COMPARATIVE SURFACE ULTRASTRUCTURE OF ADULTS AND EGGS OF *GNATHOSTOMA* OBTAINED IN JAPAN

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Abstract. As limited studies have been done on surface morphology of *Gnathostoma*, adult specimens and eggs of four kinds of species in Japan were compared by scanning electron microscopy. Worms had a subglobular head-bulb which was armed with 7-10 rows of cephalic hooks. Mutidigitate cuticular spines were spaced unevenly on transverse cuticular striations on the anterior half of the body. The lengths of the spines were variable with tridentate spines longer than bidentate ones, These tridantate spines became one of the species specific characteristics. The posterior half of the bodies of *G. doloresi* and *G. hispidum* were covered densely with long unidentate spines which were gradually shorter towards the posterior ends. Ventral sides of male terminals had different shape of papillae which so called small and caudal ones in species. Eggs recovered from the uteri of female worms were covered with cuticular pits of different sizes, shapes and depths in species

INTRODUCTION

Until the present time, there are ten distinct species of *Gnathostoma* isolated in the world. However in Japan, only four species are living: *G. spinigerum, G. doloresi, G. nipponicum* and *G. hispidum*. The first three are indigenous and the last one has been imported recently from China to Japan (Akahane *et al*, 1982). The brief life cycle of *Gnathostoma* is as follows: Cyclops eats the first stage larva swimming in fresh water that has emerged from the gnathostome egg. The early 3rd-stage larvae with head-bulb is formed in it. When fresh water fishes eat the cyclops, the larva invades the muscle, grows to the advanced 3rd-stage and remains encapsulated in the muscle. Adult worms are found in mammalian definitive hosts which eat the infected fishes. Man is an unnatural host for this helminth. When accidental infection occurs after eating raw fish containing the larvae, symptoms occur due to extensive larval migration, *eg* creeping eruption of the skin. However these four species of larvae cannot reach the adult-stage in man (Levinsen, 1889; Araki and Morita, 1981; Ando *et al*, 1988). Reported here is comparisons of the four species of adults and eggs of *Gnathostoma* by surface ultrastructure by means of scanning electron microscopy to assist differentiation of species.

MATERIALS AND METHODS

Adult specimens of *G. spinigerum* were obtained from the stomachs of dogs that were experimentally infected. The *G. doloresi* were from naturally infected wild bore, *Sus scrofa leucomystax*, *G. nipponicum* were from the esophagus tumors of naturally infected weasel, *Mustera sibirica coreana (itatsi)*, and *G. hispidum* were collected from experimentally infected pigs. Eggs were recovered from the uteri of each species of gravid female worm. Viable adult male and female worms were washed in several changes of tap water and soaked in physiological saline. The worms were fixed in 10% formalin for at least one week, washed in running tap water overnight to remove the fixative, and transferred to distilled water. Specimens were rinsed twice in Millonig's phosphate buffer and postfixed in 1% OsO_4 for 3 hours. During postfixation, worms were cut transversely into seven pieces to facilitate observations by SEM.

These pieces were dehydrated in an ascending series of ethanol concentrations, transferred into amyl acetate, and critical-point dried with a Hitachi HCP-2 critical point dryer. Specimens were sputter-coated with gold and examined with a JEOL JSM-U3 scanning microscope operated at 15kV.

RESULTS

Adult male and female specimens of every species of gnathostome have a hemispherical head-bulb at the anterior end, armed with 7-10 transverse rows of cephalic hooks (Fig 1). No distinct differences in shape of the hooks were found among these species. Their bodies are armed with varying-shaped cuticular spines which become one of the characteristics among species. *G. spinigerum* usually possesses the spines in the anterior half of the body. The posterior half has no spines except for tail extremity. The tridantate spines situated in the anterior one-fourth of the body were short and stumpy (Fig 2). Cuticular spines were present only in the anterior half of the body of *G. nipponicum*. The tridantate spines of this species located in the same part of the body were also short and in the shape of an unfolded fan (Fig 3). Cuticular spines covered whole bodies both in *G. doloresi* and *G. hispidum*. The typical three denticle spines were longer and slender in both species but the middle ones were much longer than either of the lateral two in *G. hispidum* (Figs 4, 5). Slightly elevated bilateral circular phasmids which were spineless were clearly seen at the posterior ends of these two species (Fig 6).

The ventral aspect of male tails also become important in the distinction of these four species. The distribution and length of spines and shapes of caudal papillae, which were of two types (large and small), were different from one another. Those of *G. spinigerum* (Fig 7) were similar to those of *G. nipponicum* (Fig 8), and the *G. doloresi* aspects (Fig 9) resembled those of *G. hispidum's* (Fig 10).

Eggs had a polar or bipolar plugs (*G. doloresi*) in species (Fig 1) and each has species specific surface

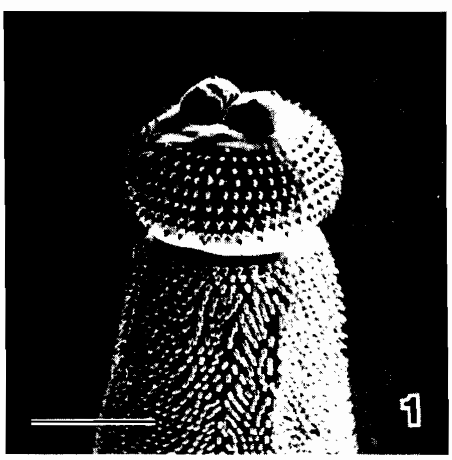
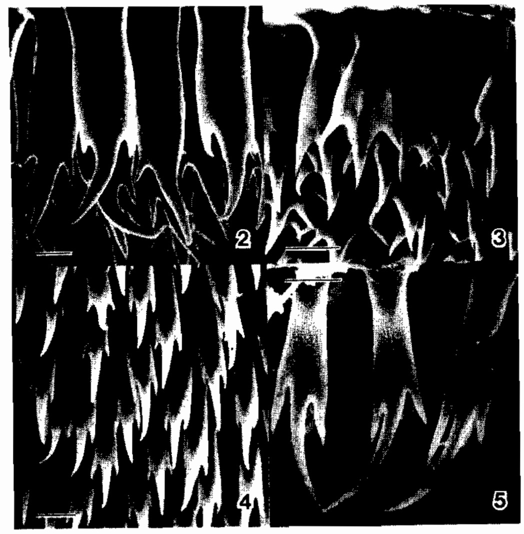


Fig 1–Lateral view of head-bulb of adult *Gnathostoma spinigerum*. The bulb has eight transverse rows of hooks. Scale bar = 200 µm.



Figs 2-5–Typical tridentate spines lying anterior one-fourth of the bodies. 2. *G. spinigerum*. 3. *G. nipponicum*. 4. *G. doloresi*. 5. *G. hispidum*. Bars = 10µm.

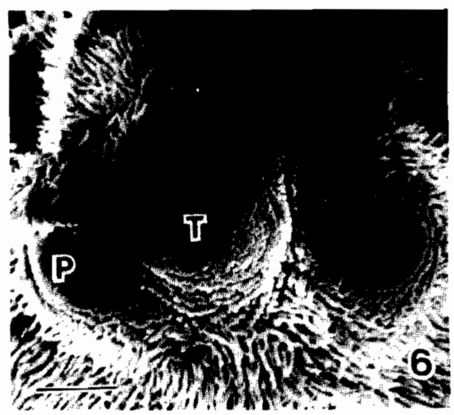
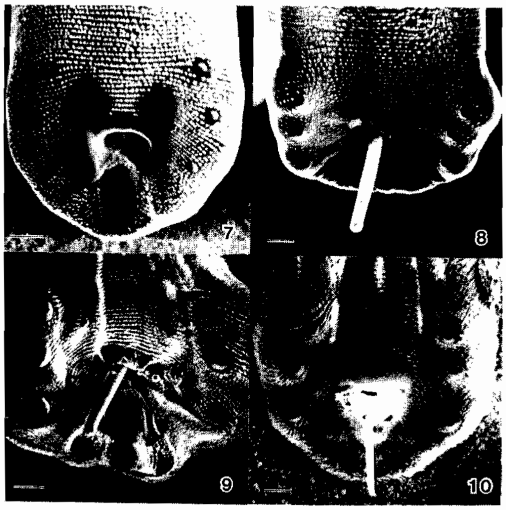
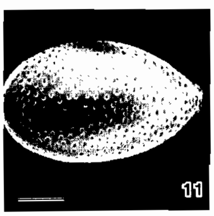


Fig 6–Extreme end of the *G. doloresi* female tail having two bare phasmids (P). The terminal projection (T) has no spines. Scale bar = 10 µm.

pits in shape on the eggshells. The pits of *G. spinigerum* (Fig 12) varied in size, shape and depth which were similar to those of *G. nipponicum* (Fig 13). The surface of eggshells had round pits of relatively equal size and depth in *G. doloresi* and *G. hispidum* (Figs 14, 15). However those of *G. hispidum* were much larger in size.



Figs 7-10–Ventral aspects of male tails. 7. *G. spinigerum*. 8. *G. nipponicum*. 9. *G. doloresi*. 10. *G. hispidum*. Scale bars = 40, 40, 20 and 50 µm respectively.



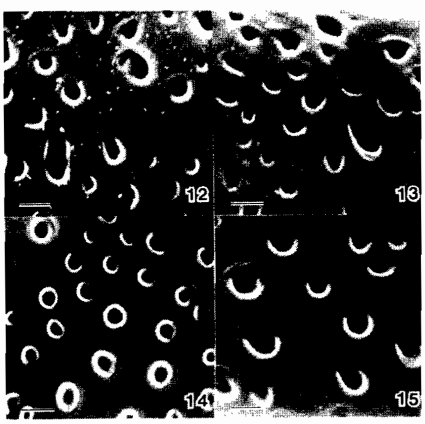


Fig 11–Whole apperence of *G. hispidum* egg. A plug is lying at one side. Scale bar = 20 µm.

Figs 12-15–Eggshell surfaces of each species having many cuticular pits. 12. *G. spinigerum*. 13. *G. nipponicum*. 14. *G. doloresi*. 15. *G. hispidum*. Scale bars = 1 µm.

DISCUSSION

The structure of adult stages of *Gnathostoma spinigerum* by SEM has been reported by Ratanarapee (1982); Ratanarapee *et al* (1988); Scholz and Ditrich(1990). For *G. doloresi*, Imai *et al* (1989), Koga and Ishii (1992) and Sakaguchi *et al* (1985), and for *G. nipponicum,* Koga and Ishii (1981) described the structures by SEM. Koga *et al* (1984) and Huang *et al* (1986) observed the surface structure of *G. hispidum*. They observed the individual species of these nematodes, however they did not compare the surface ultrastructure of these four species. Comparing between species is quite important for identifying nematodes. The body spines and ventral aspects of male tails were found having differences among species. The typically spaced body spine aspects on male tails resembled between *G. spinigerum* and *G. nipponicum*, and between *G. dololesi* and *G. hispidum*. Small papillae on the male tails were detected only in the latter two. My results agreed with earlier SEM studies.

Eggshells of *G. spinigerum* were observed by Ishii and Tokunaga (1970) and Zaman (1987) using SEM. Ishii and Tokunaga stated that the surface pits in the eggshell were large, shallow circles. Our materials had surface pits of irregular shapes and variable depths. These observations agree with those of Zaman (1987), Koga *et al* (1991) for *G. spinigerum*. The pits on eggs of *G. nipponicum* seemed irregular in shape and variable in depth and they resembled those of *G. spinigerum* (Koga and Ishii (1981). On the other hand, the shape of the pits in *G. hispidum* (Koga and Ishii, 1984) was similar to that in *G. dololesi* (Koga and Ishii, 1992) However that of *G. doloresi* seemed to be more round, smaller and of relative equal depth. Only the *G. doloresi* egg has bipolar plugs among these species.

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