한국전자현미경학회지 제 30 권 제 2 호 2000
Korean J. Electron Microscopy 30(2), 205~212(2000)

자루이형흡충(*Stictodora fuscatum*)의 표면 미세구조

손 운 목
경상대학교 의과대학 기생충학교실

Surface Ultrastructures of *Stictodora fuscatum* (Trematoda: Heterophyidae)

Woon-Mok Sohn
Department of Parasitology, College of Medicine, Gyeongsang National University, Chinju, 660-751, Korea
(Received April 22, 2000)

ABSTRACT

Present study was performed to observe the surface ultrastructures of *Stictodora fuscatum* (Trematoda: Heterophyidae). Adult worms were recovered from the cat experimentally infected with metacercariae, and were prepared for scanning electron microscopy. The body was leaf-like and ventrally concave. The oral sucker armed with prominent tegumental spines on the inner surface, and it's lips were obscure. Sensory papillae (type I) in single or grouped forms symmetrically arranged around the oral sucker. The ventrogenital opening retained protruding gonotyl spines and sperms discharged from the genital pore. The body surface was covered with scale-like multipointed tegumental spines. The density and digitated point of spines were gradually decreased toward the posterior end of the body. The digitated points of spines on the anterior portion were 9-12, on the middle were 7-8 and 5-6, and on the posterior were 2-3 and peg-like. Although the tegumental ultrastructure of *S. fuscatum* was generally similar to those of other heterophyid flukes, the oral sucker with tegumental spines on the inner surface and without obvious lips, and the ventrogenital opening with protruding gonotyl spines were suggested to be the characteristic features.

Key words : Scanning electron microscopy, *Stictodora fuscatum*, Surface ultrastructure, Tegumental spine

INTRODUCTION

Stictodora fuscatum (Trematoda: Heterophyidae) is an intestinal fluke of fish-eating birds and mammals including humans in Far Eastern countries such as Japan (Onji & Nishio, 1916) and Korea (Chai et al., 1988; Chai & Lee, 1990; Sohn et al., 1994). In the genus *Stictodora*, more than 20 species including the type species *S. sawakiensis* have been reported in the literature (Yamaguti, 1958; Velasquez, 1973; Kinsella & Heard, 1974). However, in Korea only 2 species, i.e. *S. fuscatum* and *S. lari*, have been described (Chai & Lee, 1990).

* Correspondence should be addressed to Dr. Woon-Mok Sohn, Department of Parasitology, College of Medicine, Gyeongsang National University, Chinju, 660-751, Korea. Ph.: (0591) 751-8757, FAX: (0591) 759-4022, E-mail: wmsohn@nongae.gsnu.ac.kr

Surface ultrastructure of trematodes give a clue to understand their morphological and taxonomical characteristics. Among the trematodes of the family Heterophyidae, surface ultrastructural studies were undertaken in various species representing the genera *Centrocestus* (Srisawangwong et al., 1997; Woo et al., 1998), *Cryptocotyle* (Køie, 1977), *Haplorchis* (Fujino et al., 1989), *Heterophyes* (Taraschewski, 1984; Chai et al., 1992; Uga et al., 1998), *Heterophyopsis* (Hong et al., 1991), *Metagonimus* (Lee et al., 1984; Fujino et al., 1989; Chai et al., 1998, 2000) and *Pygidiopsis* (Køie, 1992). However, any other studies on the surface ultrastructure of trematodes belonging to the genus *Stictodora* has not been yet done. Therefore, in order to know the ultrastructural characteristics of *S. fuscatum*, of which adults recovered from a kitten experimentally fed with metacercariae were observed by the scanning electron microscopy.

MATERIALS AND METHODS

The metacercariae of *S. fuscatum* were isolated from the gobies, *Acanthogobius flavimanus*, purchased at a local market in Haenam-gun, Chollanam-do, Korea, by the artificial digestion method. They were orally fed to a kitten using a gavage needle. Adult worms were recovered from the kitten 7 days after infection.

For scanning electron microscopy, collected worms were washed several times with physiological saline and 3 times with 0.1 M cacodylate buffer (pH 7.2). They were fixed in 2.5% glutaraldehyde at 4°C, and post-fixed in 1% osmium tetroxide for 2 hr at room temperature. After washing again with 0.1 M cacodylate buffer (pH 7.2), the specimens were dehydrated in graded ethanol (50%, 70%, 80%, 90%, 95% & absolute), dried in a ABT (model CP-5A) critical point dryer, and mounted on stubs. They were coated with gold using an ion sputtering coater (Bio-Rad, E 5300) and observed with a scanning electron microscope (ISI DS-130C, Korea) at an accelerating voltage of 10 kV.

RESULTS

The body of worms was leaf-like and ventrally concave and covered with scale-like multipointed tegumental spines (Fig. 1). The oral sucker armed with prominent tegumental spines on the inner surface, and it's lips were obscure. The type I sensory papillae (tegumental swelling with a short cilium at its apex) were found around the oral sucker. They symmetrically arranged in single or grouped forms around the oral sucker (Figs. 2, 3). The ventrogenital opening retained protruding gonotyl spines and sperms discharged from the genital pore (Fig. 4). Tegumental spines between the oral sucker and the genital pore were short but broad, and had 9-11 pointed tips (Fig. 5). On the ventro-mid surface, they were less densely distributed than those on the anterior portion, and had 5-6 pointed tips (Fig. 6). Two to three pointed peg-like spines and cobblestone-like cytoplasmic processes were observed on the posterior ventral surface (Fig. 7).

Whole dorsal surface was covered with scale-like multipointed tegumental spines. Their density was gradually decreased toward the posterior end of the body (Fig. 8). Tegumental spines on the dorso-anterior surface were short but broad, and had 9-11 pointed tips like those on the ventro-anterior surface (Figs. 9, 10). On the dorso-mid surface, tegumental spines had 7-8 pointed tips (Fig. 11). Tegument of dorso-posterior surface had 2-3 pointed peg-like spines and cobblestone-like cytoplasmic processes like that of ventro-posterior (Fig. 12).

DISCUSSION

Studies on the surface ultrastructure of adult heterophyid flukes were undertaken in various species i.e. *Cryptocotyle lingua* (Køie, 1977), *Metagonimus yokogawai* (Lee et al., 1984), *Metagonimus miyatai* (Chai et al., 1998) and *Metagonimus takahashii* (Chai et al.,

2000), *Heterophyes aequalis* (Taraschewski, 1984), *Heterophyes nocens* (Chai et al., 1992), *Heterophyes heterophyes* (Uga et al., 1998), *Heterophyopsis continua* (Hong et al., 1991), *Pygidiopsis ardeae* (Køie, 1992), *Centrocestus formosanus* (Srisawangwong et al., 1997), *Centrocestus armatus* (Woo et al., 1998), *Haplorchis* sp. (Fujino et al., 1989). However, the surface ultrastructure of trematodes belonging to the genus *Stictodora* is first observed by the present study.

The tegumental ultrastructure of *S. fuscatum* was generally similar to those of other heterophyids in shape of tegumental spines and cytoplasmic processes, and distribution pattern of tegumental spines and sensory papillae. However, the oral sucker with tegumental spines on the inner surface and without obvious lips, and the ventrogenital opening with protruding gonotyl spines were suggested to be the characteristic features.

The multipointed (sawtooth-shaped) tegumental spines seem to be one of the common characteristic features in the flukes of the family Heterophyidae. In present study, tegumental spines of *S. fuscatum* were multidigitated with 9-11, 5-8 and 2-3 points. Those of *H. nocens* were in most cases 12-17 points (Chai et al., 1992). Similar findings were also reported in other heterophyid flukes (Køie, 1977, 1992; Lee et al., 1984; Taraschewski, 1984; Fujino et al., 1989; Hong et al., 1991; Chai et al., 1992, 1998 & 2000; Srisawangwong et al., 1997; Uga et al., 1998; Woo et al., 1998).

The number of points and its changing pattern according to the worm maturation seems to be different by the species of worms. In case of *H. continua*, the number of points in the tegumental spines on anterior body was increased from 10-14 to 15-17 as the worm matured from metacercaria to adult (Hong et al., 1991). Similar findings were also reported in some heterophyid flukes (Chai et al., 1998, 2000). However, informations on the surface ultrastructures of metacercariae and juveniles of *S. fuscatum* is not available at present, changing pattern of tegumental spines according to the worm maturation could not be known.

In *S. fuscatum*, the number of points and density of tegumental spines were gradually decreased toward the posterior end of the body. The more pointed tegumental spines between oral sucker and the ventrogenital opening, and spines on the inner surface of the oral sucker of *S. fuscatum* seem to be related with abrasion of host intestinal epithelium for their feeding and anchorage. On the other hand, it has been generally known that two suckers in intestinal trematode play an important role against the worm elimination by peristalsis. By the way, *S. fuscatum* has no powerful adhesive organ. Not only its ventral sucker but also oral sucker is absent and/or incomplete. Especially in oral sucker, its muscular lips are inconspicuous. Whether this finding is the common characteristic feature in flukes of the genus *Stictodora* or not, the further study should be performed to verify in the future.

The sensory papillae with short cilium (type I papillae) were observed around the oral sucker, in single or grouped forms. The function of these papillae is still obscure, although functions as tango- and chemoreceptors have been inferred from their shapes and positions on the body, and from the results of transmission electron microscopy showing sensory bulbs associated with both types of the papillae (Morris and Threadgold, 1967). As speculated for other heterophyids, the papillae abundant on and around the oral sucker of *S. fuscatum* are probably tactile sensory receptors involved in mechanisms of feeding and attachment.

In conclusion, the surface ultrastructure of *S. fuscatum* was generally similar to those of other heterophyid flukes (Lee et al., 1984; Hong et al., 1991; Chai et al., 1992, 1998, 2000), however, some features were characteristic, which may be of taxonomic and bio-ecological significance.

REFERENCES

Chai JY, Chung HL, Choi MO, Sohn WM, Hong SJ, Lee SH: Surface ultrastructure of *Heterophyes nocens* (Trematoda:

Heterophyidae). Korean J Parasitol 30 : 75-82, 1992.

Chai JY, Guk SM, Han ET, Shin EH, Sohn WM, Choi SY, Lee SH: Surface ultrastructure of *Metagonimus takahashii* metacercariae and adults. Korean J Parasitol 38 : 9-15, 2000.

Chai JY, Hong SJ, Lee SH, Seo BS: *Stictodora* sp. (Trematoda: Heterophyidae) recovered from a man. Korean J Parasitol 26: 127-132, 1988.

Chai JY, Kang YJ, Choi SY, Guk SM, Yu JR, Lee SH: Surface ultrastructure of *Metagonimus miyatai* metacercariae and adults. Korean J Parasitol 30 : 75-82, 1992.

Chai JY, Lee SH: Intestinal trematodes of humans in Korea: *Metagonimus*, heterophyids and echinostomes. Korean J Parasitol 28(suppl.) : 103-122, 1992.

Fujino T, Higo H, Ishii Y, Saito S, Chen ER: Comparative studies on two similar species of *Haplorchis* and *Metagonimus* (Trematoda: Heterophyidae)-surface ultrastructure of adults and eggs. Proc Helminthol Soc Wash 56 : 35-41, 1989.

Hong SJ, Chai JY, Lee SH: Surface ultrastructure of the developmental stage of *Heterophyopsis continua* (Trematoda: Heterophyidae). J Parasitol 77 : 613-620, 1991.

Kinsella JM, Heard RWIII: Morphology and life cycle of *Stictodora cursitans* n. comb. (Trematoda: Heterophyidae) from mammals in Florida salt marshes. Tran Am Microsc Soc 93 : 408-412, 1974.

Køie M: Stereoscan studies of cercariae, metacercariae and adults of *Cryptocotyle lingua* (Creplin, 1825) Fischoeder, 1903 (Trematoda: Heterophyidae). J Parasitol 63 : 835-839, 1977.

Køie M: Scanning electron microscopy of cercariae, metacercariae and adults of *Pygidiopsis ardeae* Køie, 1990 (Digenea: Heterophyidae). Parasitol Res 78 : 469-474, 1992.

Lee SH, Seo BS, Chai JY, Hong SJ: Study on *Metagonimus yokogawai* (Katsurada, 1912) in Korean. VII. Electron microscopic observation on the tegumental structure. Korean J Parasitol 22 : 1-10, 1984. (in Korean)

Morris GP, Threadgold LT: A presumed sensory structure associated with the tegument of *Schistosoma mansoni*. J Parasitol 53 : 537-539, 1967.

Onji Y, Nishio T: On the trematodes whose second intermediate hosts are fishes from brackish water. Chiba Igaku Semmon Gakko Zasshi 81, 82 : 229-249, 1916. (in Japanese)

Sohn WM, Chai JY, Lee SH: *Stictodora fuscatum* (Heterophyidae) metacercariae encysted in gobies, *Acanthogobius flavimanus*. Korean J Parasitol 32 : 143-148, 1994.

Srisawangwong T, Pinlaor S, Kanla P, Sithithaworn, P: *Centrocestus formosanus*: surface morphology of metacercaria, adult and egg. J Helminthol 71 : 345-350, 1997.

Uga S, Morimoto M, Saito T, Rai SK: Surface ultrastructure of *Heterophyes heterophyes* (Trematoda: Heterophyidae) collected from a man. J Helminthol Soc Wash 65 : 119-122, 1998.

Velasquez CC: Observations on some Heterophydae (Trematoda: Digenea) encysted in Philippine fishes. J Parasitol 59 : 77-84, 1973.

Woo HC, Seo MD, Hong SJ: Surface ultrastructure of juvenile and adult stages of *Centrocestus armatus*. J Helminthol 72 : 215-219, 1998.

Yamaguti S: Systema Helminthum. Interscience Publishers, New York, pp. 699-724, 865-885, 1958.

FIGURE LEGENDS

Fig. 1. Whole ventral view of an adult *Stictodora fuscatum* recovered from the intestine of an experimentally infected cat, showing oral sucker (*) and ventrogenital pore (arrow mark).

Figs. 2-3. Tegument around the oral sucker. Note symmetrical distribution of sensory papillae in single or grouped forms around the oral sucker, of which lips are obscure.

Fig. 4. The ventrogenital opening showing protruding gonotyl spines (arrow marks) and sperms (white filament) discharged from the genital pore.

Fig. 5. Tegumental spines between the oral sucker and the genital pore. They are short but broad, having 9-11 pointed tips.

Fig. 6. Tegumental spines on the ventro-mid portion. They are less densely distributed than those on the anterior portion, having 5-6 pointed tips. b: Magnified view.

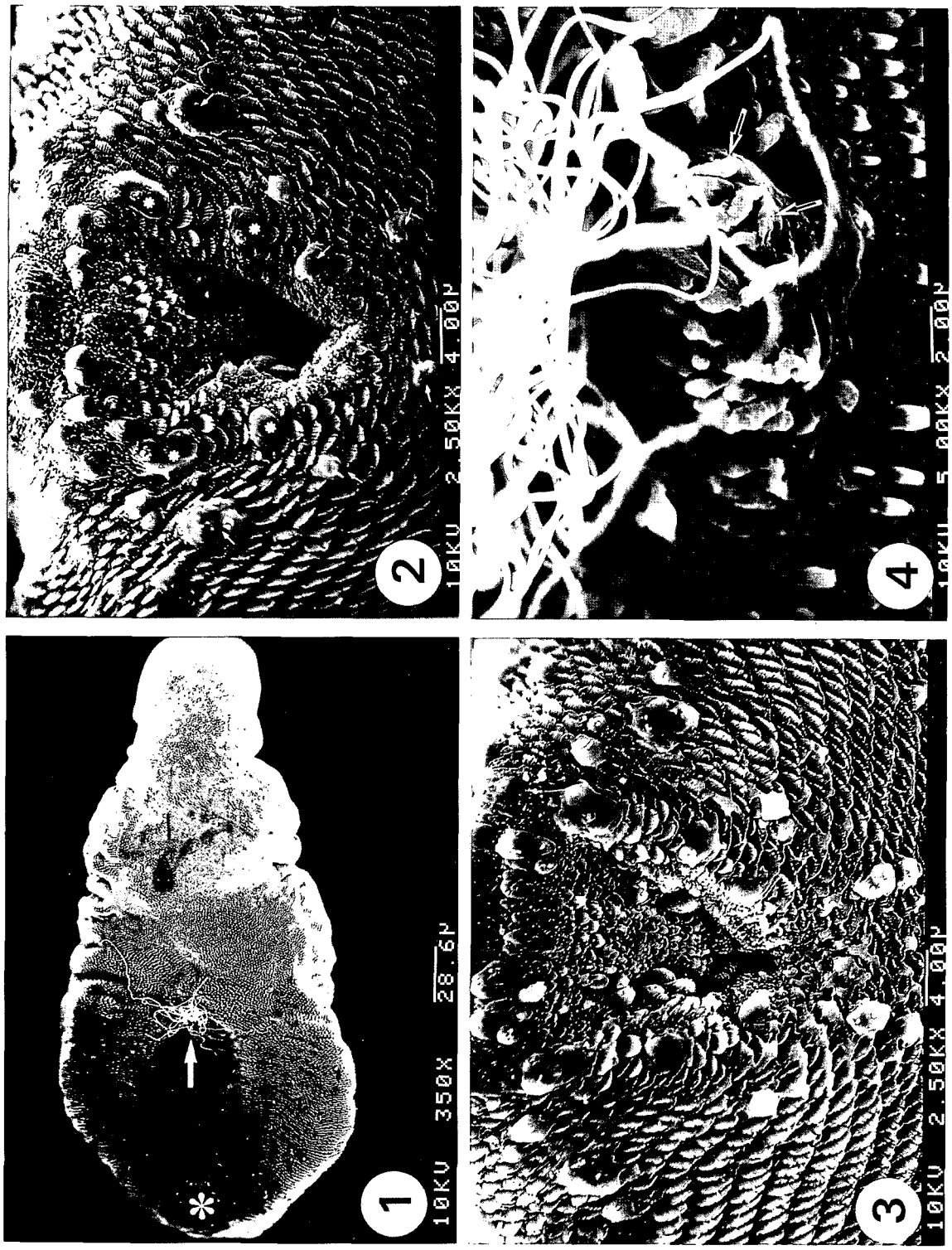
Fig. 7. Tegument of the posterior ventral surface. Two to three pointed peg-like spines and cobblestone-like cytoplasmic processes are seen. b: Magnified view.

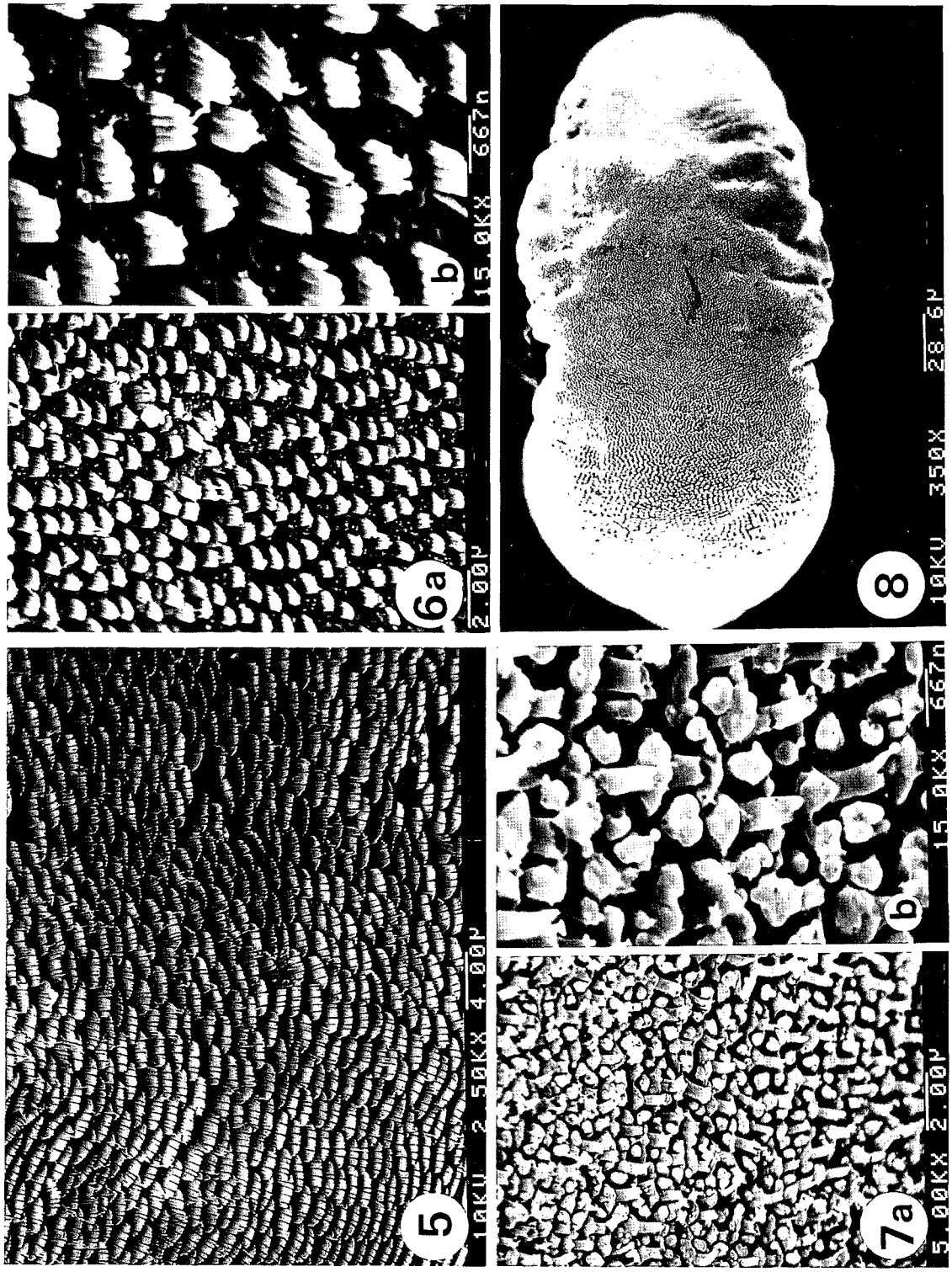
Fig. 8. Whole dorsal view, of which surface is covered with tegumental spines. The density of spines is gradually decreased toward the posterior end of the body.

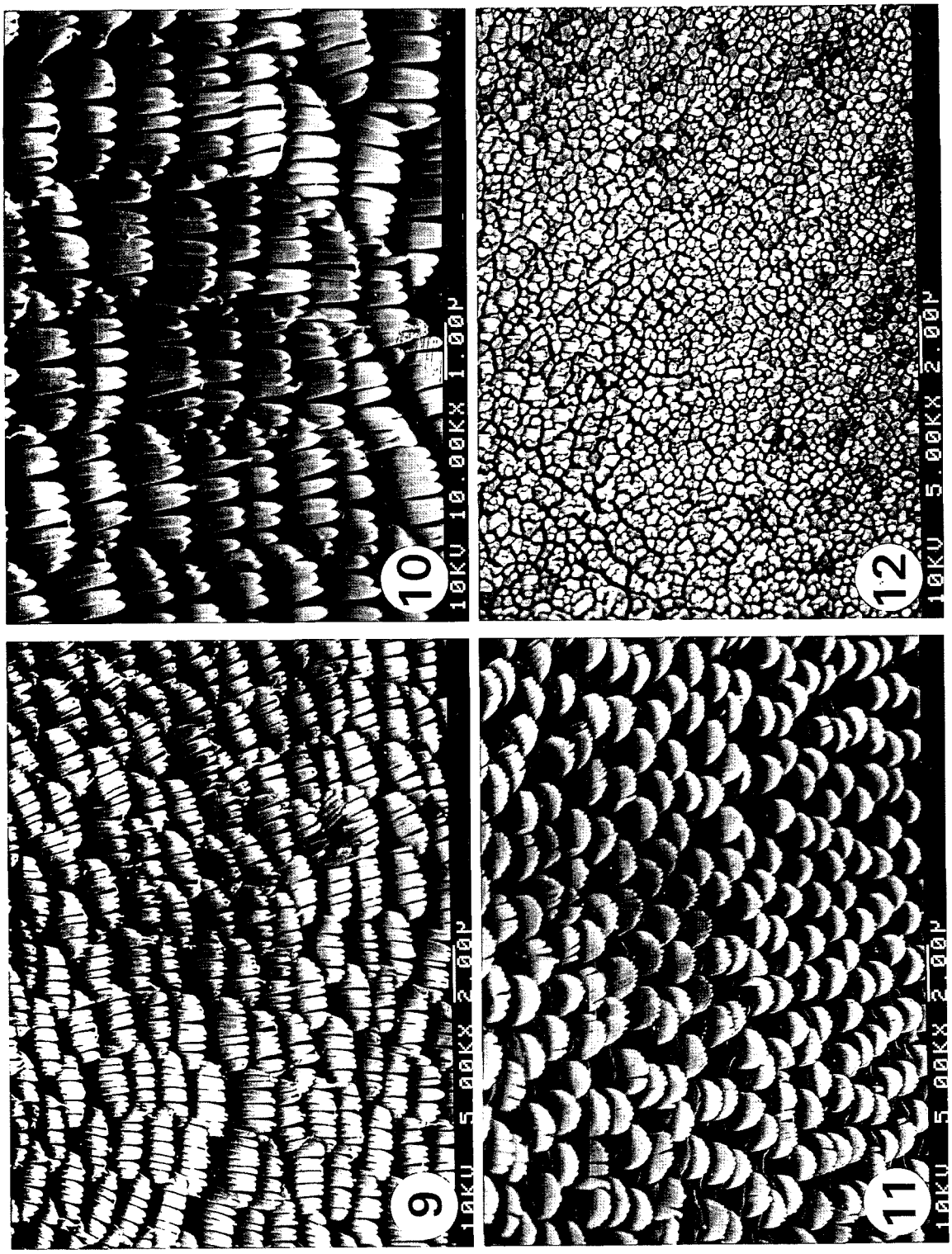
Figs. 9-10. Tegumental spines on the dorso-anterior portion. They are short but broad, having 9-11 pointed tips like those on the ventro-anterior.

Fig. 11. Tegumental spines on the dorso-mid portion. They are less densely distributed than those on the anterior portion, having 7-8 pointed tips.

Fig. 12. Tegument of the posterior dorsal surface. Two to three pointed peg-like spines and cobblestone-like cytoplasmic processes are seen.


1
10KU 350X 28.6μ
2
10KU 2.50KX 4.00μ
3
10KU 2.50KX 4.00μ
4
10KU 5.00KX 2.00μ


5
10KV 2.50KX 4.00μ
6a
2.00μ
b
15.0KX 667n
8
10KV 350X 28.6μ
7a
5.00KX 2.00μ
b
15.0KX 667n


9
10KV 5.00KX 2.00U
10
10KV 10.00KX 1.00U
11
10KV 5.00KX 2.00U
12
10KV 5.00KX 2.00U