

## Discovery of Larval *Gnathostoma nipponicum* in Frogs and Snakes from Jeju-do (Province), Republic of Korea

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**Abstract:** A survey was performed to find out the intermediate hosts of *Gnathostoma nipponicum* in Jeju-do (Province), the Republic of Korea. In August 2009 and 2010, a total of 82 tadpoles, 23 black-spotted pond frogs (*Rana nigromaculata*), 7 tiger keelback snakes (*Rhabdophis tigrinus tigrinus*), 6 red-tongue viper snakes (*Agkistrodon ussuriensis*), and 2 cat snakes (*Elaphe dione*) were collected in Jeju-do and examined by the pepsin-HCl digestion method. Total 5 gnathostome larvae were detected in 3 (50%) of 6 *A. ussuriensis*, 70 larvae in 3 of 7 (42.9%) *R. tigrinus tigrinus*, and 2 larvae in 2 of 82 (8.7%) frogs. No gnathostome larvae were detected in tadpoles and cat snakes. The larvae detected were a single species, and 2.17 × 0.22 mm in average size. They had characteristic head bulbs, muscular esophagus, and 4 cervical sacs. Three rows of hooklets were arranged in the head bulbs, and the number of hooklets in each row was 29, 33, and 36 posteriorly. All these characters were consistent with the advanced third-stage larvae of *G. nipponicum*. It has been first confirmed in Jeju-do that *R. nigromaculata*, *A. ussuriensis*, and *R. tigrinus tigrinus* play a role for intermediate and/or paratenic hosts for *G. nipponicum*.

**Key words:** *Gnathostoma nipponicum*, larval gnathostome, snake, frog, intermediate host, Jeju-do (Province)

*Gnathostoma* species nematodes are clinically important and can cause food-borne zoonotic parasitism in humans. More than 12 species, including *Gnathostoma spinigerum*, have been reported as valid species [1]. In the Republic of Korea, Kim [2] detected 2 *G. spinigerum* larvae in a snakehead, *Channa argus argus*, from Kimhae, Gyeongsangnam-do (Province) [2]. The third stage larvae of *G. hispidum* were found in loaches and snakes from China [3,4] and in pit-viper snakes, *Agkistrodon brevicaudus*, from Korea [5]. Larval *G. nipponicum* were also detected in imported Chinese loaches and also in tiger keelback snakes (*Rhabdophis tigrinus tigrinus*) from Hongcheon-gun, Gangwon-do, Korea [6,7]. On the other hand, *G. nipponicum* adults were found in the stomach of the Jeju weasel, *Mustela sibirica queipartis*, road-killed in Jeju-do [8]. In the present study, we performed a survey on intermediate hosts of *G. nipponicum*

in Jeju-do, the Republic of Korea.

We collected 82 tadpoles, 23 black-spotted pond frogs (*Rana nigromaculata*), 6 red-tongue viper snakes (*Agkistrodon ussuriensis*), 7 tiger keelback snakes, and 2 cat snakes (*Elaphe dione*) from the vicinities of Jeju National University in August 2009 and 2010. They all were transferred to the laboratory of Department of Veterinary Parasitology, Jeju National University College of Veterinary Medicine, Jeju, Korea, and examined by the pepsin-HCl digestion method. Some collected gnathostome larvae were fixed with 10% formalin, cleared in alcohol-glycerin solution, mounted in glycerin-jelly, and observed under a light microscope equipped with a micrometer. To observe the surface ultrastructure, some worms were washed several times with 0.2 M cacodylate buffer (pH 7.2) and fixed with 2.5% glutaraldehyde at 4°C. After washing 3 times with the buffer, they were dehydrated through a graded alcohol series (50%, 70%, 80%, 90%, 95%, and absolute alcohol), dried with hexamethyldisilazane, coated (JFC-1100E ion sputtering device) with gold, and observed with a scanning electron microscope (SEM, Philips XL-30S, London, UK) at a 15-kV accelerating voltage.

Total 5 gnathostome larvae were detected in 3 (50%) of 6 *A.*

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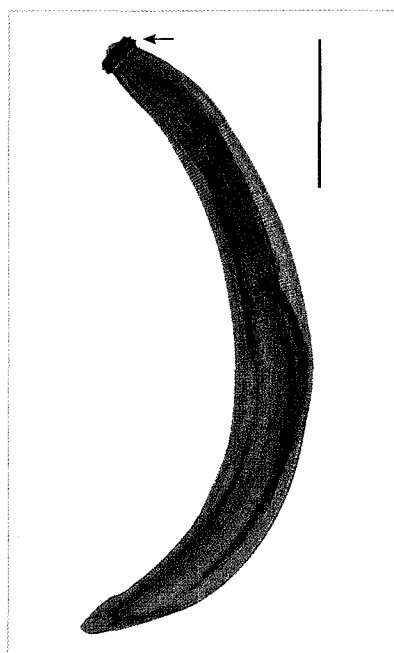
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*ussuriensis*, 70 larvae in 3 of 7 (42.9%) *R. tigrinus tigrinus*, and 2 larvae in 2 of 82 (8.7%) frogs (Table 1). However, no gnathostome larvae were detected in tadpoles and cat snakes.

The larvae from tiger keelback snakes were  $2.17 \times 0.22$  mm in average size, and they had a characteristic head bulb ( $0.05 \times 0.12$  mm in average size), muscular esophagus (0.81 mm), and 4 cervical sacs (0.40 mm). Three rows of hooklets were arranged in the head bulb, and the number of hooklets in each row was 29, 33, and 36 posteriorly. Transverse striations were regularly arranged on the tegumental surface, and their numbers were 229 in average (Table 2) (Fig. 1). In SEM observations, the head bulb bore 3 transverse rows of hooklets which were sharp-pointed and somewhat curved posteriorly, and numerous cuticular spines were regularly distributed on the transverse striations. A cervical papilla was located between the 12th and 13th transverse striations. Cuticular spines arranged on the transverse striations were sharp-pointed, and their densities were more sparse on the posterior part of the body surface. A body papilla was located on the posterior 1/3 level of the body. Near the posterior end of the larvae, smaller cuticular spines and an anus were presented (Fig. 2).



**Fig. 1.** An advanced third-stage larva (AdL<sub>3</sub>) of *G. nipponicum* detected in a tiger keelback snake, *Rhabdophis tigrinus tigrinus*, from Jeju-do. It has a characteristic head bulb (arrow mark), muscular esophagus (E), intestine (I), and 4 cervical sacs (CS) (scale bar = 0.5 mm).

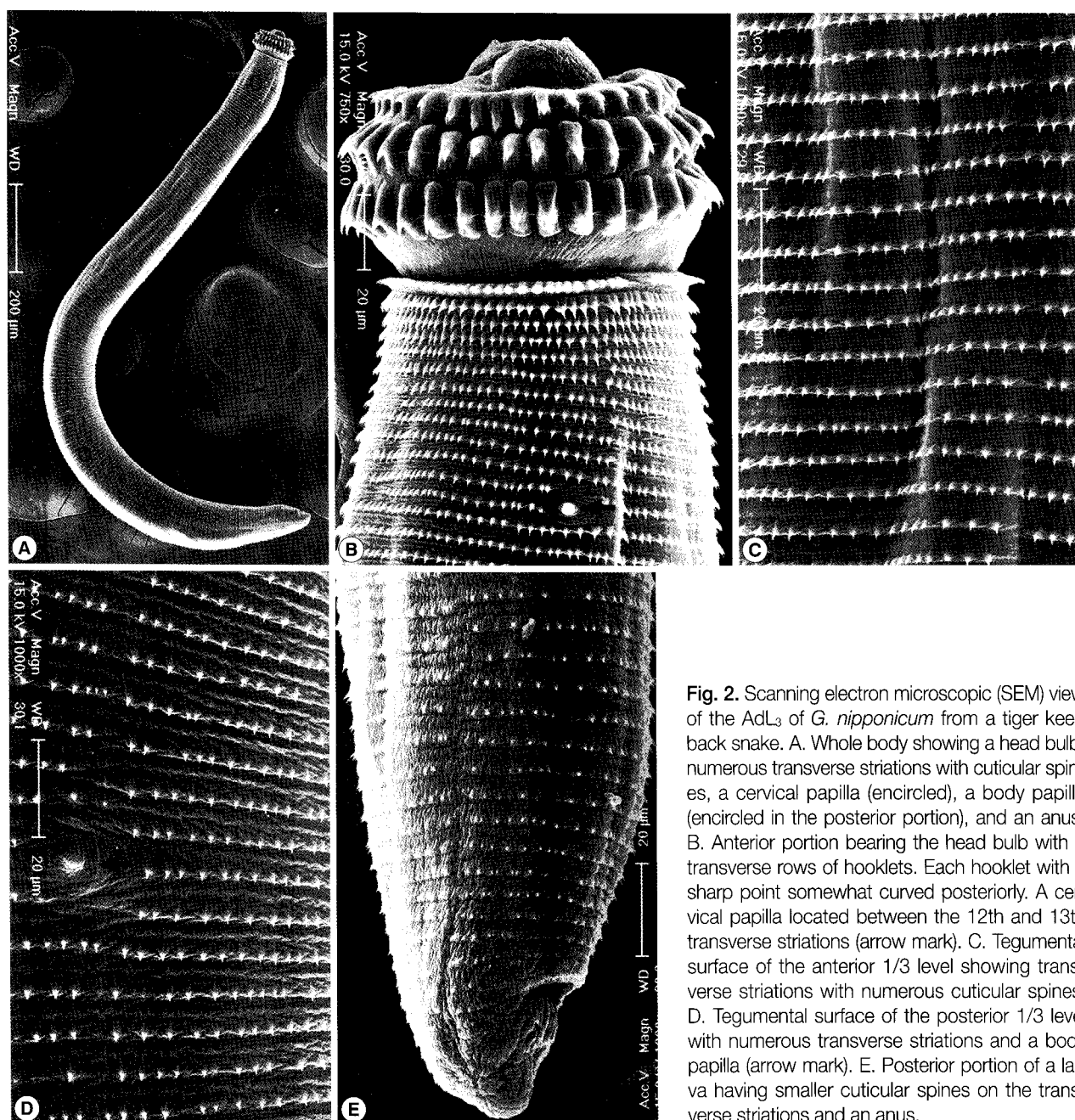
**Table 1.** The results of larval *G. nipponicum* recovery in amphibian and reptile hosts from Jeju-do (Province)

Name of host examined	No. of hosts examined	No. (%) hosts infected	No. of larvae collected		
			Total	Range	Average
<i>Agkistrodon ussuriensis</i> (Red-tongue viper snake)	6	3 (50.0)	5	1-3	1.7
<i>Rhabdophis tigrinus tigrinus</i> (Tiger keelback snake)	7	3 (42.9)	70	10-38	23.3
<i>Elaphe diene</i> (Cat snake)	2	0 (0.0)	-	-	-
<i>Rana nigromaculata</i> (Black-spotted pond frog)	23	2 (8.7)	2	-	1.0
Tadpole (species undetermined)	82	0 (0.0)	-	-	-

**Table 2.** Measurements<sup>a</sup> of the advanced third-stage larvae of *G. nipponicum* from a tiger keelback snake, *Rhabdophis tigrinus tigrinus*, and comparison with those of previous studies

Organs measured	Present study (2011) <sup>b</sup>	Han et al. (2003) <sup>c</sup>	Oyamada et al. (1996) <sup>d</sup>
Body (length)	2.0-2.4 (2.174)	1.262-1.658 (1.460)	1.068-1.872
Body (width)	0.17-0.268 (0.222)	0.180-0.185 (0.183)	0.11-0.151
Head bulb (length)	0.045-0.055 (0.049)	0.050-0.080 (0.065)	0.038-0.053
Head bulb (width)	0.103-0.13 (0.119)	0.110-0.115 (0.108)	0.085-0.107
Esophagus (length)	0.7-0.914 (0.811)	-	0.356-0.617
Cervical sac (length)	0.36-0.45 (0.403)	-	0.186-0.309
Tail (length)	0.033-0.045 (0.04)	-	0.018-0.068
No. of hooklets on head-bulb			
1st row	28-32 (29.4)	34-38 (36)	29-36
2nd row	30-36 (32.8)	37-38 (38)	31-38
3rd row	34-38 (35.6)	41-46 (43)	33-44
No. of transverse striation	200-270 (229)	213-232 (222)	214-253

<sup>a</sup>Unit in mm (average); <sup>b</sup>n=20; <sup>c</sup>10 larvae from grass snakes, *Rhabdophis tigrinus*; <sup>d</sup>measurements from 10 larvae from trouts, *Oncorhynchus masou*.



**Fig. 2.** Scanning electron microscopic (SEM) view of the AdL<sub>3</sub> of *G. nipponicum* from a tiger keel-back snake. A. Whole body showing a head bulb, numerous transverse striations with cuticular spines, a cervical papilla (encircled), a body papilla (encircled in the posterior portion), and an anus. B. Anterior portion bearing the head bulb with 3 transverse rows of hooklets. Each hooklet with a sharp point somewhat curved posteriorly. A cervical papilla located between the 12th and 13th transverse striations (arrow mark). C. Tegumental surface of the anterior 1/3 level showing transverse striations with numerous cuticular spines. D. Tegumental surface of the posterior 1/3 level with numerous transverse striations and a body papilla (arrow mark). E. Posterior portion of a larva having smaller cuticular spines on the transverse striations and an anus.

Gnathostome larvae detected in this study were identified as the advanced third-stage larvae (AdL<sub>3</sub>) of *G. nipponicum* on the basis of their morphologic characters. The general body shape of *G. nipponicum* larvae are similar to those of other species, including *G. spinigerum*. However, *G. nipponicum* can be discriminated from other species by the morphology of the head bulb. The larvae of *G. nipponicum* have a head bulb with 3 transverse rows of hooklets, whereas those of other species have 4 rows [1]. In SEM findings, most of them were corresponded with

those of Han et al. [7]. However, a cervical papilla was located between the 12th and 13th transverse striations in the present study, whereas a pair of cervical papillae was located bilaterally between the 8th and 12th transverse striations in Han et al. [7]. We could not observe a phasmid near the posterior end of the larvae.

As the second intermediate or paratenic hosts of *G. nipponicum*, snakes (*R. tigrinus* and *Elaphe quadrivirgata*), loaches (*M. anguillicaudatus*), catfish (*Silurus asotus*), sea rundaces (*Tribolo-*

*don hakonensis*), and trouts (*Oncorhynchus masou masou*) have been reported in Japan [9-13] and Korea [7]. Koga and Ishii (1981) collected 3 larval *G. nipponicum* from 3 (4.2%) of 72 *R. tigrinus* from Fukuoka Prefecture in Japan [9]. In our study, *G. nipponicum* larvae were detected from *R. nigromaculata*, *A. ussuriensis*, and *R. tigrinus tigrinus*. By this study, *R. nigromaculata* and *A. ussuriensis* have been for the first time confirmed as new second intermediate or paratenic hosts for *G. nipponicum*.

In the Republic of Korea, *G. spinigerum* larvae were found in a snakehead [2], the third stage larvae of *G. hispidum* were detected in pit-viper snakes [5], and larvae and adults of *G. nipponicum* were found in the frogs, snakes, and Jeju weasel [7,8]. Therefore, definitely 3 *Gnathostoma* species, *G. spinigerum*, *G. hispidum*, and *G. nipponicum*, are distributed indigenously in the Republic of Korea. However, no cases of indigenous gnathostomiasis have been reported yet. All gnathostomiasis cases reported by Korean workers are considered to have obtained the infection in foreign countries. In 1988, a *G. spinigerum* infection in the brain was reported from a Thai woman suffering from meningoencephalitis [14]. Later, in 2003, an outbreak of gnathostomiasis, presumably due to *G. spinigerum*, was reported among 60 Korean emigrants in Yangon, Myanmar [15]. Some patients returned to Korea after this outbreak, and one of them was reported as an imported case [16]. Recently, an imported case from China was histologically diagnosed as *G. hispidum* infection by cross sectional morphologies of the larva in skin biopsy [17]. Although, until now, human gnathostomiasis cases have been imported from foreign countries, there exists possibility of indigenous human infections. Attentions should be paid to indigenous human gnathostomiasis in the Republic of Korea.

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