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Larval *Gnathostoma nipponicum* Found in Catfish, *Silurus asotus*, in Aomori Prefecture, Japan

TAKASHI OYAMADA[1)], TAKEAKI KAWAGOE[1)], TSUYOSHI MATSUNAGA[1)], NOBORU KUDO[1)], HIROYASU YOSHIKAWA[2)] AND TAKASHI YOSHIKAWA[1)]

[1)]*Department of Veterinary Parasitology,* [2)]*Department of Veterinary Pathology, School of Veterinary Medicine and Animal Sciences, Kitasato University, Towada-shi, Aomori 034, Japan.*

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Abstract

From September 1993 to October 1994, a total of 75 catfish, *Silurus asotus*, was caught in Aomori Prefecture and was examined for gnathostome larvae. Approximately half (35) of the 75 catfish were infected, all of which were 30 cm or longer in body length. The infected catfish harbored from 1 to 13 larvae, and a total of 90 larvae was recovered. According as the body length of the catfish increased, the infection rate and intensity of infection also increased. Taxonomically, all the larvae were identified as the advanced third-stage larvae of *Gnathostoma nipponicum*. Regarding as the location of the larvae, most (88.9%) were recovered from the viscera, such as mesenterium, gastro-intestinal walls, and serosa. Under undigested examination, more than half of the larvae in visceral tissues showed partial degeneration or death.

From these findings, it suggests that although catfish are susceptible to this parasite, they may not be very suitable hosts as the second intermediate and/or paratenic hosts of *G. nipponicum*.

Key words: *Gnathostoma nipponicum*; advanced third-stage larva; catfish; *Silurus asotus*; survey.

Introduction

Gnathostoma nipponicum, a nematode found in esophageal tumors in the Japanese weasel, *Mustela sibirica itatsi*, and is widely distributed in Japan, from Kyushu to northern Honshu (Yamaguti, 1941; Miyazaki, 1960; Ashizawa *et al.*, 1978; Gyoten and Nishida, 1978; Ando *et al.*, 1988b; Oyamada *et al.*, 1995).

Recently, *G. nipponicum* has been shown to cause human gnathostomiasis, as in the case of *G. spinigerum*, *G. doloresi*, and *G. hispidum*. In all, 10 confirmed and 3 suspected cases of *G. nipponicum* infection have been recorded in Japan (Ando *et al.*, 1988a, 1991; Sato *et al.*, 1992). In the human cases, some freshwater fish, including catfish, were suspected of being the source of infection. In surveys, naturally-infected freshwater fishes such as loaches (Ando *et al.*, 1992; Oyamada *et al.*, 1994) and catfish (Ando *et al.*, 1992) were caught and demonstrated that these fishes could serve as the second intermediate hosts in the life cycle of *G. nipponicum*. In these surveys, however, only one larva was reported from a single catfish, detailed knowledge concerning the infection of catfish with the larvae of *G. nipponicum* is lacking.

The purpose of this study is to assess the prevalence of *G. nipponicum* larvae in catfish in eastern part of Aomori Prefecture where has been an endemic area.

Correspondence: Takashi Oyamada
小山田　隆[1]，川越武明[1]，松永　剛[1]，工藤　上[1]，吉川博康[2]，吉川　堯[1]（[1]北里大学獣医畜産学部獣医寄生虫学教室，[2]同獣医病理学教室）

Materials and Methods

From September 1993 to October 1994, a total of 75 catfish (*Silurus asotus*) was captured at 9 localities in Kamikita-gun located in eastern Aomori Prefecture, an endemic area of *G. nipponicum* confirmed by previous surveys (Oyamada *et al.*, 1994, 1995). Prior to dissection, each catfish was weighed and was measured its body length. The fish was then divided into several parts; the head, skin, fins, viscera, abdominal wall, and other sites of muscula-

ture. In 46 catfish, the liver, spleen, kidneys, heart, air bladder, genital organs, alimentary tracts, and mesenterium were separated from the viscera. The organs or tissues were cut into small pieces, and digested in an artificial gastric juice solution (pepsin, 1:10,000, 8 g; HCl, 8 ml in 1,000 ml distilled water) at 37°C for 50–70 minutes. After sedimentation of the digested homogenate for about 15 minutes, sediments were examined under a dissecting microscope. Furthermore, tissues from the alimentary tracts and mesenterium of 24 catfish were directly examined microscopically before artificial digestion. All larvae recovered were fixed in hot 10% formalin, cleared, and mounted in lactophenol for taxonomical observation.

Results

The prevalence of gnathostome larvae in catfish from the several localities surveyed is shown in Table 1. Of the total of 75 catfish, 35 were infected, from which 90 larvae were recovered. The infection rate in each locality was different, ranging from 0 to 100%. Each infected catfish harbored from 1 to 13 larvae. The relationship between the number of larvae and catfish body length is summarized in Table 2. Infected catfish were found to be longer than 30.0 cm, and as the body length increased, so did the infection rate and mean number of larvae per fish.

The location of larvae in the catfish is shown in Tables 3, 4, and 5. The vast majority, 80 (88.9%) of 90, were recovered from the viscera, with only 9 (10%) and 1 (1.0%) found in the muscles and head, respectively (Table 3). In the viscera in 25 of 35 infected catfish, 57 (89.1%) of 64 larvae were recovered from the alimentary tracts and mesenterium (Table 4). And, in 12 of 35 infected catfish, 24

Table 1 Prevalence of larval *Gnathostoma nipponicum* in catfish from eastern Aomori Prefecture

Locality	No. of catfish		Prevalence (%)	No. of larvae recovered	Intensity (mean)
	examined	infected			
Toyota	32	17	53.1	43	1– 6 (2.5)
Hanamukai	14	2	14.3	3	1– 2 (1.5)
Futatsumori	12	9	75.0	25	1–13 (2.8)
Tsukuta	7	1	14.3	1	1 (1.0)
Toriguchi	6	5	83.3	17	1– 5 (3.4)
Sakainuma	1	1	100.0	1	1 (1.0)
Yojyokai	1	0	0		
Terasawa	1	0	0		
Tanosawa	1	0	0		
Total	75	35	46.7	90	1–13 (2.6)

Table 2 Relationship between body length of catfish and number of larval *Gnathostoma nipponicum*

Body length of catfish (cm)	≤30.0	30.1–40.0	40.1–50.0	50.1≤	Total
No. infected/examined	1/11	13/33	10/17	11/14	35/75
Prevalence (%)	9.1	46.0	58.8	78.6	46.7
No. of larvae recovered	1	24	27	38	90
Mean of intensity	1.0	1.8	2.7	3.5	2.6

Table 3 Location and number of larval *Gnathostoma nipponicum* in 35 infected catfishes

No. of larvae (%) recovered from				
Head	Viscera	Muscles of		Total
		Abdomen	Others	
1 (1.1)	80 (88.9)	8 (8.9)	1 (1.1)	90

Table 4 Location and number (%) of larval *Gnathostoma nipponicum* in the viscera in 25 of 35 infected catfishes

Li	Sp	Ge	Al & Me	Total
2 (3.1)	2 (3.1)	3 (4.7)	57 (89.1)	64

Li, Liver; Sp, Spleen; Ge, Genital organs; Al, Alimentary tracts; Me, Mesenterium.

Table 5. Location and number (%) of larval *Gnathostoma nipponicum* in the alimentary tracts and mesenterium in 12 of 35 infected catfishes

Stomach		Intestinal		Mesenterium	Total
wall	serosa	wall	serosa		
2 (5.7)	0 (0)	8 (22.9)	1 (2.9)	24 (68.6)	35

(68.6%), 10 (28.6%), and 1 (2.9%) of 35 larvae, were recovered from the mesenterium, gastro-intestinal walls, and serosa, respectively (Table 5). By light microscopy of fresh (undigested) tissues, all larvae were found within minute nodular foci. The foci were irregular in shape, and the larvae were enclosed by an abundant cellular or fibrous tissues (Figs. 1 and 2). Since the appearance of the larvae within the foci varied, they were conveniently classified into 3 types, i.e., normal larvae (type 1): showing sluggish movement and normal architecture (Fig. 1), degenerated larvae (type 2): with lack of movement and partial degeneration (Fig. 2), and

Table 6 Incidence of larval *Gnathostoma nipponicum* based on the morphological characteristics in undigested tissues of the alimentary tracts and mesenterium in 12 of 35 infected catfishes

No. of larvae (%) classified as			
Normal (type 1)	Degenerated (type 2)	Dead (type 3)	Total
20 (48.8)	5 (12.2)	16 (39.0)	41

Of 25 larvae recovered from the mesenterium and serosa of the alimentary tracts, 16 were from the gastro-intestinal walls.

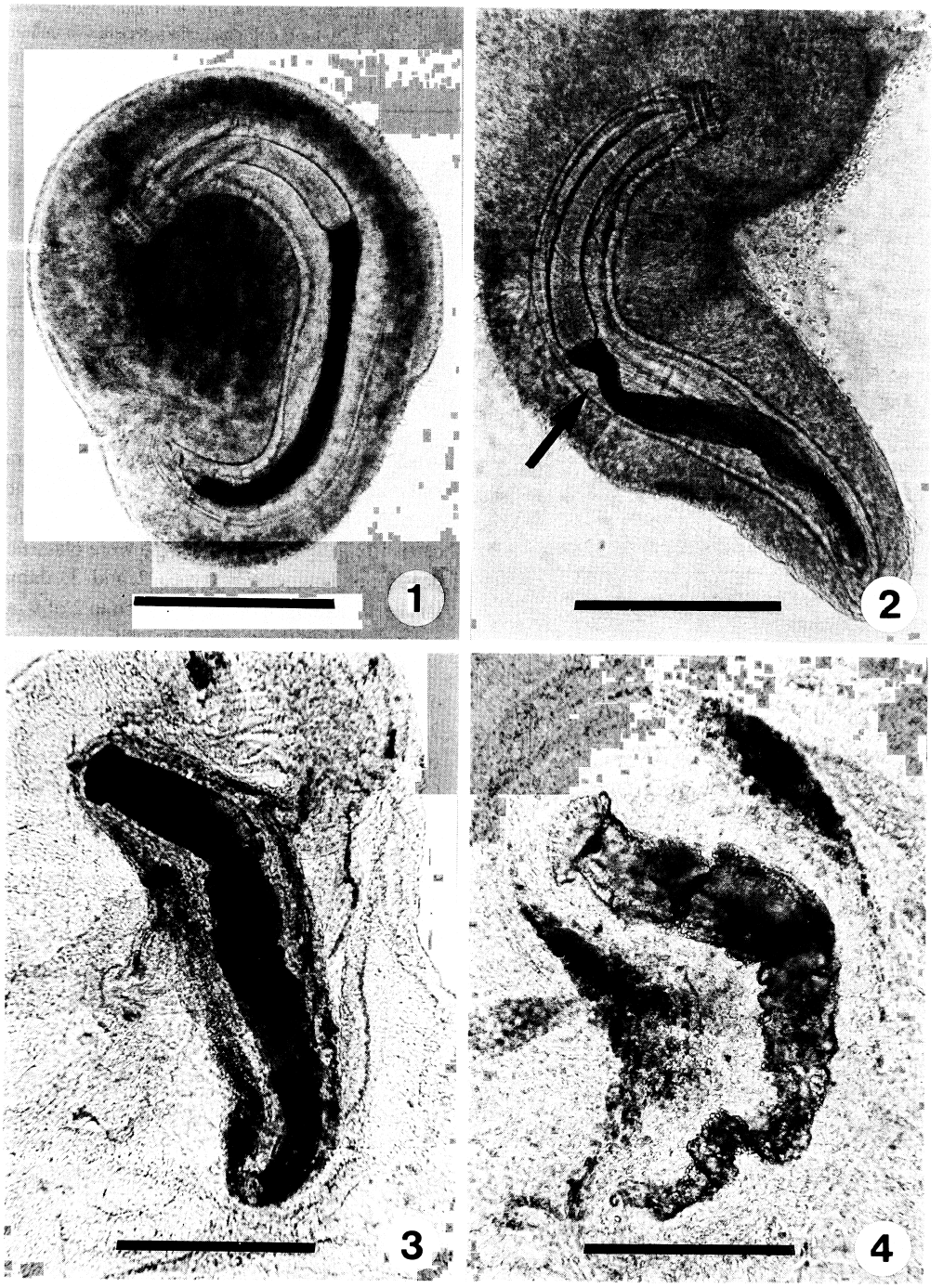
dead larvae (type 3): with advanced degeneration and calcification (Figs. 3 and 4). The incidence of each 3 type is shown in Table 6. In 12 infected catfish, 21 (51.2%) of 41 larvae were classified as dead and degenerated (types 2 and 3: damaged larvae). These types were also seen in 2 of 9 larvae recovered from the muscles by artificial digestions.

The morphological architecture and dimensions of the larvae in the catfish are summarized in Table 7, and compared with those of larval *G. nipponicum* from loaches previously reported by Oyamada *et al.* (1994). In the present case, the bodies were 508–1,675 μm in length and 90–170 μm in width. The larvae had three rows of hooklets on head-bulb (Fig. 5), and the number of hooklets on the first, second, and third rows were 26–41, 25–40, and 34–45. The body was encircled by 212–246 transverse striations consisting of single-pointed minute spines. Extending posteriorly, the spines gradually decreased in size and density, and finally disappeared near the tip of the tail.

In this study, no relationship was detected between the morphology and location of the larvae. Furthermore, there were no relations between the incidence of damaged larvae and the body length of the infected catfishes.

Discussion

A few reports dealing with the catfish as the second intermediate and/or paratenic hosts of *G. nipponicum* are present or seen. In previous reports, four catfishes examined in Aich Prefecture were



Figs. 1–4 *G. nipponicum* larvae in fresh (undigested) tissues of catfish (Bar = 300 μm).
Fig. 1 Apparently normal larva within a nodular focus in the stomach wall.
Fig. 2 Larva within a mesenteric nodular focus, showing partial degeneration of the intestine (arrow).
Figs. 3, 4 Dead larvae undergoing calcification within fibrous mesenteric nodular foci.

Table 7 Comparison of measurements of larval *Gnathostoma nipponicum* from catfishes and loaches in eastern Aomori Prefecture

	Present paper	Oyamada *et al.* (1994)
Host	Catfish*	Loach
Body length	508–1,675	745–1,684
width	90–170	98–186
Head-bulb height	32–66	28–60
width	78–108	54–108
Esophagus length	334–600	321–594
Cervical sac length	113–360	156–363
Tail length	21–63	15–62
Number of transverse striation	212–246	188–267
Number of hooklets on head-bulb†		
1st row	26–41	28–39
2nd row	25–40	30–42
3rd row	34–45	25–46

*Worm size (μm) based on 79 specimens; †No. of fooklets based on 72 specimens.

negative (Kato, 1956) whereas in Mie Prefecture, one of six one caught was positive (Ando *et al.*, 1992). The latter report is the first case of naturally-infected catfish.

In the present study, we collected a total of 90 gnathostome larvae from 35 of 75 catfishes. The taxonomical features of the present larvae coincide well with the descriptions of the advanced third-stage larvae (AdL3) of *G. nipponicum* reported by previous investigators (Miyazaki, 1954, 1960; Koga and Ishii, 1981; Ando *et al.*, 1992), and are also similar to those of AdL3 from loaches (Table 6) taken in Aomori Prefecture. Morphologically, even in severely damaged larvae in viscera, the architecture of hooklets on head-bulb were relatively well preserved and it was possible to distinguish *G. nipponicum* from the other species of gnathostome larvae indigenous in Japan. Miyazaki (1960) emphasized that the row number and shape of the head-bulb hooklets of the third-stage larvae were very useful in making a specific identification of the genus *Gnathostoma*.

The high prevalence of *G. nipponicum* larvae in catfish, as well as in loaches, clearly indicates that Kamikita-gun in Aomori Prefecture is an endemic area for this nematode. As the body length of the catfish increased, the prevalence and the mean number of larvae per catfish showed higher level. This fact attributes to the feeding habits of the catfish. Neonatal catfish eats copepods and wheel-animalcules for 3–8 days after hatching, then the juveniles feed chiefly on small freshwater animals, such as bitterlings, loaches, shrimps, and snails. The catfish become the adult (about 30cm in body length) in 2 years (Tomoda, 1962). Therefore, because of its feeding habits as a neonate, juvenile and adult, it seems likely that catfish can become infected with *G. nipponicum* larvae by eating both copepods and small freshwater animals mentioned above which are known to serve as the first and second intermediate hosts of this nematode. Nevertheless, catfish less than 29.0 cm in body length showed to be uninfected. This relationship might be related to the quantity of foods, including the first and/or second intermediate host animals. Moreover, as to the higher prevalence and intensity in adult catfishes, a probable explanation of this fact might be that the adult catfish was secondarily infected with larvae by

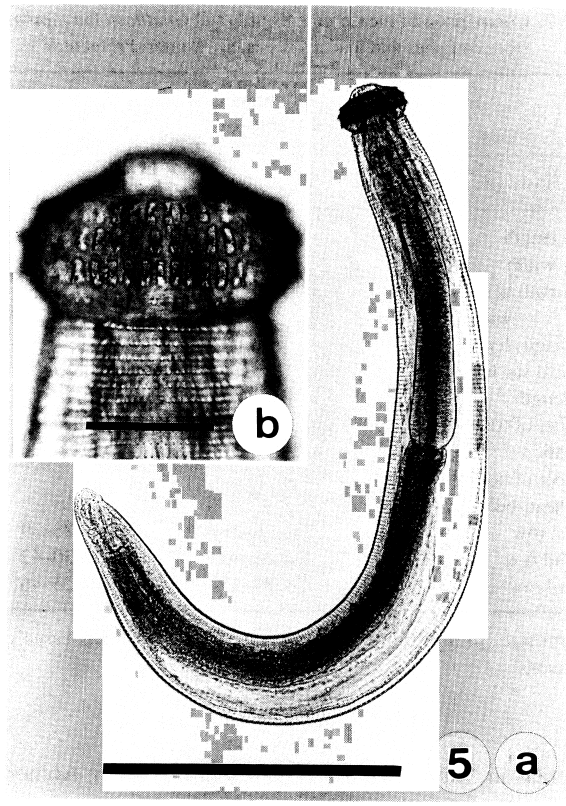


Fig. 5a and b a: Lateral view of a larva recovered from the mesenterium (Bar = 500 μm). b: Head-bulb showing three rows of hooklets on the surface (Bar = 50 μm).

eating the second intermediate host such as loaches than the first intermediate host such as copepods due to the feeding habits in their adult stage. The higher prevalence of the larvae in loaches reported by previous survey in the same areas (Oyamada *et al.*, 1994) and, the active and ferocious feeding habits of adult catfish may help explain the observed relationship between the body length of the catfish and the higher prevalence.

Most (88.9%) of the AdL3 were recovered from the viscera, such as mesenterium and alimentary tracts. This finding differs from previous reports in which *G. nipponicum* larvae generally were recovered from the musculature of naturally- and experimentally-infected animals (Koga and Ishii, 1981; Ando *et al.*, 1988a, 1992; Oyamada *et al.*, 1994). In 12 infected catfish, 41 larvae were found within minute nodular foci in the viscera and were surrounded by a cellular or fibrous tissues. More than half (51.2%) of the larvae were dead and degenerating. These larval foci differs from the encapsulations found in the muscles of other animals. It was considered that the nodular foci in the catfish were formed as a result of an inflammatory defense reactions of the host against the larvae. Moreover, it was supposed that the reactions might be related either to the location of the most of larvae or the high incidence of damaged larvae in the mesnterium and alimentary tracts of the catfish. Until now, the damaged larvae of *G. nipponicum* as in the case of these catfishes were not reported from the other animals. Similarly, we could not to find them in examined 160 larvae collected from naturally-infected loaches (unpublished data). It is necessary to clarify the relationship

between the high incidence of the damaged larvae and biological natures of the catfish as the host of larval *G. nipponicum*.

On the other hand, 9 (10%) larvae were only recovered from the muscles. Ando *et al.* (1988a) found the encapsulated AdL3 of *G. nipponicum* in the muscles of experimentally-infected loaches. However, it was uncertain whether the larvae were encapsulated or not in this study, because we made artificial digestion to recover larvae from the muscles.

It is clear that although catfish are susceptible to this parasite, they may not be very suitable hosts. Further study is necessary to clarify the role of catfish in the natural life cycle of *G. nipponicum*. In addition, to prevent the further occurrence of human gnathostomiasis, the public should be informed of the danger of eating raw catfish, prepared as "sashimi" or "sushi".

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References

1) Ando, K., Hatsushika, R., Akahane, H., Matsuoka, H., Taylor, D., Miura, K. and Chinzei, Y. (1991): *Gnathostoma nipponicum* infection in the past human case in Japan. Jpn. J. Parasitol., 40, 184–186.
2) Ando, K., Tanaka, H., Taniguti, Y., Shimizu, M. and Kondo, K. (1988a): Two human cases of gnathostomiasis and discovery of a second intermediate host of *Gnathostoma nipponicum* in Japan. J. Parasitol., 74, 623–627.
3) Ando, K., Tanaka, H. and Ohkawa, C. (1988b): A survey of geographical distribution of *Gnathostoma nipponicum* in Mie, Nara, Kyoto and Shiga Prefectures, Japan. Jpn. J. Parasitol., 37, 263–267 (in Japanese with English Abstract).
4) Ando, K., Tokura, H., Matsuoka, H., Taylor, D. and Chinzei, Y. (1992): Life cycle of *Gnathostoma nipponicum* Yamaguti, 1941. J. Helminthol., 66, 53–61.
5) Ashizawa, H., Kugi, G., Nosaka, D., Tateyama, S. and Yanai, T. (1978): Natural infection of weasels with *Gnathostoma nipponicum* in Oita Prefecture, Japan. Bull. Fac. Agr. Miyazaki Univ., 25, 85–92 (in Japanese with English Abstract).
6) Gyoten, J. and Nishida, H. (1978): On the *Gnathostoma nipponicum* in Kagawa Prefecture. Jpn. J. Parasitol., 27, 411–416 (in Japanese with English Abstract).
7) Kato, W. (1956): Study on *Gnathostoma nipponicum* Yamaguti, 1941. Nagoyaigaku, 74, 659–676 (in Japanese with English Abstract).
8) Koga, M. and Ishii, Y. (1981): Larval gnathostomes found in reptiles in Japan and experimental life cycle of *Gnathostoma nipponicum*. J. Parasitol., 67, 565–570.
9) Miyazaki, I. (1954): Studies on *Gnathostoma* occurring in Japan (Nematoda: Gnathostomidae) II. Life history of *Gnathostoma* and morphological comparison of its larval forms. Kyushu Mem. Med. Sci., 5, 123–139.
10) Miyazaki, I. (1960): On the genus *Gnathostoma* and human gnathostomiasis, with special reference to Japan. Exp. Parasitol., 9, 338–370.
11) Oyamada, T., Kudo, N., Sakashita, H. and Yoshikawa, T. (1995): The first record of *Gnathostoma nipponicum* in Aomori Prefecture. Jpn. J. Parasitol., 44, 128–132.
12) Oyamada, T., Kudo, N., Sano, T. and Narai, H. (1994): An epidemiological survey of the third-stage larva of *Gnathostoma nipponicum* in loaches, *Misgurnus anguillicaudatus* in eastern district. Aomori Prefecture. Jpn. J. Parasitol., 43(Suppl.), 73 (in Japanese).
13) Sato, H., Kamiya, H. and Hanada, K. (1992): Five confirmed human cases of gnathostomiasis nipponica recently found in northern Japan. J. Parasitol., 78, 1006–1010.
14) Tomoda, Y. (1962): Studies on the fishes of Lake Biwa-ko – I. Morphological study of the three species of catfishes of the genus *Parasilurus* from Lake Biwa-ko, with reference to their life. Jpn. J. Ichthyol., 8, 126–146 (in Japanese with English Abstract).
15) Yamaguti, S. (1941): Studies on helminth fauna of Japan. Part 35. Mammalian nematodes, II. Jpn. J. Zool., 9, 409–438.