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Research Note

Gnathostoma doloresi Larvae Found in *Lepomis macrochirus* RAFINESQUE, a Freshwater Fish (common name: blue-gill), Captured in the Central Part of Miyazaki Prefecture, Japan

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Since we reported the first confirmed human case of infection with *Gnathostoma doloresi* (Nawa *et al.*, 1989), 19 cases (two unpublished case) including 4 confirmed cases of human gnathostomiasis doloresi have been found in the central part of Miyazaki Prefecture (Ogata *et al.*, 1988; 1992). Thus, *G. doloresi* is now considered as one of the important zoonotic parasites (Nawa, 1991). All except one case had a common past history of eating raw flesh of freshwater fishes as "Sashimi" or "Segoshi". To determine the exact route of infection to human beings and to elucidate the natural life cycle of *G. doloresi* in the endemic area, we have been conducting surveys for *G. doloresi* infection in freshwater fishes for about 3 years since 1990. Eventually the third stage larvae of *Gnathostoma doloresi* were found from *Lepomis macrochirus* RAFINESQUE, a freshwater fish (common name: blue-gill, Fig. 1), captured at the Hitotsuse-Dam located in the central part of Miyazaki Prefecture. This is the first record of natural infection of a freshwater fish with *G. doloresi* larvae.

During May 1990–September 1992, a total of about 300 blue-gills were captured at the Hitotsuse-Dam located in the central part of Miyazaki Prefecture whereabouts the majority of the patients caught fishes, their head was cut off and the viscera were removed. The skin was peeled and the muscles were cut into small pieces. The muscles were homogenized by a blender, and digested in an artificial gastric juice (pepsin, Difco, 1:10,000 1 g, conc. HCl 7 ml in 1,000 ml distilled water) at 37°C for 3–5 hr, or occasionally overnight. The homogenate was passed through a stainless mesh and the residues were examined under a dissecting microscope. The larvae were fixed in 4% buffered formalin. For counting and morphological observation of the hooklets on the head bulb, the heads of the larvae were cut off from body and mounted in lactophenol solution.

After unsuccessful trial surveys of about 250 fishes for over 2 years, a total of 11 *G. doloresi* larvae (Fig. 2) were obtained from the homogenate of the pooled flesh of 51 blue-gills which were caught on September 1992. Morphometric observations were done on all 11 larvae which were excysted and their internal structures were partially damaged probably because of overdigestion. Four relatively intact larvae were kept aside as representative samples

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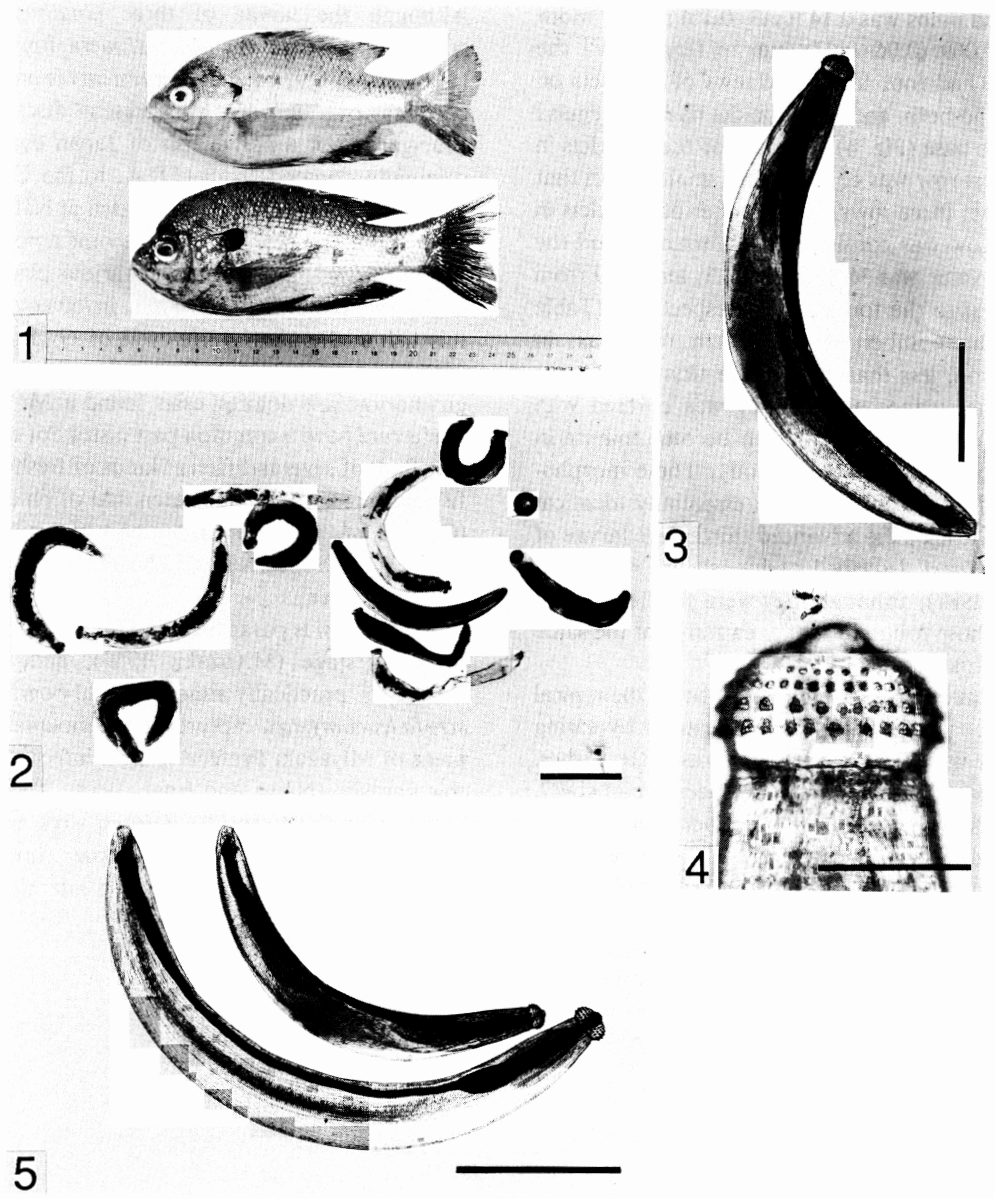

Fig. 1 Blue-gills captured at the Hitotsuse-Dam, Miyazaki Prefecture.
Fig. 2 The larvae recovered from blue-gills. Scale bar = 1.0 mm.
Fig. 3 A representative larva. Scale bar = 0.5 mm.
Fig. 4 Head bulb of the larva in Fig. 2. Scale bar = 0.1 mm.
Fig. 5 Comparison of *G. doloresi* larvae recovered from blue-gills (the upper smaller one) and from snakes (the lower larger one). Scale bar = 1.0 mm.

(Fig. 3) and the head bulb of the rest of 7 larvae were, to count the number of hooklets, cut off after measurement of their body size. The average body length was 1.95 mm, ranging from 1.5 to 2.4 mm, and the average width 0.21 mm, ranging from 0.18 to 0.27 mm. The average size

of head bulbs was 0.14 (0.13–0.15) mm in width and 0.069 (0.06–0.07) mm in length. All the larvae had four transverse raws of hooklets on the head-bulb, and each hooklet had an irregular square base (Fig. 4). The size of the hooklets in the first row was considerably smaller than that of other three rows. The number of hooklets in each row was consistently less than 40 and the mean value was 36.1, 34.8, 33.1, and 33.0 from the first to the fourth rows, respectively (Table 1). The number in the fourth row was, in common, less than that in the first row (Table 1). The surface of the body was covered with small cuticular spines which become minute in the posterior part of the body. These morphological characteristics were essentially identical with those of the advanced third stage larvae of *G. doloresi* described previously by Koga and Ishii (1987), although they were smaller (Fig. 5) than those found in snakes captured at the same area (Imai *et al.*, 1988).

Human gnathostomiasis is one of the typical food-borne parasitic diseases caused by eating raw or under-cooked flesh of freshwater fishes. In Japan, four *Gnathostoma* species, namely *G. spinigerum, G. hispidum, G. nipponicum* and *G. doloresi,* are now known as the causative agents for human gnathostomiasis (Nawa, 1991). Although the larvae of three *Gnathostoma* species other than *G. doloresi* were found in freshwater fishes, only *G. doloresi* larvae had not been discovered until our present discovery. Blue-gill is not a native fish of Japan but was originally donated from U.S.A. to the Crown Prince of Japan on 1960, cultivated at National Freshwater Fishery, and now become a popular fish in freshwater reservoirs of various places in Japan (Nakamura, 1971). Thus, involvement of this fish in the natural life cycle of *G. doloresi* is rather a recent event. Since 18 of 19 human gnathostomiasis doloresi cases found in Miyazaki Prefecture have a common past history of eating raw flesh of several different kinds of freshwater fishes and 3 of them had eaten that of blue-gills (Ogata *et al.*, 1988; 1992), not only blue-gills but also other fish species should be infected with *G. doloresi* larvae.

G. doloresi is parasitic to wild boars and pigs in adult stage (Miyazaki, 1960), and, even nowadays, practically almost all wild boars, *Sus scrofa leucomystax,* captured in the mountainous areas of Miyazaki Prefecture were infected with this parasite (Nawa and Imai, 1989). The first intermediate hosts for *G. doloresi* were experimentally determined to be some copepods (Miyazaki, 1952). As to the second intermediate

Table 1 Number of hooklets on the head-bulb of 7 larval *Gnathostoma doloresi* obtained from blue-gills

Larva No.	1st row	2nd row	3rd row	4th row	4th–1st*
1	36	38	31	32	–4
2	33	35	33	31	–2
3	38	32	34	33	–5
4	36	37	34	33	–3
5	34	32	33	30	–4
6	38	34	33	37	–1
7	38	36	34	35	–3
Mean ± SD	36.1 ± 1.8	36.9 ± 1.8	34.0 ± 2.0	34.4 ± 1.5	–3.1

*Number of hooklets on the 4th row minus that on the 1st row

host, Miyazaki and Ishii (1952) first found the third stage larvae in salamanders. Subsequently, various reptiles and amphibians were added as the intermediate and/or paratenic hosts (reviewed by Imai *et al.*, 1988). Our results is the first direct evidence that freshwater fishes are, like in the life cycle of other *Gnathostoma* species, included in the natural life cycle of *G. doloresi.*

In the present study, all larvae recovered from blue-gills were identified as the advanced third stage larvae of *G. doloresi,* although their size was smaller than those found from snakes captured in the same area (Imai *et al.*, 1988). It should be clarified in future by examining larger numbers of specimens whether or not this size difference between fish and reptile hosts is a constant phenomenon.

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References

1) Imai, J., Asada, Y., Horii, Y. and Nawa, Y. (1988): *Gnathostoma doloresi* larvae found in snakes, *Agkistrodon halys,* captured in the central part of Miyazaki Prefecture. Jpn. J. Parasitol., 37, 444–450.
2) Koga, M. and Ishii, Y. (1987): Surface morphology of the advanced third-stage larva of *Gnathostoma doloresi* – An electron microscopic study –. Jpn. J. Parasitol., 36, 231–235.
3) Miyazaki, I. (1952): *Mesocyclops leuckarti* and *Eucyclops serrulatus* in the first intermediate host of *Gnathostoma* in Japan. Igaku-to-Seibutsugaku (Med. Biol.), 24, 35–37. (in Japanese)
4) Miyazaki, I. (1960): On the genus *Gnathostoma* and human gnathostomiasis, with special reference to Japan. Exp. Parasitol., 9, 338–370.
5) Miyazaki, I. and Ishii, Y. (1952): On a gnathosome larva encysted in the muscle of salamander, *Hynobius.* Igaku Kenkyu (Acta Med.), 22, 467–473. (in Japanese with English abstract)
6) Nakamura, M. (1971): Keys to the Freshwater Fishes of Japan Fully Illustrated in Colors. 2nd edn. Hokuryukan, Tokyo, p.225. (in Japanese)
7) Nawa, Y. (1991): Historical review and current status of gnathostomiasis in Asia. Southeast Asian J. Trop. Med. Publ. Hlth., 22 (Suppl.), 217–219.
8) Nawa, Y. and Imai, J. (1989): Current status of *Gnathostoma doloresi* infection in wild boars captured in Miyazaki Prefecture, Japan. Jpn. J. Parasitol., 38, 385–387.
9) Nawa, Y., Imai, J., Ogata, K. and Otsuka, K. (1989): The first record of confirmed human case of *Gnathostoma doloresi* infection. J. Parasitol., 75, 146–149.
10) Ogata, K., Imai, J. and Nawa, Y. (1988): Three confirmed and five suspected human cases of *Gnathostoma doloresi* infection found in Miyazaki Prefecture, Kyushu. Jpn. J. Parasitol., 37, 358–364.
11) Ogata, K., Sakaguchi, E., Idemori, M., Inoue, S., Nawa, Y., Imai, J. and Otsuka, K. (1992): Creeping disease – gnathostomiasis doloresi – Rinsho Derma (Tokyo), 34, 1313–1320. (in Japanese)