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Further Observation on the Larva of the Suborder Spirurina Suspected as the Causative Agent of Creeping Eruption

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Abstract

Prevalence of the type X larva of Hasegawa (1978) (Nematoda: Spirurina), the estimated causative agent of creeping eruption, was studied in some marine animals. The larva was detected for the first time from *Watasenia scintillans*, a squid suspected as one of the sources of infection. External morphology of the larva was observed using a scanning electron microscope. A discussion was made on the systematic position of the type X larva.

Key words: larva migrans, Spirurina, scanning electron microscopy, marine animals, epidemiology

Introduction

In Japan, the number of cases of creeping eruption due to larval nematodes of the suborder Spirurina other than *Gnathostoma* spp. has increased recently (Kagei, 1991; Tanaka *et al.*, 1992; Takahashi *et al.*, 1992; Fujihira *et al.*, 1992; Okazaki *et al.*, 1992; Akao *et al.*, Ando *et al.*, and Nakamoto *et al.*, unpublished data). In addition, three human cases of ileus caused by these spirurin larvae were also reported (Otsuru *et al.*, 1974; Kagei *et al.*, 1992a). The patients seem to have acquired the infections by eating marine fish or squid raw.

The morphological appearance of the sectioned worms in these human cases closely resembled the "type X larva of the superfamily Spiruroidea" of Hasegawa (1978), who proved that this type larva from marine fish had strong tissue migrating activity in experimental animals. In fact, the type X larva was detected in the anterior chamber of the eye of a man (Chung *et al.*, 1990). Morphological features in cross section of type X larva were reported previously to facilitate the identification of larva in human tissue (Ando *et al.*, 1992).

In this report, infection rate of marine animals with type X larva is reported. The external morphology of type X larva is also studied by scanning electron microscopy in order to obtain information for identification.

Materials and Methods

Marine fish, shrimp and squid were purchased from city markets in Tsu City, Mie Prefecture, Japan. They were digested with artificial gastric juice at 37°C. After digestion, residues were examined under a dissecting microscope. Detected type X larvae were then fixed in 10% formalin for one week and washed with 0.1M phosphate buffer (pH 7.2) to remove the formalin. The larvae were post-fixed in 1% osmium tetroxide in phosphate buffer (pH 7.2) for 2 hrs and washed with Tyrode's solution. Following dehydration in a graded series of ethanol, the larvae were dried in liquid carbon dioxide using a critical-point drying apparatus (Hitachi HCP-2). They were coated with gold in a ion-sputtering apparatus (JFC-1100) and observed under a JSM-T200 scanning electron microscope (SEM) operated at 10KV.

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Results

Fourty four type X larvae (about 1.0 mm in diameter of cyst) were detected from the stomach and intestinal wall of 77 cods, *Theragra chalcogramma*. However, the number of infected fish were unknown because several larvae were detected from a batch of fish. Four larvae were also detected from the viscera of 4 squid (one each), *Watasenia scintillans* (Table 1). They were 6.5–8.5 mm long and 80–105 μm wide, and had almost transparent muscular esophagus (about 20 μm wide), slightly light brown glandular esophagus (about 45 μm wide) and almost transparent intestine (about 20 μm wide).

Living larva moved vigorously (Fig. 1) but dead ones were bent ventrally forming a coil or fishhook shape (Fig. 2). Head ornamented with two triangular lateral pseudolabia pointed apically (Figs. 4–6). Each lateral pseudolabia had two submedian papillae, and an amphidial pore was located anterior to the submedian papillae (Figs. 4 and 6). The mouth was elongated and enlarged dorsoventrally. The dorsal and ventral rims of the mouth were slightly thickened (Figs. 4–6). A pair of cilium-like cervical papillae was located laterally at a distance of 143 μm from cephalic apex (Figs. 3 and 7). The

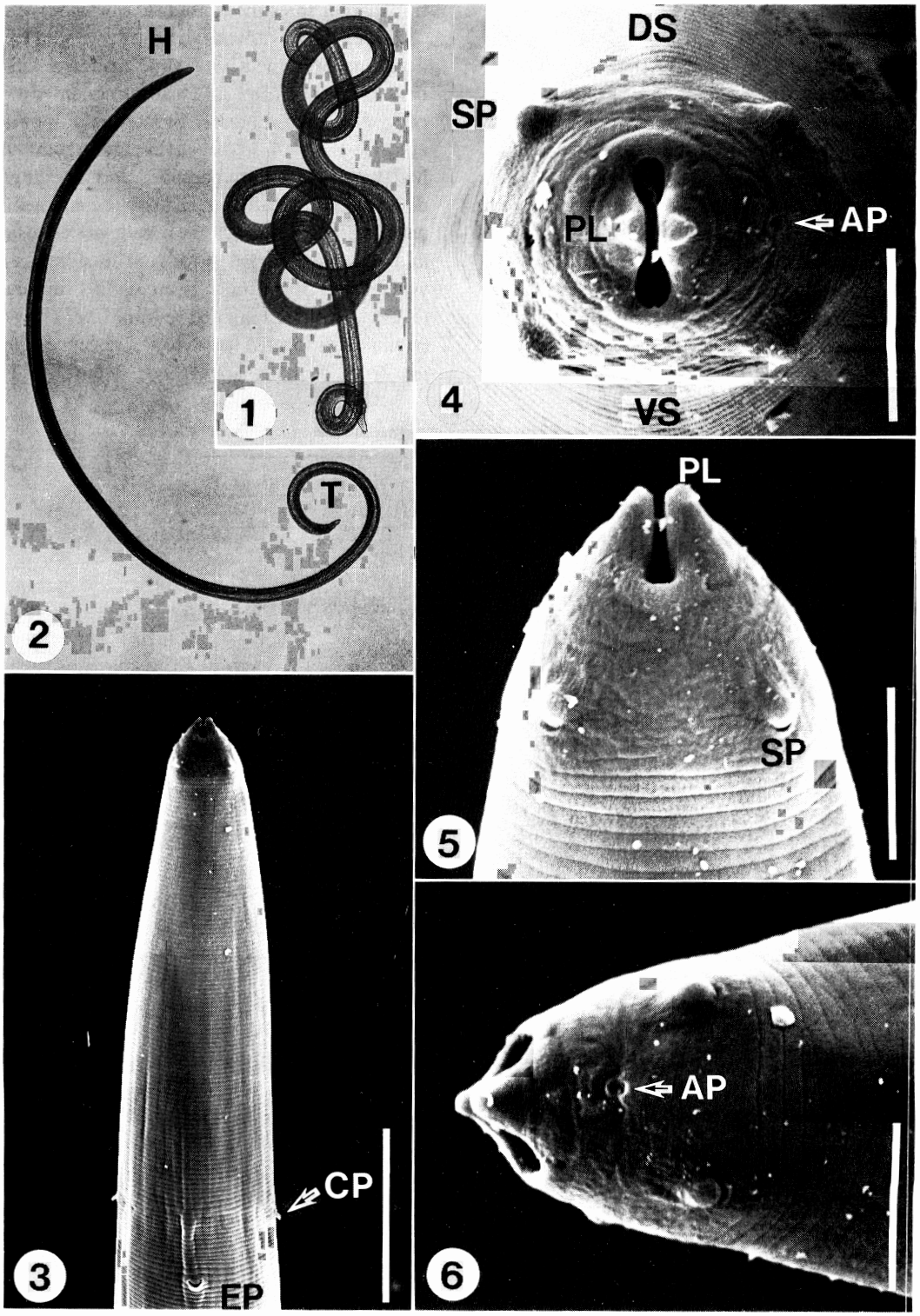
Table 1 Infection rate of marine animals with type X larva

species (Japanese name)	no. examined	no. of larva detected	
		muscle	viscera
Fish			
Theragra chalcogramma (Suketou-dara)	77	not examined	44
Salangichthys microdon (Shira-uo)	162	0	0
Leucopsarian petersi (Shiro-uo)	242	0	0
Shrimp			
Pandalus borealis (Hokkoku aka-ebi)	126	0	0
Pasiphaea sivado (Shira-ebi)	230	0	0
Squid			
Watasenia scintillans (Hotaru-ika)	162	0	4

Figs. 1–12 Light microscopy (1–2) and scanning electron microscopy (3–12) of type X larva of the suborder Spirurina.
Fig. 1 Living larva.
Fig. 2 Dead larva.
Fig. 3 Ventral view of anterior part of the body. Bar = 50 μm.
Fig. 4 Front view of head. Bar = 10 μm.
Fig. 5 Ventral view of head. Bar = 10 μm.
Fig. 6 Lateral view of head. Bar = 10 μm.
Fig. 7 Cervical papilla on lateral surface. Bar = 2 μm.
Fig. 8 Excretory pore on ventral surface. Bar = 2.5 μm.
Fig. 9 Transverse striation of middle part of the body. Bar = 10 μm.
Fig. 10 Lateral view of tail. Bar = 20 μm.
Fig. 11 Ventral view of tail. Bar = 20 μm.
Fig. 12 Enlargement of tail. Bar = 10 μm.

Abbreviations

A: anus, AP: amphidial pore, CP: cervical papilla, DS: dorsal surface, EP: excretory pore, H: head, PL: pseudolabia, PP: phasmidial pore, SP: submedian papilla, T: tail, TU: tubercle, VS: ventral surface


H
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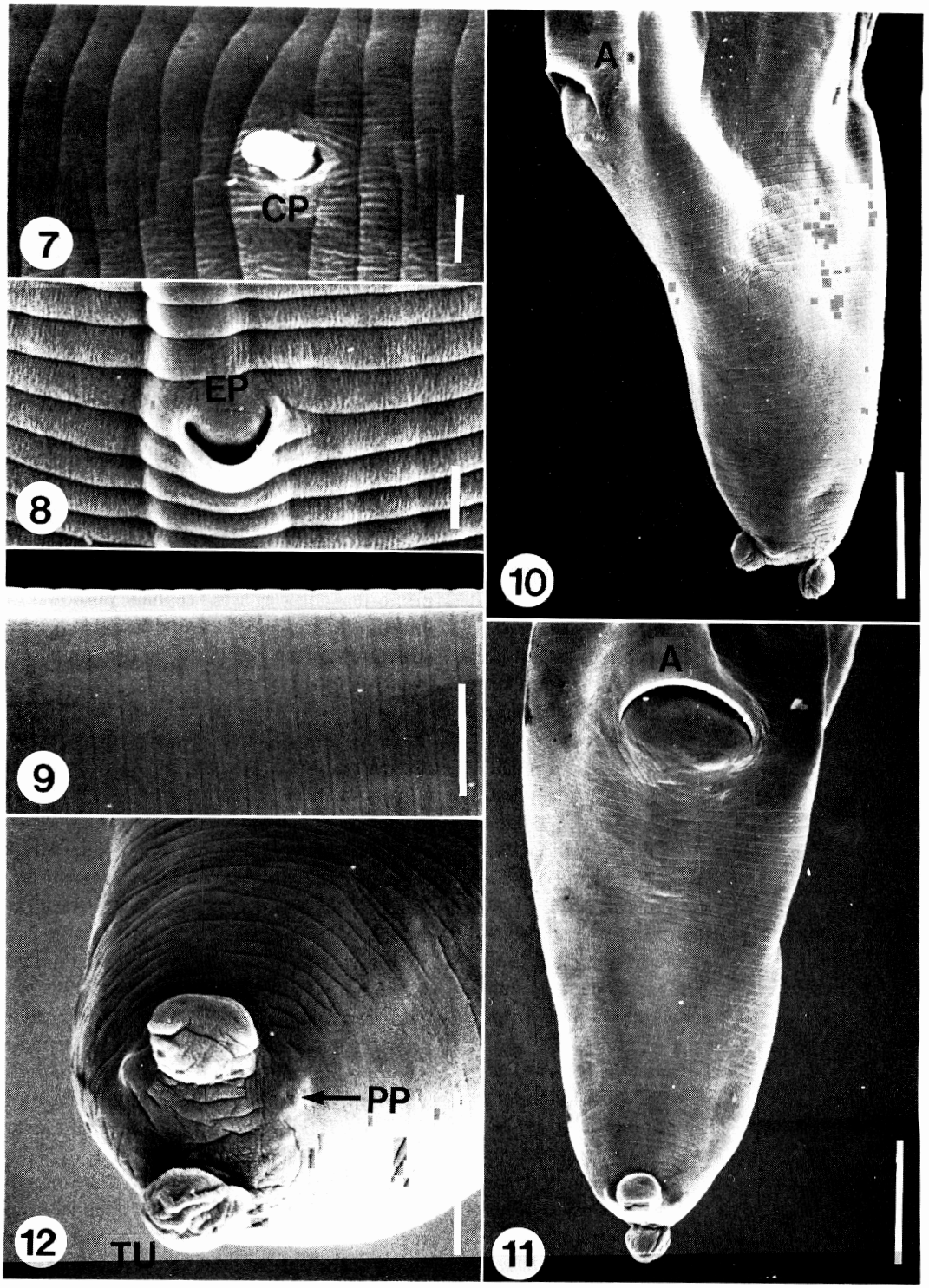
CP
7
EP
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A
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9
A
PP
12
TU
11

papilla was surrounded by a rim at the base (Fig. 7). The excretory pore was located on the ventral midline at a distance of 164 μm from cephalic apex (Figs. 3 and 8). Anus opened at a distance of 90 μm from tail tip (Figs. 10 and 11). Two large tubercles were present, one (about 7 μm in diameter) at the tail tip and the other slightly smaller than the former on the ventral surface (Figs. 10–12). A pair of phasmidial pores was clearly visible near the smaller tubercle on the ventral surface (Fig. 12). Transverse striation was visible from the end of the pseudolabia to the tail tip. However, striations were often broken laterally (Figs. 7 and 12). The intervals of the transverse striation was 1.4 μm at the anterior part of the body, 2.5 μm at the middle (Fig. 9) and 1.8 μm near the anus. Lateral ala was absent.

Discussion

The type X larva has been detected from the stomach and intestinal wall of cod, *T. chalcogramma* (Hasegawa, 1978; Ando *et al.*, 1992) and sandfish, *Arctoscopus japonicus,* (Otsuru *et al.*, 1974). However, the viscera of these fish are very rarely eaten raw. It is of special interest that the type X larva was detected for the first time from *Watasenia scintillans,* a small squid (about 9 cm long), which is often eaten whole as a delicacy. This squid is sold from late winter to early summer and 8 out of 14 cases, in which the date of onset of symptoms was clearly recorded, occurred within this period. The source of human infection to date remains unclear. However, it is strongly suggested that this squid is a source of infection because one patient had actually eaten the squid raw. (Okazaki *et al.*, 1992)

The natural final host of the type X larva has been considered to be marine birds or mammals (Hasegawa, 1978; Hasegawa and Otsuru, 1982). However, with the absence of knowledge on the adult stage, its superfamily has not been specified. The suborder Spirurina consists of 10 superfamilies, namely Gnathostomatoidea, Physalopteroidea, Rictularioidea, Thelazioidea, Spiruroidea, Habronematoidea, Acuarioidea, Filarioidea, Aploctoidea and Diplotriaenoidea (Chabaud, 1974). Representatives of the last three superfamilies apparently differ from the type X larva in that they lack pseudolabium (Chabaud, 1974; Anderson and Bain, 1976). The superfamily Gnathostomatoidea has trilobed pseudolabia, Physalopteroidea has unlobed pseudolabia with a variable number of teeth, Rictularioidea has two rows of subventral cuticular spines along the length of the body and Thelazioidea has a variable buccal cavity (Chabaud, 1974). Morphological characteristics of these four superfamilies differ from that of type X larva. Most of the members of Spiruroidea have more or less lobed pseudolabium in the infective third-stage larva (Chabaud, 1954; Quentin, 1970), distinguishing them from the type X larva.

Most members of Acuarioidea and some representatives of Habronematoidea resemble the type X larva in having two lateral pseudolabia and a slender buccal cavity (Chabaud, 1975). However, most of acuarioid nematodes have primordial cordons in the cephalic portion of the infective larval stage (Chabaud, 1954), while such structures are not recognized in the type X larva even by SEM observation.

In Habronematoidea, members of the subfamily Crassicaudiinae have been detected from marine mammals, namely whales and dolphins (Chabaud, 1975). *Crassicauda* spp. are parasitic in various organs such as the urogenital system, mammary glands, body muscles, cranial sinuses, stomach and blubber tissues (Lambersten, 1985; Raga, 1987; Araki *et al.*, 1989; Raga and Balbuena, 1990), indicating that they cause tissue migration in their development in the final host (Lambersten, 1985). Although the life history of *Crassicauda* has not been elucidated, it is highly probable that cetaceans acquire the infection by ingesting intermediate or paratenic hosts in the marine environment. From Japanese waters, *C. grampicola* has been recorded recently (Araki and Machida, 1992; Kagei and Morimitsu, 1992b). Unfortunately, the morphology of the infective larva of crassicaudiins is unknown, and it is thus impossible to assign the type X larva to this subfamily at the present time. However, more attention should be payed to this poorly-known spirurin nematode group because some of the

cetacean helminths are actually zoonotic.

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