[原著] Helminth Fauna of the Ryukyu Archipelago, Japan : 3. Gnathostoma doloresi larvae from Rana (Babina) subaspera in Amami-oshima Island

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Helminth Fauna of the Ryukyu Archipelago, Japan: 3. *Gnathostoma doloresi* larvae from *Rana (Babina) subaspera* in Amami-oshima Island

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Gnathostomiasis is a famous zoonotic disease caused by the larval nematodes of the genus *Gnathostoma*. Almost all of the causative agents recovered from the human cases in the past Japan have been identified with *Gnathostoma spinigerum* Owen, 1936 . However, some of the recent cases were demonstrated to be due to some other gnathostome species, and attentions have been attracted to these non-*spinigerum* species.

This report will cover the larvae of a non-*spinigerum* gnathostome collected from a species of frog in Amami-oshima Island, Japan, last year. Included in this study is the description of the morphological characteristics of the worms which have not yet been reported from a frog. The life history and pathogenicity of this species will be discussed.

Materials and Methods

The frogs, *Rana (Babina) subaspera,* were captured in the mountainous area of Amami - oshima Island, July 1981 . They were killed with ether. The muscles and viscera were dissected out and pressed between two thick glass plates and searched for parasites under a dissecting microscope. Then, the muscles and viscera were digested with artificial gastric juice (saccharized pepsine 50 g: HCl 7ml: distilled water 1000 ml), and the residues were examined for remaining worms. Detected larvae were fixed in hot 70% ethanol, cleared in glycerin alcohol solution and mounted in 50% glycerin jelly. All worms were measured by using a ocular micrometer. Then, the heads of four larvae were cut off from body and mounted in gum-chloral solution in order to count the number of hooks on the cephalic bulb.

Results

Three *Rana (Babina) subaspera* were examined and 2 , 2 and 5 larval gnathostomes were obtained, respectively. Most of them were recovered from the muscles while one larva was collected from the viscera. Five larvae from the muscles were found to be encysted in those of the hind thigh (Fig. 2). The cysts were subspherical, each contained one larva, and 0.9 − 1.3 mm in diameter (Fig. 3.) .

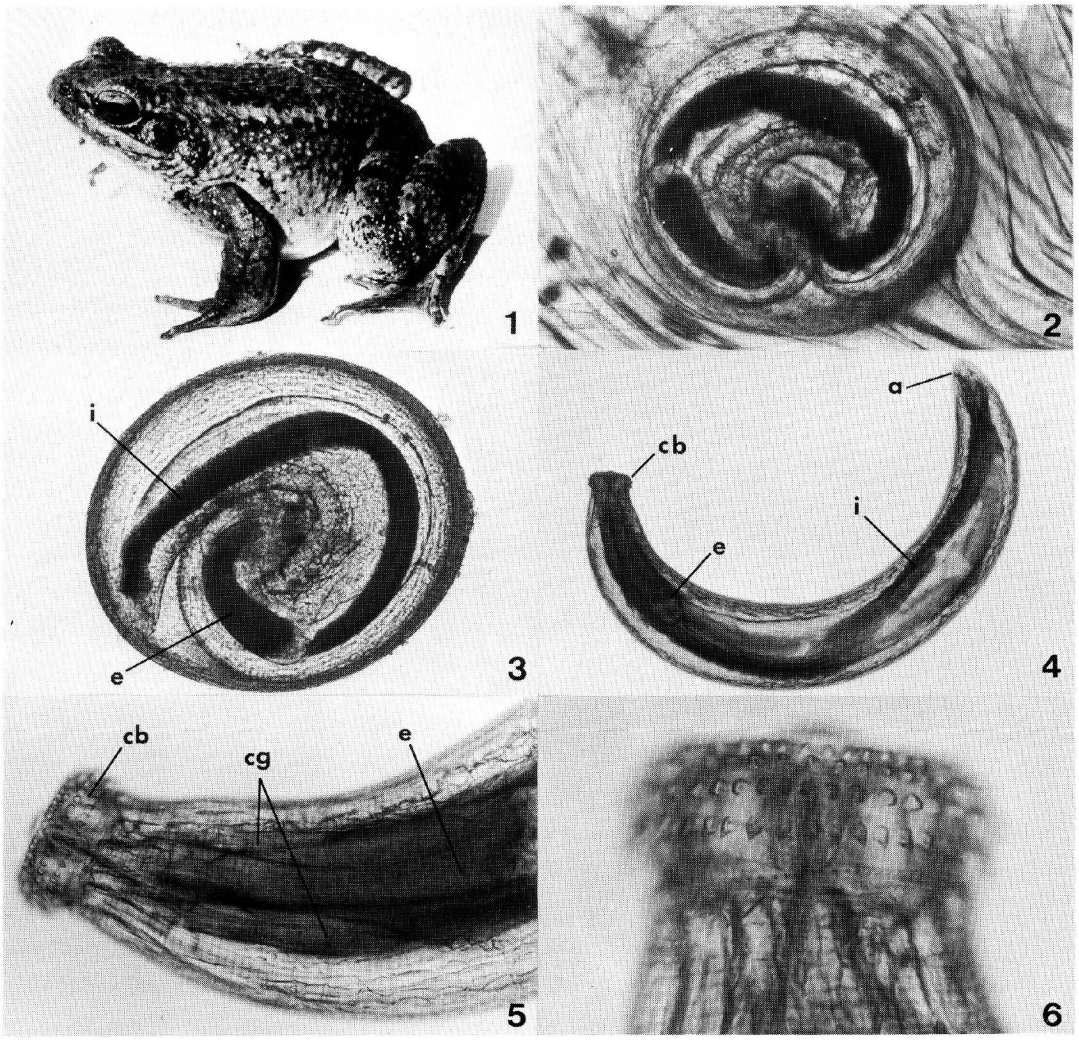

Fig. 1. *Rana (Babina) subaspera.* Photograph, courtesy of Mr. S. Katsuren. (x 0.5)
Fig. 2. Gnathostome cyst in the muscle of hind thigh. (x 45)
Fig. 3. Gnathostome cyst excised from the muscle, slightly compressed. (x 45)
Fig. 4. Total view of larva. (x 60)
Fig. 5. Anterior part of worm, lateral view. (x 95)
Fig. 6. Cephalic bulb showing four transverse rows of hooks. (x 220)
Abbreviations used : a, anus ; cb, cephalic bulb; cg, cervical glands; e, esophagus; i, intestine.

Description of larvae : The worm is almost transparent except dark intestine when alive but whitish after fixation. The body is robust and 2.43 – 3.23 mm long and 0.24 – 0.39 mm wide at middle of body (Fig. 4) . The mouth elongated dorso - ventrally. Each lateral pseudolabium has a pair of

papillae and an amphid. The cephalic bulb is 0.07－0.10 mm long and 0.15－0.20 mm in diameter, and ornamented with 4 transverse rows of cuticular hooks (Figs. 5,6) . Number of these hooks is 36－39 in 1st row, 35－40 in 2nd, 32－38 in 3rd and 32－36 in 4th. Minute cuticular spinules are present on whole body except cephalic bulb forming 176－213 transverse rows. These spinules are decreasing in sizes near the caudal end. The esophagus is club-shaped and divided into anterior muscular and posterior glandular portions. Distances from the cephalic apex to the nerve ring, deirids, posterior end of the esophagus and posterior end of cervical glands are 115－160 μm, 113－213 μm, 0.66－0.77 mm and 0.42－0.59 mm, respectively. The tail is round and distance from the caudal end to the anus is 55－65 μm.

Discussion

The morphological features of the worms,such as the number and arrangement of the hooks on the cephalic bulb, number of the spinule rows on the body and dimensions of body parts, are identical with those of the third-stage larvae of *Gnathostoma doloresi* Tubangui,1925 (Gnathostomatidae) reported by earlier researchers [1) 2) 3)]. Therefore, the authors identified them with *G. doloresi.* This gnathstome species is parasitic to wild boars and pigs in adult stage,and distributed in the Far East, Southeast Asia and Oceania [2) 4)]. The occurrence of this species in Amami-oshima Island has been already well documented [5) 6) 7)].

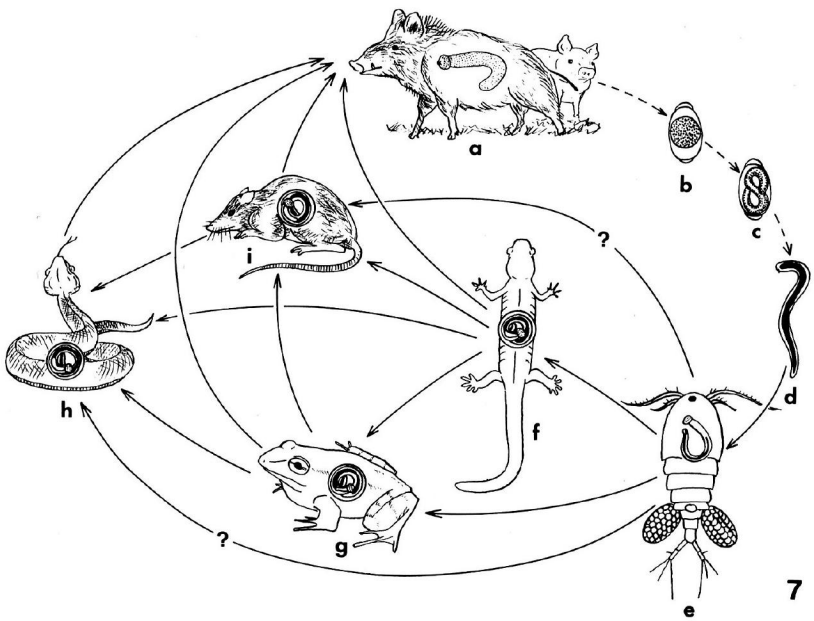

Fig. 7. Schematic illustration of life history of *G. doloresi.* a, Definitive host, wild boar or pig, with adult worms in the stomach; b and c, Development of eggs in water; d, Sheathed first-stage larva hatched from egg; e, Copepod, first intermediate host, with free second-stage larva in hemocoelom; f and g, Amphibian second intermediate hosts with encysted third-stage larvae; h and i, snake and mammal as paratenic hosts (their role as second intermediate hosts are open to question) .

Natural infections of the third-stage larvae of *G. doloresi* have been observed so far in salamanders[8) 9)], newt[3)] and snakes[3) 5) 6) 10)]. However, none of the anuran amphibians has been known as the second intermediate host for *G. doloresi*.

The life history of *G. doloresi* has not been completely elucidated, but is supposed to be as in Fig. 7. The first intermediate hosts were experimentally determined to be some copepods[9)]. Recently, the advanced third-stage larvae were successfully reared in the rats, guinea pigs and dog[11)], demonstrating the possible role of mammals as paratenic hosts. Therefore, the life history of *G. doloresi* is almost same as that of *G. spinigerum* studied extensively by earlier workers [1) 2)], though the participation of fish and birds, the well-known intermediate and paratenic hosts for *G. spinigerum*, in the life history of *G. doloresi* remains unknown.

No human case of larva migrans due to *G. doloresi* has been reported. However, Koga and Ishii (1981)[11)] found that the experimentally-infected larvae persisted alive within the tissues of monkey, suggesting potential pathogenicity of *G. doloresi* larvae for human. Since the frog, *R.(B.) subaspera* is occasionally eaten raw by the local inhabitants in Amami-oshima Island (Uchida, personnal communication), the occurrence of human infection with this gnathostome is presumed in this area.

Summary

Third-stage larvae of *Gnathostoma doloresi* Tubangui, 1925 (Nematoda : Gnathostomatidae) were collected from the frogs, *Rana (Babina) subaspera* in Amami-oshima Island. This is the first record of *G. doloresi* larvae from anuran amphibians. The life history of this species was discussed and illustrated. The role of *G. doloresi* as a human pathogen was also suggested.

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References

1) Miyazaki, I. : On the genus *Gnathostoma* and human gnathostomiasis, with special reference to Japan. Exp. Parasit. 9, 338 – 370, 1960.
2) Miyazaki, I. : *Gnathostoma* and gnathostomiasis. Prog. Med. Parasit. Japan. 3, 275 –309, 1963. (In Japanese.)
3) Hasegawa. H. , Otsuru, M. , Miyagi, I. : Larval *Gnathostoma* recovered from amphibian and reptilian hosts in Okinawa Island, Japan. Ryukyu Univ. J. Hlth. Sci. Med. 4 (2), 103 – 108, 1981 .
4) Miyazaki, I. :*Gnathostoma doloresi* Tubangui, 1925 from pigs in New Guinea. J. Parasit. 54, 186 – 187, 1968.
5) Tada, I. , Sato, A. , Nagano, K. : On the larval *Gnathostoma doloresi* found in snakes,

Trimeresurus flavoviridis flavoviridis, from Amami - oshima Is. , Kagoshima, Japan. Jap. J. Parasit. 18 (3), 289 – 293, 1969.

6) Toshioka, S. : On the larval *Gnathostoma doloresi* found in the himehabu, *Trimersurus okinavensis*, from Amami Islands, Kagoshima Prefecture, Japan. Snake 2, 57 –58, 1970.

7) Uchida, K. , Uchida, A. , Itagaki, H. : Helminth fauna of the Amami Islands. (10) *Gnathostoma doloresi* from a wild boar. Jap. J. Parasit. 30 (Sup.) , 100, 1981. (In Japanese.)

8) Miyazaki, I., Ishii, Y. : On a gnathostome larva encysted in the muscle of salamander, *Hynobius*. Acta Med. 22 (4) , 467 – 473, 1952. (In Japanese.)

9) Ishii, Y. : Studies on the life history of *Gnathostoma doloresi* Tubangui, 1925 in Japan. Fukuoka Acta Med. 47, 1474 – 1494, 1956. (In Japanese.)

10) Miyazaki, I. , Kawashima, K. : On the larval *Gnathostoma doloresi* Tubangui found in a snake from Ishigaki - jima, the Ryukyu Islands (Namatoda : Gnathostomidae.). Kyushu J. Med. Sci. 13, 165 – 169, 1962.

11) Koga, M. , Ishii, Y. : Susceptibility of mammalian hosts to larvae of *Gnathostoma doloresi* Tubangui, 1925. J. Parasit. 67 (6) , 965 –966, 1981.