a larval stage of the genus *Brachylaimus* a distinctive surface structure has been observed. This structure of net-like interconnected ridges of the tegument was present on the whole body surface with the exception of the anterior part. Beside this structure scanning electron microscopy revealed five types of papillae. Three types, dome-like papillae, papillae with a finger-like process, and hollow papillae with a short cilium, were localized mainly in the suckers. Hollow papillae without a cilium were arranged in groups or singly around the ventral sucker and genital pore. Ribbed papillae were observed on the ventral body surface.

The metacercaria of *Brachylaimus fuscatus* (Rud., 1819) is unencysted, as are metacercariae of other members of the family Brachylaimidae Joyeux et Foley, 1930. Detailed scanning electron microscopic (SEM) studies of this metacercaria revealed a distinctive net-like structure corresponding with the interlacing network of ridges of the metacercaria of *Postharmostomum gallinum* Witenberg, 1923, earlier studied by us (Ždárská et al. 1990). This species belongs to the same family as *B. fuscatus*. The first description of such superficial structure was given by Font and Wittrock (1980) in the metacercaria of *Leucochloridiomorpha constantiae* (Mueller, 1935) Allison, 1943 from the family Leucochloridiomorphidae.

The aim of the present study was to compare at the scanning electron microscopical level the external surface of the unencysted metacercariae of the family Brachylaimidae.

MATERIALS AND METHODS

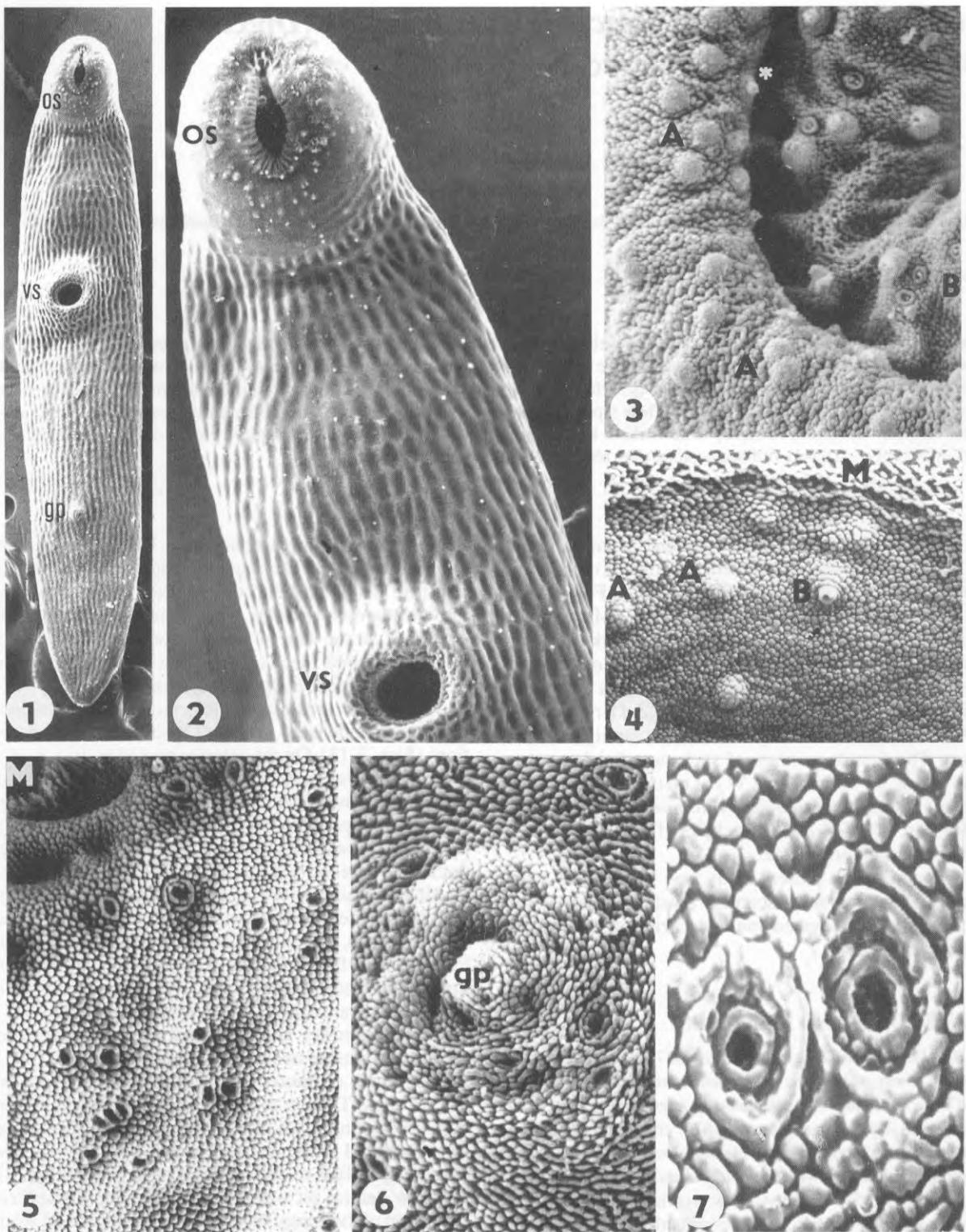
Brachylaimus fuscatus metacercariae (0.70 - 1.62 mm long and 0.30 - 0.35 mm wide) at the stage of intensive feeding were recovered from the kidney of spontaneously infected terrestrial snails *Ponsadenia duplocincta* (Martens, 1879) collected by Dr. T. A. Soboleva in 1988 in the vicinity of Alma-Ata (Kazakhstan). After washing in saline they were fixed in cold and hot Baker's solution (Pearse 1985). Then the metacercariae were dehydrated through a graded ethylalcohol series and dried in CO₂ at critical point, mounted on stubs with silver paste, coated with gold and examined in a Tesla 300 BS scanning electron microscope at an accelerating voltage of 9 kV.

RESULTS

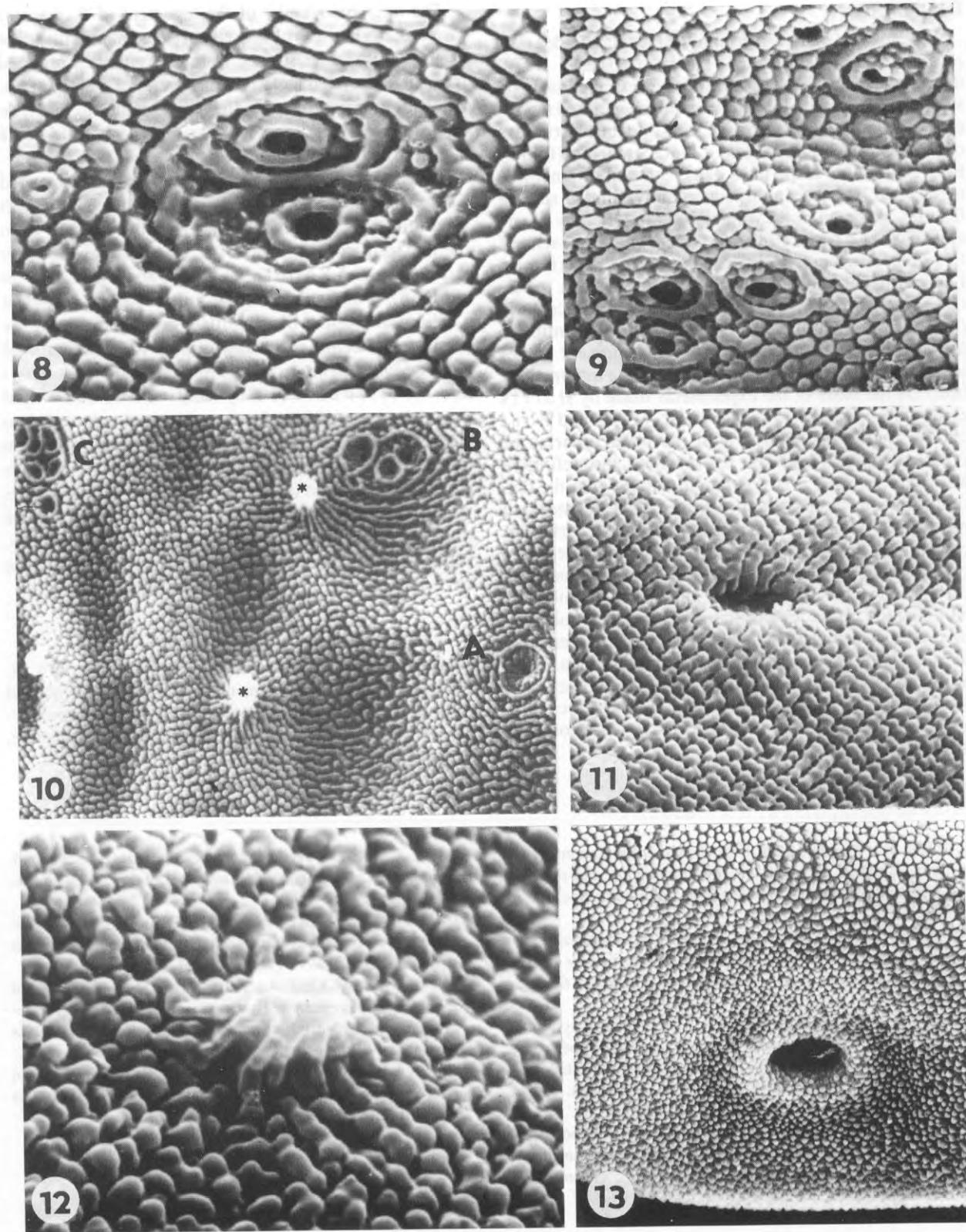
The tegument of the metacercaria (Figs. 1, 2) formed interconnected ridges over the whole body surface with

the exception of the anterior part. The ridges arranged longitudinally and transversally thus formed a net-like structure and divided the body surface of the metacercaria into mostly oblong fields. The orientation of these fields depended on the state of contraction or dilatation of the body. In dilated metacercariae the orientation of these fields was longitudinal, in contracted transverse.

The surface area of the metacercarial body is highly increased by densely arranged microvillus-like projections (Figs. 3-13). On the anterior part of body, i. e. at the level of the oral sucker, small pointed spines irregularly distributed in rows were present on the lateral sides only. Five types of papillae, presumed to be sensory, were dispersed on the surface of the metacercarial body. Elevated dome-like papillae (Figs. 3, 4) with a rugose surface were distributed mainly in the tegument of both the ventral and oral suckers, few of them were localized in the tegument of the ventral, lateral and dorsal surfaces of the body. Papillae with finger-like projections (Figs. 3, 4), sometimes with a short cilium visible, were present on both the ventral and oral suckers. There was a type of papillae on the ventral surface not described in digeneans. These papillae we designated as ribbed papillae (Figs. 10, 12). The tegument of these papillae formed ribs running from base to apex. The top of the ribbed papillae was smooth. Papillae bearing a hollow central pit and a short cilium were localized in the oral sucker only (Fig. 3). The most frequent papillae of this metacercaria were those with a hollow central pit without a cilium. The highest concentration of these papillae has been demonstrated around the ventral sucker (Figs. 5, 8, 10) and genital pore (Fig. 6). In the area around the ventral sucker these papillae were distributed in groups of 2 to 5 or single (Figs. 5, 9, 10). The central pits of these hollow papillae were surrounded by one (Figs. 5, 6), two (Fig. 7) or three (Fig. 8) circles of elevated tegument. Around the genital pore hollow, pitted papillae were distributed singly (Fig. 6). Papillae were not detected on the surface around the opening of Lauder's canal (Fig. 11) and excretory pore (Fig. 13).



Figs. 1-7. *Brachylaimus fuscatus* metacercaria. **Fig. 1.** Note the well visible net-like interconnected ridges of the tegument of the whole body surface with the exception of the anterior part. os - oral sucker; vs - ventral sucker; gp - genital pore. x 65. **Fig. 2.** Detail of Fig. 1. x 175. **Fig. 3.** Detail of oral sucker papillae. A - dome-like papillae, B - hollow papillae with a short cilium, * - papilla with a finger-like process. x 1560. **Fig. 4.** Papillae on the inside of the ventral sucker. A - dome-like papillae; B - papilla with finger-like process; M - margin of the ventral sucker. x 1850. **Fig. 5.** Hollow papillae without a cilium localized around the ventral sucker. M - margin of the sucker. x 1720. **Fig. 6.** Hollow papillae without a cilium distributed around the genital pore (gp). x 1630. **Fig. 7.** Detail of two hollow papillae with two circles of elevated tegument. x 5920.



Figs. 8-13. *Brachylaimus fuscatus* metacercaria. **Fig. 8.** Detail of two hollow papillae surrounded with three circles of elevated tegument. x 6215. **Fig. 9.** Detail of a group of hollow papillae without a cilium. x 4700. **Fig. 10.** Detail of the body surface near the ventral sucker. Note two ribbed papillae (*) and groups of one (A), three (B) and five (C) hollow papillae without a cilium. x 1720. **Fig. 11.** Tegument structure in the region of Laurer's canal. Note the microvillus-like projections. x 2825. **Fig. 12.** Detail of a ribbed papilla. x 6280. **Fig. 13.** Tegument structure around the excretory pore. x 1720.

DISCUSSION

It seems that in the superfamily Brachylaimoidea (last revision made by Gvozdev and Soboleva 1978) the distinctive net-like structure of the metacercarial body surface is common to the families with unencysted metacercaria. The observation of this surface structure observed in *B. fuscatus* metacercaria (family Brachylaimidae) is the third demonstration of a net-like tegumental structure in this superfamily. Similar surface features observed in SEM have been reported by Font and Wittrock (1980) from the metacercaria of *Leucochloridiomorpha constantiae* (family Leucochloridiomorphidae) and by Žárská et al. (1990) from *Postharmostomum gallinum* metacercaria (family Brachylaimidae). The three above mentioned metacercariae do not encyst and their tegument comes into immediate contact with the tissue of the intermediate host. On the basis of the experiments made by Harris et al. (1974), Contos and Fried (1976) and Font and Wittrock (1980) with the metacercariae of *L. constantiae* in both the intermediate and definitive hosts and *in vitro*, it is possible to assume that the distinctive superficial structure protects the unencysted metacercariae to come with their whole body surface in contact with the intermediate hosts tissue, and later, when swallowed by the definitive host, it protects the metacercaria passing through the host gut. In my opinion the modification of the tegumental structure of the metacercaria of *B. fuscatus*, as in other unencysted metacercariae of species of the superfamily Brachylaimoidea, is an adaptation to the environment. The rapidity of the transformation of the ridged tegument of the metacercaria to a smooth one in the definitive host, and *in vitro*, confirms this opinion.

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The microtopography of the tegument of *B. fuscatus* metacercaria consists of densely arranged microvillus-like projections. Similar surface features have been reported from all unencysted metacercaria species of the superfamily Brachylaimoidea studied by scanning electron microscopy (Font and Wittrock 1980, Žárská and Soboleva 1980, Žárská et al. 1990).

Detailed studies of *B. fuscatus* metacercaria revealed five, presumably sensory types of papillae. Those with finger-like processes correspond with the papillae from the metacercaria and adult of *B. aequans* described by Žárská and Soboleva (1980) and Žárská et al. (1988). Two types, hollow, pitted papillae without a cilium and dome-like papillae, correspond with the papillae in *Leucochloridiomorpha constantiae* and *Brachylaimus aequans* metacercariae shown by Font and Wittrock (1980) and Žárská and Soboleva (1980). In the above mentioned two species hollow pitted papillae without a cilium have been described as distributed singly. In *B. fuscatus* metacercaria, we assume, on the basis of the concentration of this papillae in groups around the ventral sucker, that this type of papillae could represent openings of some gland cells. Ribbed papillae and hollow, pitted papillae with a cilium have not been reported previously in Brachylaimoidea.

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