

Research Note

Surface Ultrastructure of *Heterophyes heterophyes*
(Trematoda: Heterophyidae) Collected from a Man

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ABSTRACT: Surface ultrastructure of *Heterophyes heterophyes* recovered from a man by treatment with praziquantel is reported. Whole surface of the body was covered with saw-toothed or alternatively brush-shaped tegumental spines with an average density of 42 per square micrometer. Sensory papillae were not identified. The gonotyl was posterosinistal to the ventral sucker and was protruded in 28% flukes. The rodlets were arranged radially along the gonotyl, occupying about 85% of the gonotyl circumference. Rodlets were seen to be composed of 3 to 6 spines in a row appearing as "cockscomb."

KEY WORDS: *Heterophyes heterophyes*, surface ultrastructure, scanning electron microscopy, rodlets.

Trematodes of the family Heterophyidae are minute flukes distributed in various regions of Middle and East Asia. The second intermediate hosts of these parasites are fresh and/or brackish water fishes. Adult flukes are found in fish-eating animals including humans (Beaver et al., 1984). *Heterophyes heterophyes* is distributed primarily in Egypt (particularly in the lower Nile Valley), Greece, and Israel. Human cases of *H. heterophyes* infection were obtained by eating brackish water fishes caught in the endemic areas (Kagei et al., 1980; Adams et al., 1986; Chai et al., 1986).

Heterophyes heterophyes and *H. nocens* have very similar morphological features. Taxonomically, there have been debates about the validity of *H. nocens* in contrast to the type species, *H. heterophyes*. Chai et al. (1986) suggested that they are 2 distinct species from the number of rodlets, 50 to 63 in *H. nocens* and 70 to 90 in *H. heterophyes*. Observations on the surface ultrastructure of the Heterophyidae were made in *Metagonimus* spp. (Saito, 1972; Fujino et al.,

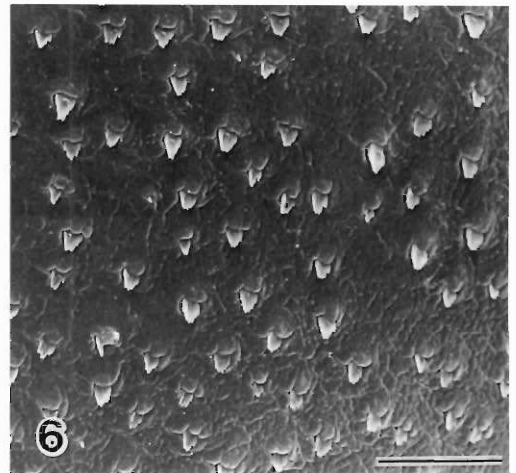
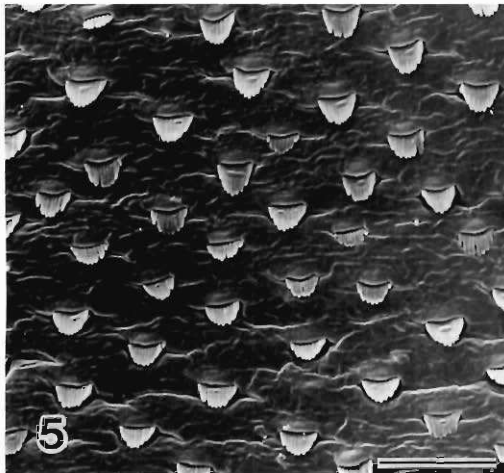
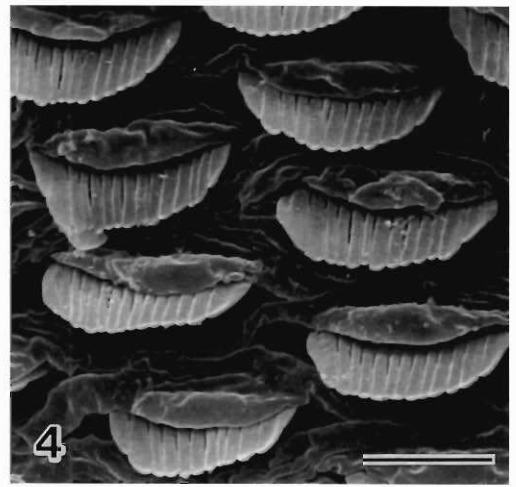
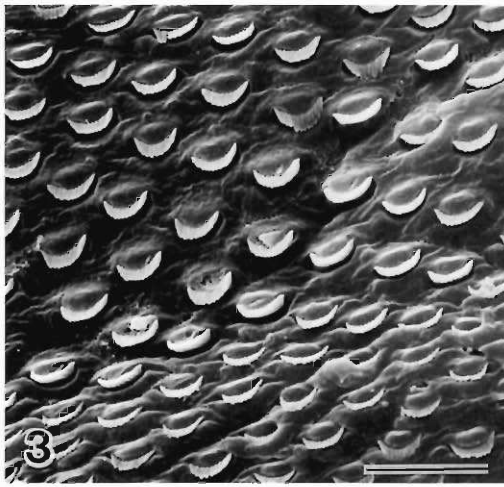
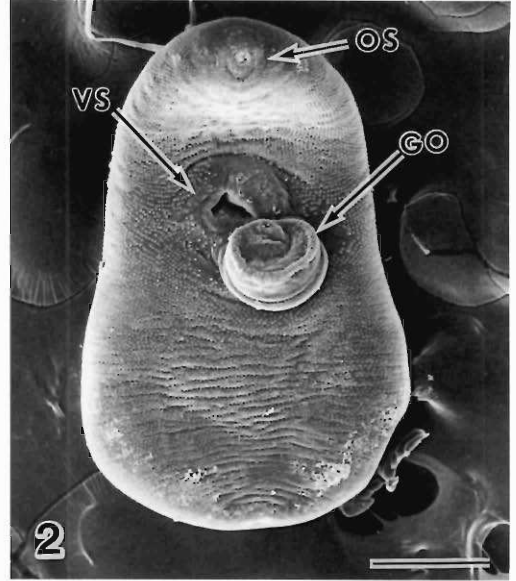
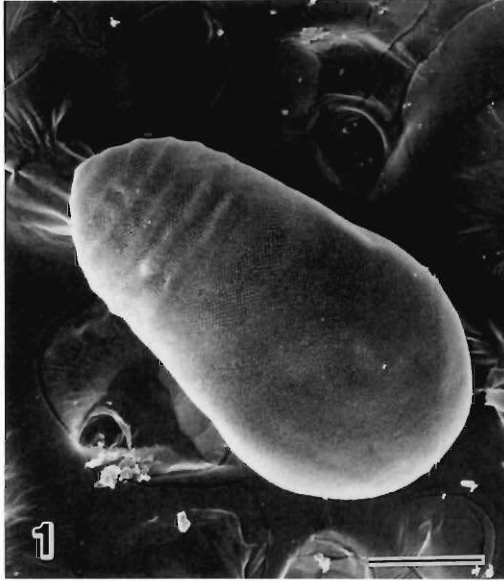
1989), *Haplorchis* (Fujino et al., 1989), and *Heterophyopsis continua* (Hong et al., 1991). However, the morphological details of the rodlets are not reported. The present study was undertaken to establish basic knowledge on the morphology of *H. heterophyes*, especially on the rodlets, by scanning electron microscopy.

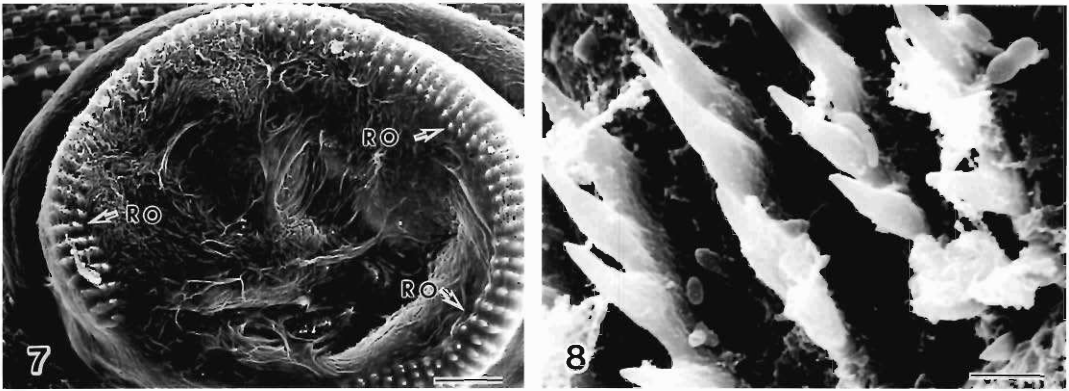
A total of 138 *H. heterophyes* were obtained from a 40-yr-old Japanese man after praziquantel administration. He returned to Japan in November 1994 after a 14-mo stay in Egypt as an engineer. Parasites were identified as *H. heterophyes* by light microscopy on the basis of their size, shape, and characteristic feature of both suckers. The details on the parasite collection will be reported elsewhere.

A total of 15 *H. heterophyes* were fixed in 10% formalin buffered with phosphate-buffered saline (pH 7.2), rinsed with PBS for 3 times at room temperature, and dehydrated with a graded series of ethyl alcohol. The specimens were transferred to absolute ethyl alcohol and to isoamyl acetate. The specimen was dried in a critical-point dryer (HCP-2, Hitachi, Tokyo, Japan), coated with gold (Ion Coater IB-3, Eiko, Tokyo, Japan), and observed under a scanning electron microscope (SEM) (JSM-T330A, Jeol, Tokyo, Japan). Several flukes were frozen in liquid nitrogen and fractured for observation of intrauterine eggs. Some of the remaining parasites in formalin have been deposited in the Meguro Parasite Museum, Tokyo, Japan (MPM Collection #19712).

Whole surface of the body was covered with tegumental spines (Figs. 1, 2). In the anterior part, tegumental spines were sawtoothed or alternatively brush shaped (mean length, 2 μ m), with a mean density of 42 per square micrometer (Fig. 3). The tegumental spines in the anterior part of the body were digitated into 14 to 17

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Figures 7, 8. Rodlets along the gonotyl tip of the genital sucker (7). Rodlets (RO). Higher magnification of rodlets (8). Bars = 10 μm in 7 and 1 μm in 8.

points (Fig. 4). The density and size of the tegumental spines decreased posteriorly (Figs. 5, 6). The number of points (or digits) of the tegumental spines in the posterior part was 7 to 10. The morphology and distribution of tegumental spines on the ventral surface were similar to those of the dorsal surface. Sensory papillae were not identified on either surface. The gonotyl was posterosinistal to the ventral sucker (Fig. 2). Of 108 flukes observed under a stereomicroscope, the gonotyl was protruded in 30 (28%) flukes, but not in 78 (72%). The rodlets were arranged radially along the gonotyl of the genital sucker (Fig. 7). Each rodlet was linear and consisted of 3 to 6 sharp spines (Fig. 8). The rodlets were 72–77 in number (mean 74) and occupied about 85% of the gonotyl circumference.

The uterus contained many oval-shaped eggs, with a mean length of 26.4 μm (24.5–28.0 μm ; $n = 16$) and mean width of 15.2 μm (15.0–15.6 μm ; $n = 16$). One end of the eggs was round and the other end had an operculum (5.2–6.4 μm). The basal edge of the operculum and the edge it fit into were both raised, forming narrow ridges. The entire egg surface was smooth.

The heterophyid trematodes comprise many species, and their morphologies have been studied extensively. Hong et al. (1991) observed sur-

face ultrastructures of *Heterophyopsis continua* and Chai et al. (1992) reported those of *Heterophyes nocens*, and our results agree with their findings in the following ways. First, tegumental spines are dense on anterior part of the body, whereas they are thin on the posterior part. Second, tegumental spines between the oral and ventral suckers have tips with 10 to 17 points in *H. continua*, but 12 to 17 points in *H. nocens* and 14 to 17 points in *H. heterophyes*. Sensory papillae were observed on the ventral surface of *Metagonimus* spp., *H. continua*, and *H. nocens* (Fujino et al. 1989; Hong et al. 1991; Chai et al. 1992), but we could not find any papillae in the *H. heterophyes* studied.

The number of rodlets on a gonotyl is important in the identification of species of the genus *Heterophyes*. *Heterophyes* with 50 to 63 rodlets are identified as *H. nocens* (*H. heterophyes nocens*), those with 52 to 57 as *H. katuradai*, and those with 70 to 90 as *H. heterophyes*. In the report by Chai et al. (1992), the gonotyl of *H. nocens* did not show the spines. They explain that “the tegument and spines were destroyed due to the effect of bithionol.” Kagei et al. (1980) reported light microscopic morphology of rodlets of *H. heterophyes* recovered from patients infected in Egypt. Miyazaki and Toh (1988) reported that rodlets are shaped like an

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Figures 1–6. Dorsal (1) and ventral (2) surfaces of *H. heterophyes*. Oral sucker (OS); ventral sucker (VS); gonotyl (GO). Tegumental spines on anterior dorsal surface (3). Higher magnification of dorsoanterior tegumental spines (4). Tegumental spine on middle (5) and posterior dorsal surface (6). Bars = 200 μm in 1 and 2; 5 μm in 3, 5, and 6; and 1 μm in 4.

antler, and an illustration was given, but the rodlets were not clear. In this study, rodlets could be photographed clearly. The rodlets were observed running around not 100% but about 85% of the gonotyl circumference radially, and this observation agrees with that reported by Chai et al. (1986). The present study revealed that the rodlets are not a single rod but are composed of 3 to 6 spines in a row appearing as "cockscomb."

Acknowledgments

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Research Note

Applicability of Crude Extracts of Adult *Spirometra erinacei* for Serodiagnosis of Sparganosis

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ABSTRACT: Antigenicity of crude extracts of adult *Spirometra erinacei* was evaluated in comparison to those of the plerocercoid (sparganum) for serodiagnosis of human sparganosis. Patients' sera from 39 sparganosis, 77 other helminthic diseases, and 50 uninfected controls were tested by enzyme-linked immunosorbent assay (ELISA). When both extracts were used as antigen, specific antibody levels in sparganosis sera were highly correlated ($r = 0.83$). The sensitivity and specificity of the adult worm extracts were 92.3 and 98.3%, while those of the sparganum were 94.3 and 96.6%, respectively. This result showed that crude

extracts of adult *S. erinacei* could be used as a diagnostic antigen of sparganosis.

KEY WORDS: *Spirometra erinacei*, sparganum, sparganosis, antigen, immunodiagnosis.

Human sparganosis is a parasitic disease caused by tissue-invading plerocercoids of *Spirometra* spp. (sparganum) such as *S. erinacei* Faust, Campbell, and Kellogg 1929 or *S. mansonioides* (Mueller 1935) Wardle, McLeod and