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Ultrastructure of Sensory Receptors of Adult *Echinostoma hortense* (Trematoda: Echinostomatidae)

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Abstract

Four types of sensory structures were studied in adult *Echinostoma hortense* by scanning and transmission electron microscopy. Two types of ciliated papillae and two types of aciliate bulbs occurred around the oral sucker. Type 1 ciliated papillae were predominant and had a cilium projecting above the tegumental surface of the papillae. This type of papilla was also common in the spinous tegument and around the ventral sucker. Type 2 ciliated papillae had a modified short cilium. Aciliate nerve bulbs with an electron-dense body (type 3) and aciliate nerve bulbs with a rootlet (type 4) were situated within the tegument. The fine structure of these sensory structures is described and their possible functions are discussed.

Key words: *Echinostoma hortense,* sensory receptor, trematoda, ultrastructure

Introduction

The ultrastructure of trematode sensory receptors has been described in miracidia (Wilson, 1970; Pan, 1980), cercariae (Nuttman, 1971; Page *et al.*, 1980), and juveniles and/or adults (Morris and Threadgold, 1967; Nollen and Nadakavukaren, 1974; Bakke, 1976; Edwards *et al.*, 1977; Fujino *et al.*, 1979; Font and Wittrock, 1980; Hoole and Mitchell, 1981; Ip and Desser, 1984). In the family Echinostomatidae (Trematoda), Chapman and Wilson (1970) observed ciliated papillae in the tegument of the cercaria of *Himasthla secunda* by scanning and transmission electron microscopy (SEM and TEM). Recently, the sensory structures of newly excysted or adult worms have been described for *Echinostoma revolutum* by Fried and Fujino (1984), *H. leptosoma* by Irwin *et al.* (1984), *E. revolutum* and *Isthmiophora melis* by Smales and Blankespoor (1984), and *Mesorchis denticulatus* by Køie (1987). While a number of studies by SEM have described ciliated and/or aciliate papillae on the tegumental surface of echinostomes, there have been no detailed studies by TEM of these structures in adult worms. The present investigation was undertaken to obtain more detailed observations on the tegumental sensory receptors of the *E. hortense* by correlating SEM and TEM.

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Materials and Methods

Metacercariae of *Echinostoma hortense* Asada, 1926 were obtained from soft tissues adjacent to the gills of loaches, *Misgurnus anguillicaudatus*. They were orally administered to Wistar rats, and adult worms were collected from the small intestine of the rats at 30 days postinfection.

For SEM, worms were treated in phosphate buffered Ringer's solution (pH 7.2) containing 2.5 mg/ml trypsin (DIFCO 1:250) for about 15 min at 37°C, fixed in 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). The specimens were post-fixed in 2% osmium tetroxide, dehydrated through a graded series of ethanol and immersed in isoamyl-acetate. After drying by the critical-point method and sputter-coating with platinum, the specimens were examined with a Hitachi S-500A scanning electron microscope at 20 kV.

Specimens for TEM were fixed immediately in 4% glutaraldehyde in 0.1 M phosphate buffer

(pH 7.4), washed in the same buffer and post-fixed in 2% osmium tetroxide, dehydrated in a graded series of ethanol and embedded in Epon 812 (SERVA, Heidelberg). Ultrathin sections were cut with a Porter-Blum MT-2 microtome, double-stained with uranyl acetate and lead citrate, and viewed with a Hitachi HU-12A electron microscope at 75 kV.

Results

Sensory structures in the anterior region

The anterior region of adult *Ehinostoma hortense,* including the oral sucker and spiny collar, had an aspinous tegument composed of cobblestone-like or somewhat parallel rows of rounded protuberances. Two types of ciliated papillae and two types of aciliate bulbs occurred in this region. A large number of ciliated papillae were arranged in two concentric circles around the oral sucker. The inner circle was situated on the rim of the oral sucker while the outer one surrounded its base. Clusters of ciliated papillae were observed on the tegument between the oral sucker and the collar spines. Ciliated papillae were also scattered inside the edge of spiny collar (Fig. 1).

Type 1 ciliated papillae This type of papilla formed a smooth-surfaced mound on the tegumental surface and had a centrally situated cilium (Fig. 2). Longitudinal sections through individual papillae revealed that each cilium emerged from the bulbous extension of a sensory process. The cilium, 1.5 to 2.0 μm in length, arose from a basal body with an attached rootlet. The bulb was attached to the tegument by a septate desmosome. Immediately beneath the desmosome on the inner surface of the bulb plasma membrane were two rings of electron-dense material. The bulb contained mitochondria, small electron-lucent vesicles and microtubules (Figs. 4, 5).

Type 2 ciliated papillae Viewed by SEM, these papillae appeared as smooth surfaced mounds which bore a centrally situated knob-like structure (Fig. 3). TEM demonstrated that this apical knob was a short cilium. The cilium, 1.0 to 1.2 μm in length, was mostly embedded in the tegument. The mid-portion of the cilium was somewhat swollen and had randomly arranged microtubules. The nerve bulb contained a concentric electron-dense ring, electron-lucent vesicles, mitochondria and microtubules. The rootlet attached to the basal body was not prominent (Fig. 6).

Type 3 aciliate nerve bulbs Bulbous extensions of nerve processes were located beneath the tegumental surface and were not distinguishable by SEM. By TEM, bulbs measured 5.2 to 5.8 μm in diameter and 4.9 to 6.0 μm in height and contained a large electron-dense body that was not bounded by a membrane. The electron-dense body was connected to an electron-dense undercoating on the inner surface of the nerve bulb membrane by fine fibrils. The bulb also contained mitochondria, small electron-lucent vesicles, small electron-dense vesicles and microtubules (Figs. 7, 8).

Type 4 aciliate nerve bulbs This type of sensory structure was located in the tegument just above the basal lamina, and did not extend to

Fig. 1 Tegumental surface around the oral sucker (OS). Ciliated papillae are distributed on the rim of oral sucker (arrowhead), around the oral sucker (*), on the spiny collar (arrow) and between the oral sucker and the collar spines (SP). ×460.

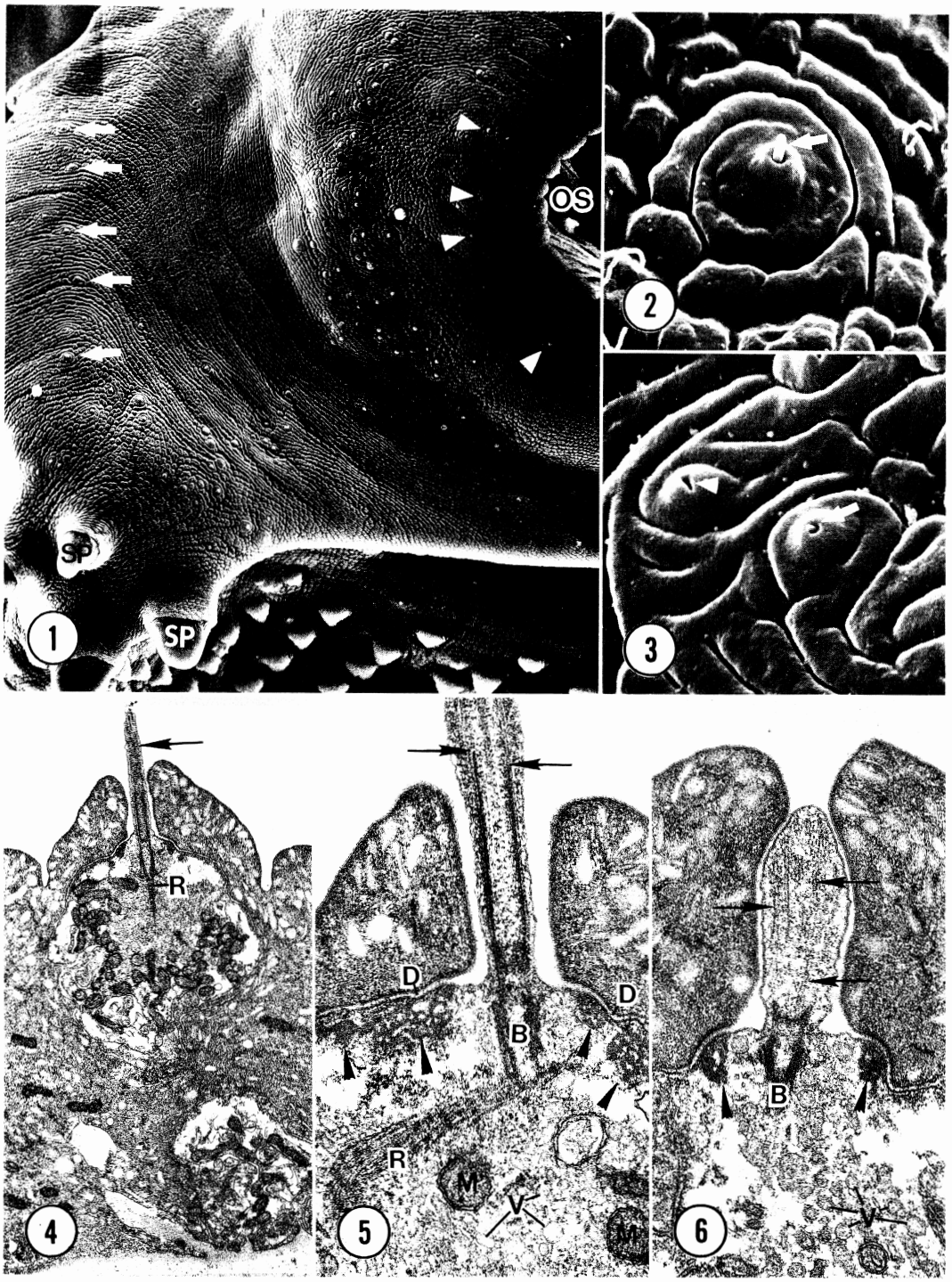
Fig. 2 A type 1 papilla with a cilium (arrow) in the center. ×6800.

Fig. 3 Type 2 papillae with a small knob-like cilium (arrow) and with an opening of the apical pit (arrowhead). ×6000.

Fig. 4 Longitudinal section of a type 1 papilla with a cilium (arrow) and rootlet (R). ×12000.

Fig. 5 Enlarged view of a type 1 papilla showing the microtubules of a cilium (arrow), basal body (B), rootlet (R), electron-dense rings (arrowhead), desmosome (D), mitochondria (M), and electron-lucent vesicles (V). ×31000.

Fig. 6 Longitudinal section through a type 2 papilla showing a short cilium which contains microtubules (arrow), a basal body (B), an electron-dense ring (arrowhead), and electron-lucent vesicles (V). ×28000.


OS
SP
SP
1
2
3
R
4
D
D
B
R
M
V
M
5
B
V
6

the surface of the worm. By TEM, bulbs measured 3.0 to 3.4 μm in diameter and 3.5 to 4.1 μm in height, had an inverted pear-like appearance and contained a prominent central rootlet. Instead of a cilium, a small bell-shaped hollow was observed in the tegument above the basal body of the rootlet. The rootlet had alternating electron-dense and electron-lucent bands and was surrounded by mitochondria and microtubules. Two electron-dense rings were closely associated with the apical membrane of the bulb (Figs. 9, 10).

Sensory structures posterior to the cephalic collar

Type 1 ciliated papillae were also common around the ventral sucker and on the spinous tegument of the anterior half of the body. They usually occurred singly (Figs. 11, 12, 13, 14), but occasionally occurred doubly, triply, or in quadruples around the ventral sucker (Fig. 14).

Discussion

Two types of ciliated papillae were observed in *Echinostoma hortense*: type 1 papillae with a cilium that projected above the tegumental surface as described previously in other trematodes (Morris and Threadgold, 1967; Chapman and Wilson, 1970; Edwards *et al.*, 1977; Fujino *et al.*, 1979; Page *et al.*, 1980) and type 2 papillae with a short cilium that barely projected above the top of papillae as a knob-like structure. Longitudinal sections of type 1 cilia demonstrated that microtubules ran from the basal body to the tip of the cilium in an orderly manner. On the other hand, microtubules in type 2 cilia appeared randomly arranged. The total number of microtubules and their exact relationships to one another could not be determined in either type 1 or type 2 cilia because cross sections of these structures were not obtained. Other differences between these cilia were evident in structures associated with their basal bodies; type 1 cilia had two electron-dense rings which encircled their basal bodies while type 2 cilia had only a single ring. Rootlets in type 1 cilia were well developed, while those in type 2 cilia were not.

Among echinostomatids that have been studied by SEM, Smales and Blankespoor (1984) described aciliate "domed papillae" in *E. revolutum* and *Isthmiophora melis*. Observations of papillae on *E. revolutum* demonstrated a central pore at their apex, but cilia or knob-like structures were not found. TEM observations of *E. hortense* demonstrated that the short, knob-like cilia of type 2 papillae sometimes failed to reach the surface of the tegument. By SEM, these papillae appeared to be aciliate. It is possible that the "domed papillae" observed in *E. revolutum* are identical to type 2 papillae of *E. hortense*.

Fujino *et al.* (1979) observed short, swollen, club-shaped cilia in "Type D" papillae of juvenile *Clonorchis sinensis*. Unlike the type 2 papillae of *E. hortense*, "Type D" papillae were almost completely covered by tegumental extensions and were not detected by SEM. Their internal structure was similar to that of type 2 papillae with the exception that the cilia contained vesicles. Fujino *et al.* (1979) suggested that these are modified cilia which may function as chemoreceptors (Lyons, 1969).

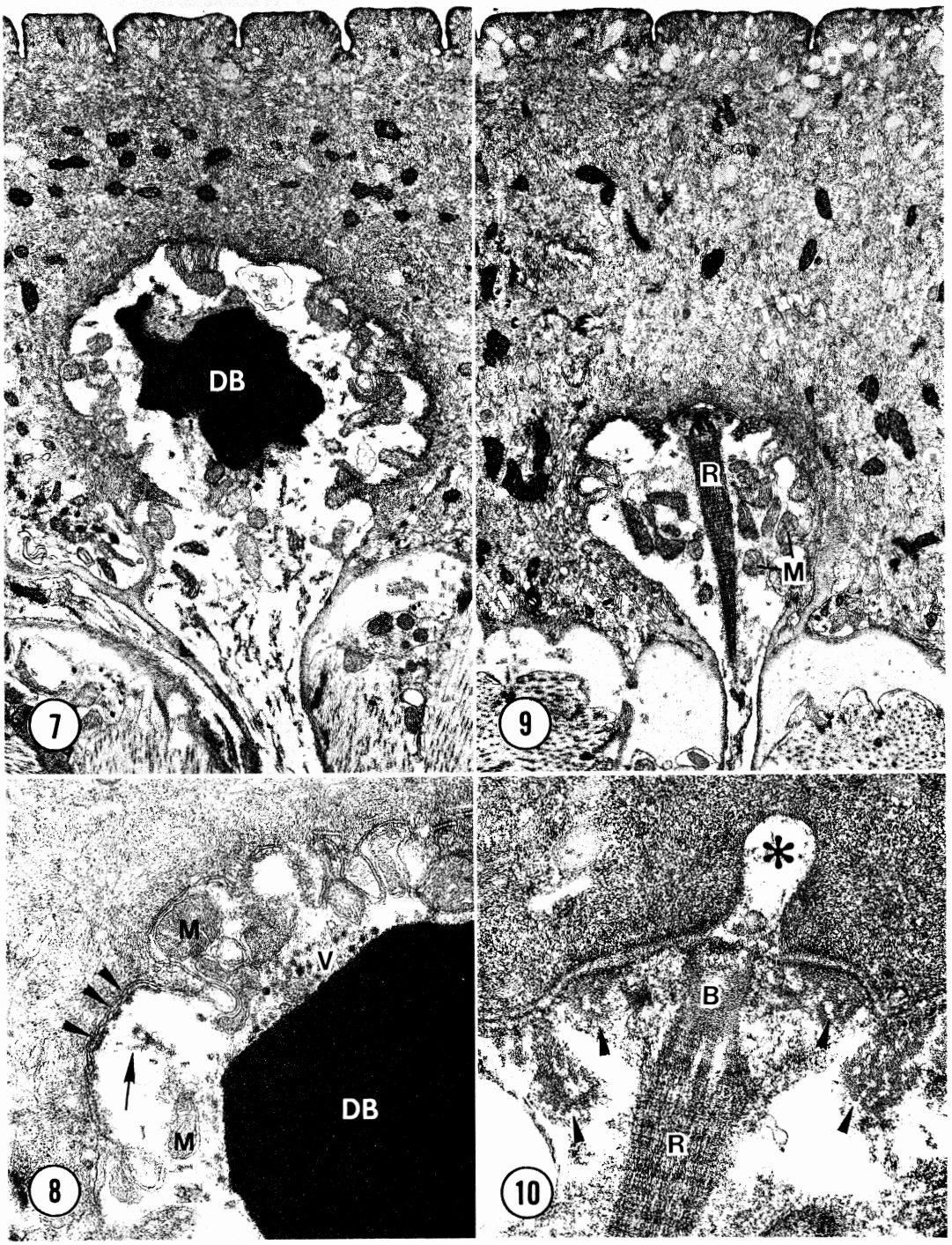
Two other trematode sensory structures are similar to the aciliate nerve bulbs (type 3) with electron-dense bodies of *E. hortense*. Fujino *et al.* (1979) described "Type C" papillae in newly excysted juvenile *C. sinensis* and Hoole and Mitchell (1981) observed "domed papillae" in juvenile and adult *Gorgoderina vitelliloba*. Both of these papillae contained a round, electron-dense body in the center of the sensory bulbs. Fujino *et al.* (1979) and Hoole and Mitchell (1981) observed tegumental protuberances by SEM which corresponded to these sensory structures. Although the aciliate nerve bulbs (type

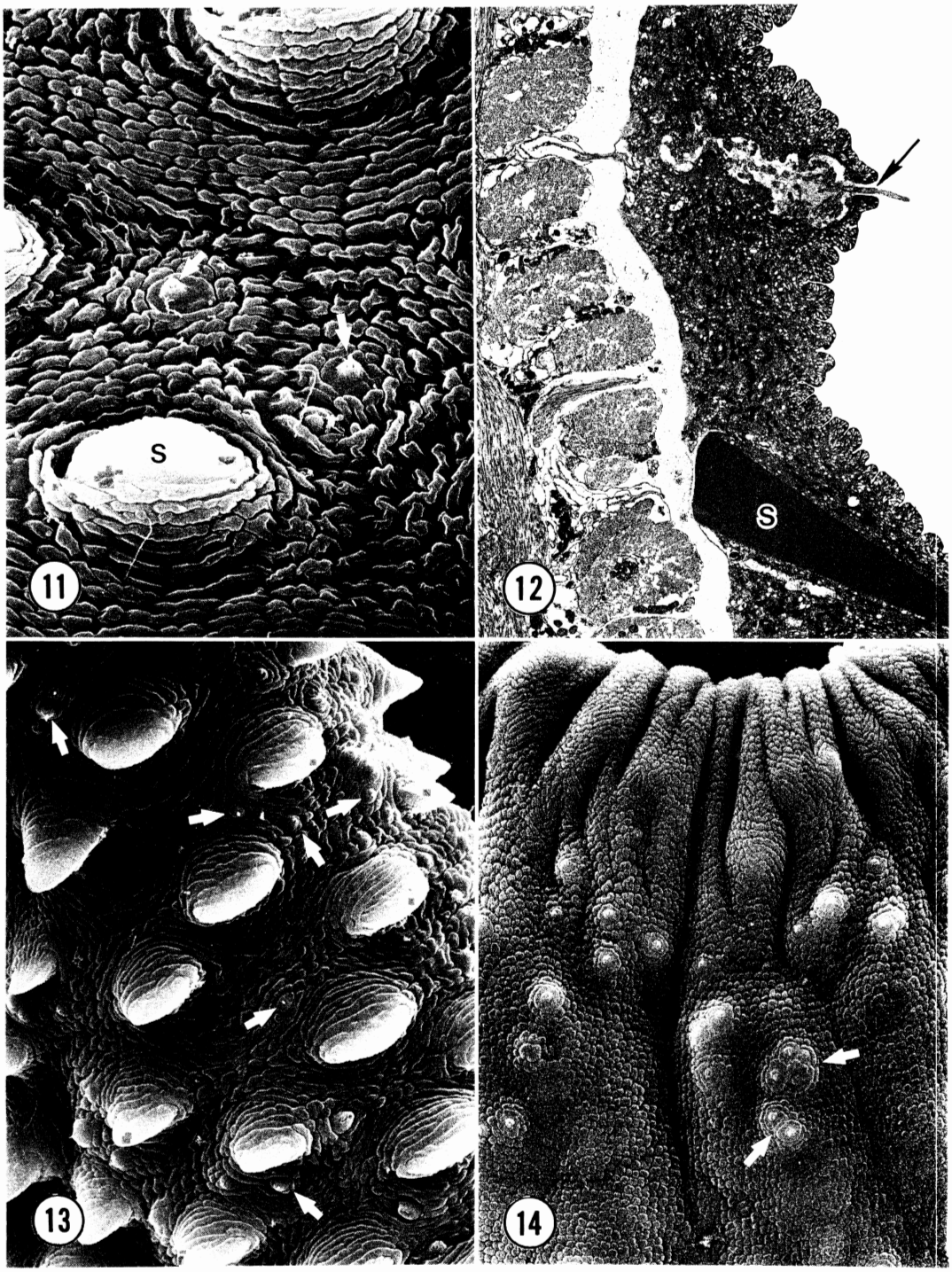
Fig. 7 Longitudinal section through a type 3 aciliate bulb with an electron-dense body (DB). ×11000.

Fig. 8 Enlarged view of a type 3 aciliate bulb with an electron-dense body (DB) containing mitochondria (M), small electron-dense and electron-lucent vesicles (V), an electron-dense undercoating (arrowhead), and fine fibrils (arrow). ×22000.

Fig. 9 Longitudinal section through a type 4 aciliate bulb, showing a rootlet (R) and mitochondria (M). ×11000.

Fig. 10 Enlarged view of a type 4 aciliate bulb showing a rootlet (R), electron-dense rings (arrowhead) and small bell-shaped hollow (*) above the basal body (B). ×44000.


DB
7
R
M
9
M
V
DB
M
8
B
R
10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3) of *E. hortense* were similar to these sensory receptors, the tegumental surface covering the bulbs did not form protuberances and had a normal cobblestone-like pattern. "Type C" papillae occur on the lip of oral and ventral suckers of *C. sinensis,* while "domed papillae" occur on the internal surface of the ventral sucker of *G. vitelliloba.* Aciliate nerve bulbs (type 3) are found around the oral sucker of *E. hortense.* Based on their distribution and structural features, these sensory structures may function as tangoreceptors.

Previous SEM observations have demonstrated aciliate papillae on the tegumental surface which were called "button" papillae (Nadakavukaren and Nollen, 1975; Hoole and Mitchell, 1981) or "domed" papillae (Bennett, 1975; Bakke, 1976; Bakke and Lien, 1978; Font and Wittrock, 1980; Page *et al.*, 1980). TEM observations have shown that these papillae are bulbous extensions of nervous processes which contain a prominent central rootlet (Bennett, 1975; Hoole and Mitchell, 1981). In the present study, *E. hortense* had similar aciliate nerve bulbs (type 4) with central rootlets, but no corresponding tegumental protrusions were detected by SEM. Similar aciliate sensory bulbs have recently been described in other trematodes (Ip and Desser, 1984) and also in cestodes (Webb and Davey, 1975; Richards and Arme, 1982; Torii, 1983), indicating that aciliate nerve bulbs (type 4) of *E. hortense* can be included in the same category. "Button" or "domed" papillae are situated on or around the oral and/or ventral suckers (Bennett, 1975; Bakke, 1976; Bakke and Lien, 1978; Font and Wittrock, 1980; Page *et al.*, 1980; Hoole and Mitchell, 1981; Smales and Blankespoor, 1984). The aciliate nerve bulbs (type 4) of *E. hortense* were found mostly around the oral sucker. Although electrophysiological studies of these sensory structures have not been done, most authors have speculated that the morphological features and patterns of distribution of these papillae indicate that they function as contact or stretch receptors during attachment and feeding of worms.

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Fig. 11, 12 SEM (Fig. 11) and TEM (Fig. 12) of the dorsal surface between spiny collar and ventral sucker. Type 1 papillae (arrow) and spines (S) are observed. Fig. 11; ×2800. Fig. 12: ×3800.

Fig. 13 The antero-lateral body surface showing ciliated papillae (arrow). ×1300.

Fig. 14 Tegumental surface around the ventral sucker showing many ciliated papillae. Double and quadruple ciliated papillae located on the postero-ventral surface of the ventral sucker (arrow) are seen. ×960.

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