

Research Note

**Prevalence and Habitat of the Type X Larvae of the  
Suborder Spirurina in Squids**

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Recently, several cases of creeping eruption and ileus have been attributed to larvae of the suborder Spirurina other than *Gnathostoma* spp. (Kagei, 1991; Fujihira *et al.*, 1992; Murata *et al.*, 1992; Takahashi *et al.*, 1992; Tanaka *et al.*, 1992; Otsuru *et al.*, 1974; Kagei *et al.*, 1992). Similarities in the diagnostic morphology of the worms in the pathological sections have implicated the “type X larva of the superfamily Spiruroidea”, as described by Hasegawa (1978) (Fujihira *et al.*, 1992; Takahashi *et al.*, 1992; Ando *et al.*, 1992a). The superfamily Spiruroidea used by Hasegawa (1978) corresponds to the suborder Spirurina in recent nematode systematics (cf. Ando *et al.*, 1992a, b). Detection of a type X larva in the anterior chamber of the human eye (Chang *et al.*, 1990) suggested its high migration ability and the possibility of cutaneous and visceral larva migrans by this larva. Shinozaki *et al.* (1993) gave definite evidence that the type X larva was actually the causative agent of creeping eruption by detection of an intact worm tail in the affected part of a patient. They observed 2 hemispheric tubercles at the posterior

end of the tail, which are characteristic of this larva (Hasegawa 1978). Although they were able to identify the causative parasite, the source of infection remained uncertain. Thus far, type X larva has been obtained from two fish species, *Arctoscopus japonicus* (Otsuru *et al.*, 1974: as “Spiruridea G”) and *Theragra chalcogramma* (Hasegawa, 1978). Recently, type X larva has been found from the viscera of *Watasenia scintillans*, known as the “firefly squid” in Japan (Ando *et al.*, 1992b). In the case reported by Shinozaki *et al.*, the patient had ingested raw *W. scintillans*, at 2 and 9 days prior to the eruption. Okazaki *et al.* (1992) also reported a case of creeping eruption in which the patient had ingested raw *W. scintillans*. Since whole body of *W. scintillans* is often eaten raw in Japan, it is likely that this squid was the source of infection in those cases.

In the present report, we studied the natural infection of some marine animals with type X larva, and investigated the larval habitat in *W. scintillans*. We also conducted morphological observations of the larvae using light and scanning electron microscopes.

*W. scintillans*, *Todarodes pacificus* (sagittated calamary), *Hemirhamphus sajori* (halfbeak), and *Pandalus borealis* (shrimp) were obtained from a market in Kanazawa, Ishikawa Prefecture, Japan, in May 1992. Raw *W. scintillans* usually arrive in the market in early spring to early summer (i.e., March to June). The squids used

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in the present study were captured in the Japan Sea, landed at Shinminato, Toyama Prefecture. The other species examined were also caught in the Japan Sea and in the market in the same season as *W. scintillans*. In order to determine the habitat of the larvae, the squids were dissected into several body parts. Five examinations were conducted, using various combinations of the viscera. Each body part was digested separately in a solution containing pepsin and hydrochloric acid (pH 1.5). For the other species, only the viscera were examined. All larvae were collected using sieves with mesh sized of 210, 149, 105 and 74  $\mu\text{m}$ .

A total of 37 type X larvae were obtained from a sample of 1,109 *W. scintillans*, and 1 larva was obtained from a sample of 30 *T. pacificus* (Table 1). No type X larva was found in the sample of *H. sajori* or *P. borealis*. The habitat of the type X larva was determined to be the stomach and intestine of the *W. scintillans* in the examination V (Table 2). This determination was consistent with the results of the other four examinations conducted.

Although the infection rate is relatively low in *W. scintillans* (at most 3.3%), there is always

a risk of human infection with type X associated with the consumption of raw squids. To minimize this risk, raw *W. scintillans* should be prepared by removing the stomach and intestine before consumption.

The larvae from *W. scintillans* were killed with hot water and fixed with 10% buffered formaldehyde solution. Measurement of 12 intact larvae was conducted using a video-micrometer (Olym-

Table 1 Prevalence of type X larvae in squid, fish and shrimp

Host species	No. hosts examined	No. Type X larvae collected
<i>Watasenia scintillans</i> (Hotaru-ika)	1,109	37
<i>Todarodes pacificus</i> (Surume-ika)	30	1
<i>Hemirhamphus sajori</i> (Sayori)	10	0
<i>Pandalus borealis</i> (Hokkoku aka-ebi)	16	0

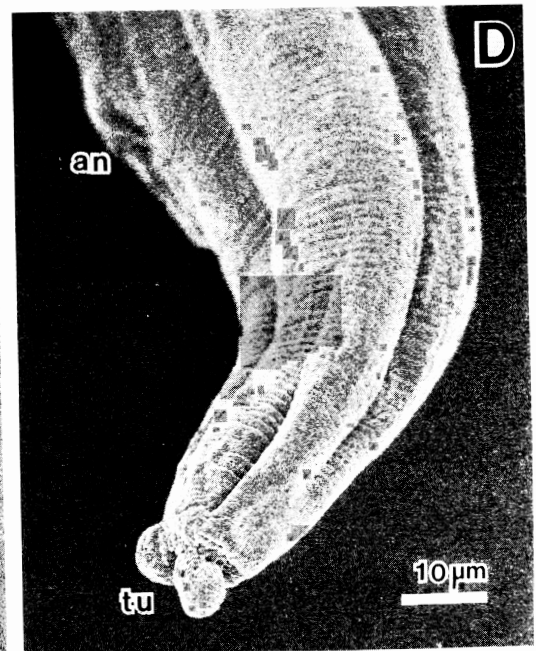
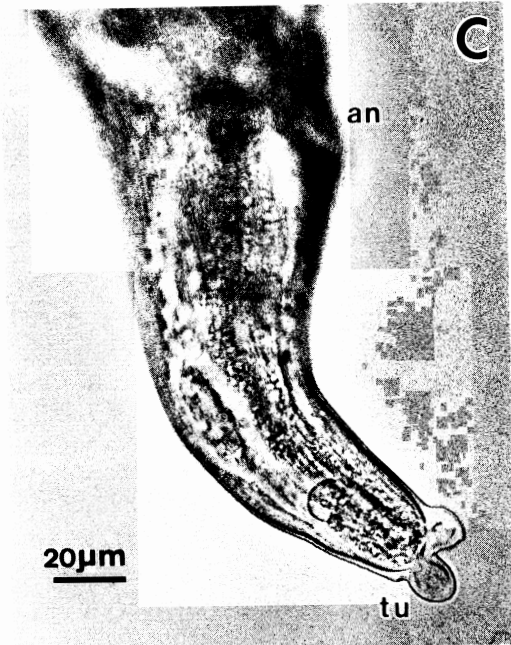
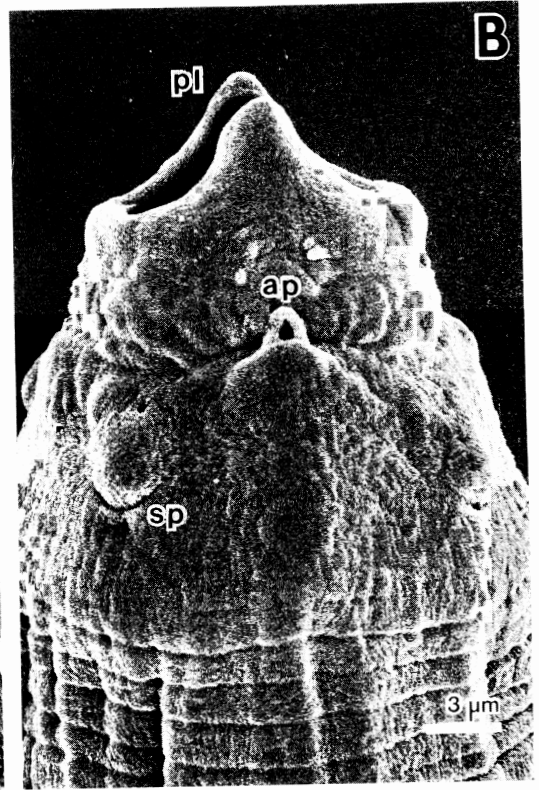
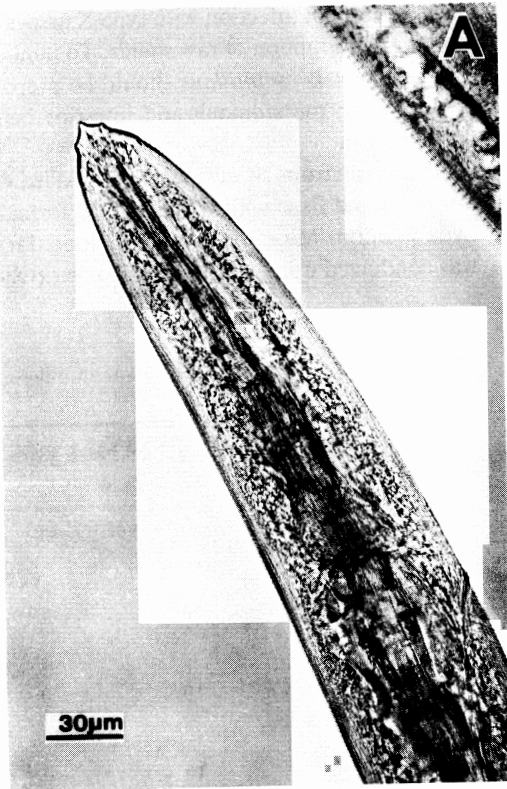
Table 2 Habitat of type X larvae in *Watasenia scintillans*

Examination (date)	No. examined	Mantle + head + arms	Viscera*						
			Ovary	Liver	Esop.	Inte.	Stom.	Others	
I (May 1, '92)	315	NE†	[		7			]	
II (May 7, '92)	315	0	0	[	7	]	[	3	]
III (May 14, '92)	315	NE	0	0	[	14		]	
IV (May 20, '92)	17	NE	0	0	[	1	]	0	
V (May 22, '92)	147	NE	0	0	0	2	3	0	

\*: Esop.: Esophagus, Inte.: Intestine, Stom.: Stomach.

† Not examined.

‡ Combined.



pus VM-31, Tokyo). In preparation for electron microscopy, 5 larvae were post-fixed with 0.1% osmic acid, and were observed with a Hitachi S-900 at 6 kv of accelerating voltage.

The larvae from *W. scintillans* had 2 medially projected pseudolabia (Figs. 1A, B), and 2 hemispheric tubercles at the posterior end of the tail (Figs. 1C, D). The 2 tubercles were situated closely to each other. The amphidial pores were located between the anterior extremity and submedian cephalic papillae (Fig. 1B). The glandular esophagus occupied 61.2% of total body length, and the intestine was approximately half the length of the glandular esophagus.

The features of the head and the tail are identical to those described by Hasegawa (1978), and are consistent with the observation made by

Ando *et al.* (1992b) on the specimens from *T. chalcogramma*. However, the larvae from *W. scintillans* were longer than those reported for *T. chalcogramma* (Hasegawa, 1978; Ando *et al.*, 1992b) (Table 3), though they were of similar width. It is still uncertain whether the type X represents a sole species or consists of several species. Further observation on the morphology of the type X larvae from various hosts is necessary.

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Table 3 Measurements of type X larvae obtained from *Watasenia scintillans* in comparison with those from *Theregra chalcogramma*

Measurements ( $\mu\text{m}$ )	Host species			
	<i>W. scintillans</i>			<i>T. chalcogramma</i> *
	Range†	Mean $\pm$ SD†	%‡	Range
Body length	8,660–12,006	10,549 $\pm$ 1,045		5,430–9,800 (6,500–8,500)§
Maximum width	95–108	99 $\pm$ 4.7		74–110 (80–105)
Muscular esophagus length	599–892	689 $\pm$ 97.1	6.5	481–703
Glandular esophagus length	5,150–7,516	6,453 $\pm$ 710.1	61.2	2,870–5,190
Intestine length	2,548–4,009	3,257 $\pm$ 437.6	30.9	
Tail length	130–170	150 $\pm$ 10.9	1.4	84–114
Nerve ring from anterior apex	189–210	201 $\pm$ 7.1	1.9	154–176

\* Hasegawa (1978).

† N = 12

‡ % of total length.

§ Ando *et al.* (1982b).

Fig. 1 Light microscopy (A and C) and scanning electron microscopy (B and D) of type X larvae of the suborder Spirurina recovered from *Watasenia scintillans*. A: Lateral view of the anterior part of the body. B: Lateral view of the head. C, D: Lateral view of the tail. an: anus, ap: amphidial pore, sp: submedian cephalic papilla, pl: pseudolabia, tu: tubercles at the end of tail.

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