

Description of *Neodiplostomum (Neodiplostomum) brachypteris nipponense* subsp. nov. with Notes on Its Metacercariae Parasitic in a Frog, *Rana nigromaculata* Hallowell (Trematoda : Strigeoidea : Diplostomidae)

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The frogs are well known not only as the definitive hosts but also as the intermediate ones of various trematodes. In Japan, however, no species of the trematode metacercariae has been found from the liver of frogs up to present (Komiya, 1965).

We recently found many metacercariae of an unknown trematode species in the livers of several adults of *Rana nigromaculata* Hallowell, one of the commonest frogs in Japan, which had been collected in the paddy fields near Ezu Lake, in the suburbs of Kumamoto City, Kyushu, and obtained 33 adult trematodes by feeding newborn chickens with those metacercariae.

The adult obtained is closely allied to *Neodiplostomum (Neodiplostomum) brachypteris* Chatterji, 1942, which was first found in *Brachypterus benghalensis benghalensis* (Linnaeus) (Aves: Pici) and then in *Lobivanellus indicus* (Boddaert) (Aves: Charadrii) from India, but is distinguishable from it by some minute differences of organizations as stated below. We, therefore, describe here the Japanese materials as a new subspecies of the Indian species.

Materials and Methods

The frogs, *Rana nigromaculata*, used in this study are composed of three groups of two different sources. Both of the first and the second groups were obtained through an

animal trader in Kumamoto City, in June and July, 1970. Since they were estimated to be collected in paddy fields near Ezu Lake in the suburbs of Kumamoto City by an inquiry to the trader, we surveyed approximately the same locality and collected some adult frogs in September 1970, which form the third group.

Each frog was autopsied and the visceral organs were separately examined. The metacercariae freed from the liver of the frog were orally fed by newborn chickens (White Leghorn) with a drop of tap water. The chickens were autopsied one by one at a certain intervals such as 1 to 14 hours or 1 to 14 days after feeding. The digestive tract of those chickens were thoroughly detected and adults recovered were prepared into mounted specimens *in toto*. Bouin's fluid, borax carmine, and Eukitt were used for fixation, staining, and mounting, respectively. Fecal examination of the chickens was also occasionally performed.

Results and Discussion

Detection of metacercaria

Results of the examination of the frogs are summarized in Table 1. Almost all of the frogs harbored numerous metacercariae but some frogs did a few ones such as only 3, 5, 10, or 14. The metacercariae were only in liver and evenly distributed in it.

On the other hand, no one of this kind of metacercariae has been found from the frog collected in Kurume City and its adjacent

Table 1 Incidence of metacercaria (MC) of *Neodiplostomum* (*Neodiplostomum*) *brachypteris nipponense* subsp. nov. in *Rana nigromaculata* collected in Kumamoto City

Date of collection	No. of <i>R. nigromaculata</i>		Maximum no. of MC in a single host	Remarks
	Examined	Infected (%)		
June, 1970	9	8 (88.9)	More than 20	From an animal trader
July, 1970	8	6 (75.0)	More than 50	Same as above
Sept. 6, 1970	4	4 (100.0)	Ca. 100	Collected by the authors
	Total 21	18 (85.7)		

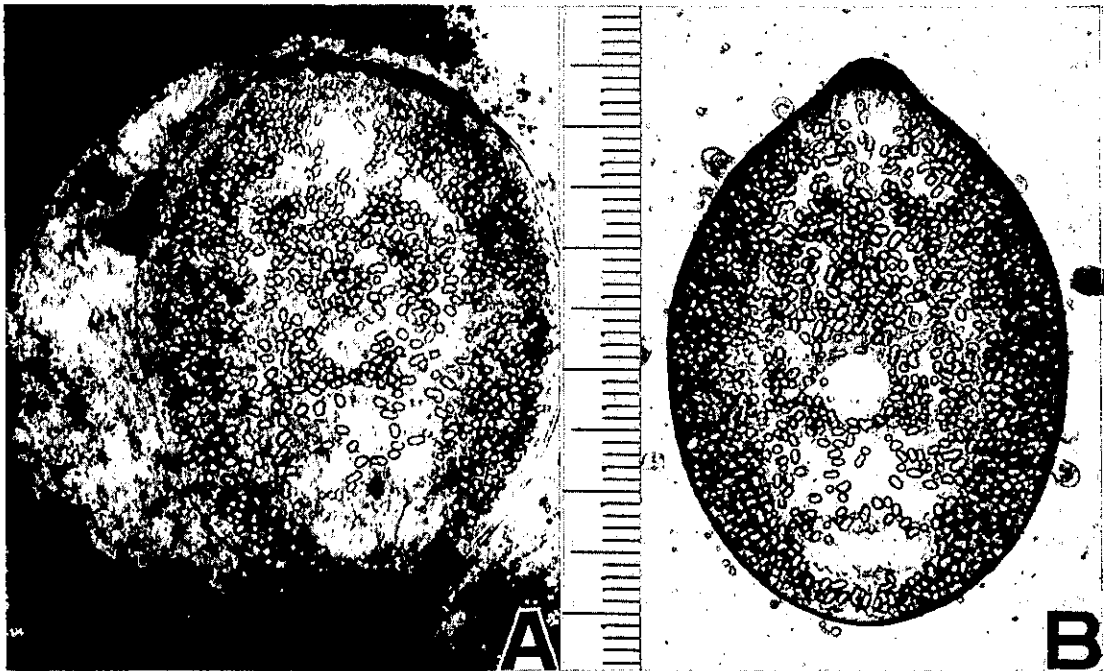


Fig. 1 Metacercaria of *Neodiplostomum* (*Neodiplostomum*) *brachypteris nipponense* subsp. nov.

A. An encysted metacercaria in liver of *Rana nigromaculata*.

B. An excysted metacercaria.

Scale: 0.01 mm

areas in Fukuoka and Saga Prefectures*.

Metacercaria (Fig. 1 A & B)

Cyst: Oval, 0.34-0.39×0.31-0.38 mm. Cyst

* Both of these two groups of the frog (from Kumamoto and from Kurume) were found to be evenly infected with the metacercariae of *Encyclometra japonica* in the muscle and with the adults of *Pleurogenes japonicus*, *Glyphelmis rugicaudata*, and/or *Diplodiscus japonicus* in the digestive tract.

membrane composed of a single layer of moderate thickness; a thin additional membrane usually enclosing the cyst in the frog liver (Fig. 1 A). Easily excysted by a soft compression or 1 to 2 hours' treatment with artificial gastric juice (Fig. 1 B).

Excysted metacercaria: Oval or elliptical, oral sucker and its adjacent region bluntly protruded anteriorly; 0.36-0.52×0.34-0.43 mm under a flattened condition. Oral sucker 51-74×46-69 μ ; acetabulum 40-51×43-57 μ .

Table 2 Results of feeding experiments of chickens with the metacercariae (MC) from liver of *Rana nigromaculata* collected in Kumamoto City

Experiments	Chicken				No. of MC fed per a host	No. of worms recovered
	No. of examined	Age at feeding	Duration till examination	No. of infected		
Part 1	11 (Nos. 1-11)	1 day	1, 2, 3, 5, & 14 hrs., 1, 2, 5, 7, 9, & 14 days	1/11 (9 days)	ca. 10	2
Part 2	10 (Nos. 12-21)	5-11 days	7, 10, & 11 days	0/10	ca. 10	—
Part 3	10 (Nos. 22-31)	1 day	8 & 9 days	3/10 (9 days)	ca. 15	20, 10, & 1, respectively
Total				4/31 (19.9 %)		33

Almost wholly filled up with numerous macrogranules as a characteristic of the strigeoid metacercaria.

Recovery of adults from chicken (Table 2)

The metacercariae separated from the frog liver were orally fed to newborn chickens (White Leghorn) as soon as possible. Each chicken was fed about 10 to 15 metacercariae. According to the grouping of the frogs, the feeding experiments were also divided into three parts.

(Experiment Part 1)

Eleven chickens (Nos. 1-11) of 1 day after hatching were subjected. Each was fed with about 10 metacercariae, and sacrificed one by one 1, 2, 3, 5, 14 hours, 1, 2, 5, 7, 9, and 14 days after feeding. Only one chicken (No. 10) sacrificed 9 days after feeding harbored 2 adult worms in duodenum. Other 10 chickens were free from the trematode.

(Experiment Part 2)

Each of ten chickens (Nos. 12-21) of 5 to 11 days after hatching was fed with about 10 metacercariae. They were sacrificed 7, 10, or 11 days after feeding, because a duration of 10 days is estimated to be enough for obtaining gravid adults of this trematode by the preceding experiment (Part 1). None of the chickens, however, was parasitized by the trematode. This may suggest that even a few days old chickens are already inadequate for the experimental host of the present trematode.

(Experiment Part 3)

Each of ten newborn chickens (Nos. 22-31) was fed with about 15 metacercariae which were separated from frogs collected by ourselves on September 6, 1970, in paddy fields near Ezu Lake. Two of them (Nos. 22 and 23) were sacrificed 8 days after feeding and found to be free from the parasite. On the following day (9 days after feeding), one of the rest chickens (No. 24) was found to be excreting the diplostomid eggs in its stool. Then, all surviving chickens (Nos. 24-31) were autopsied and 20 adult worms were recovered from the chicken No. 24, and 10 and 1 adult worms from Nos. 25 and 26, respectively. Other 5 chickens (Nos. 27-31) did not harbor the worm.

From these results obtained in experimental infections, it is possible to estimate that the chicken is not so adequate for the experimental host of the present trematode, but the very newly hatched chicken may eventually serve as a host, and that the present trematode matures within 10 days in the definitive host after infection.

Description of adult

Examining the adults obtained from the duodenum of the chickens, we conclude that the present materials are closely allied to *Neodiplostomum* (*Neodiplostomum*) *brachypteris* Chatterji, 1942, but differ from it by some minute organizations as stated below. Therefore, the Japanese material is described as a new subspecies of the above-mentioned Indian species as follows.

Neodiplostomum (*Neodiplostomum*)
brachypteris nipponense
 subsp. nov.

Intermediate host: *Rana nigromaculata* Hal-
 lowell

Habitat in the intermediate host: Liver

Locality: Kumamoto City, Kyushu

Definitive host: Newborn chicken (White Leg-
 horn) (exp.)

Habitat in the definitive host: Duodenum

Description is based on 25 gravid adult spec-
 imens. One example was destroyed in the course
 of mounting. Other 7 ungravid specimens were
 also examined but excluded from the measurements

of organs and from the type specimens.

Body divided into flat, oval forebody and
 rather thick conical hindbody; total length
 1.03–2.03 mm. Forebody oval, easily flattened
 and spread by compression, 0.49–1.11 mm in
 length and 0.64–1.01 mm in maximum breadth
 at level of tribocytic organ or a little pos-
 teriorly; hindbody 0.54–1.00 mm long, 0.35–
 0.67 mm broad at level of testicular area.
 Oral sucker broader than long, 60–91×71–
 117 μ. Prepharynx invisible. Pharynx almost
 circular, 51–66×43–71 μ. Esophagus very
 short, almost absent. Bifurcated ceca slender,
 outwardly curved in forebody, most distant

Table 3 Comparisons of measurements and other data between two
 subspecies of *Neodiplostomum brachypteris*

(mm)		
	<i>N. b. nipponense</i>	<i>N. b. brachypteris</i>
Authority	New record	Dubois, 1970*
No. of specimens examined	26 out of 33	
Host	New born chicken (exp.) (9 days after infection)	<i>Brachypteris b.</i> <i>benghalensis</i>
Habitat	Duodenum	Small intestine
Locality	Kumamoto, Kyushu, Japan	near Allahabad, India
Total length	1.02–2.03 (1.54)	2.4
Forebody	(L 0.49–1.11 (0.73) (W 0.64–1.01 (0.81)	0.59–1.44 0.56–1.17
Hindbody	(L 0.54–1.00 (0.82) (W 0.35–0.67 (0.53)	0.35–1.05 0.30–0.68
Oral sucker	(L 0.060–0.091 (0.075) (W 0.071–0.117 (0.096)	0.057–0.085 0.063–0.100
Acetabulum	(L 0.057–0.126 (0.094) (W 0.106–0.131 (0.118)	0.065–0.091 0.087–0.115
Pharynx	(L 0.051–0.066 (0.058) (W 0.043–0.071 (0.056)	0.050–0.091 0.043–0.068
Ovary	(L 0.095–0.150 (0.120) (W 0.172–0.289 (0.214)	0.053–0.153 0.183–0.289
Anterior testis	(L 0.109–0.259 (0.176) (W 0.300–0.600 (0.433)	0.088–0.300 0.163–0.470
Posterior testis	(L 0.177–0.327 (0.251) (W 0.290–0.535 (0.438)	0.100–0.310 0.238–0.510
Eggs	(L 0.087–0.096 (0.091†) (W 0.050–0.057 (0.053†)	0.092–0.117 0.045–0.062
Maximum no. of uterine eggs	14	6

* The original description by Chatterji was not referable for ourselves.

† Average of 20 eggs delivered into saline solution.

to each other at level of tribocytic organ, running into hindbody, terminating at the middle of posttesticular area. Acetabulum transversely elongated, elliptical, slightly larger than oral sucker, $57-126 \times 106-131 \mu$, located at the posterior end of the anterior third of forebody. Tribocytic organ transversely elongated, elliptical, located at the center of forebody, immediately posterior to acetabulum or sometimes covered by it. Vitellaria distributed in forebody except peripheral portion of it and almost all of hindbody except a small transverse space near the caudal end; anterior margin of vitellaria reaching pharynx. Ovary transversely elongated, elliptical, $95-$

$150 \times 172-289 \mu$; its anterior margin almost in contact with posterior margin of forebody. Testes tandem, both transversely elongated, often constricted at the middle portions, their lateral portions swollen, claviform; anterior testis usually asymmetrical, left half larger than right, $109-259 \times 300-600 \mu$; posterior testis usually symmetrical, $88-300 \times 163-470 \mu$; anterior testis sometimes broader but usually smaller in volume than posterior one.

Measurements are summarized in Table 3 with comparison to the nominate subspecies from India.

The present new subspecies differs from

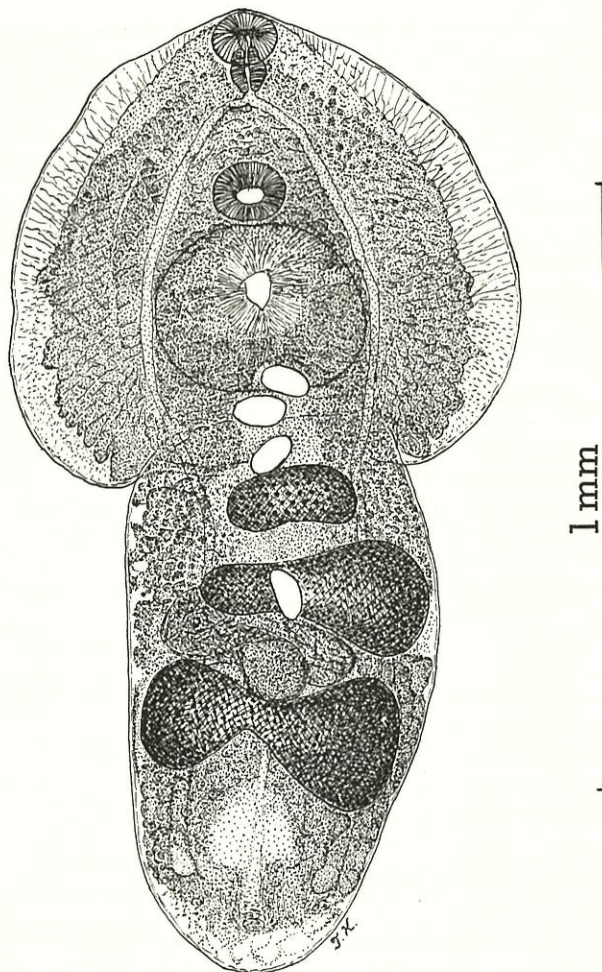


Fig. 2 *Neodiplostomum (Neodiplostomum) brachypteris nipponense* subsp. nov., holotype, ventral view.

the nominate subspecies in the following characters: Ovary not overlapped by forebody; forebody and hindbody subequal in length; anterior part of vitellaria reaching pharynx, etc.

Type materials: 1 holotype (slide no. 260, Fig. 2) and 24 paratypes (slide nos. 261-284).

Other specimens examined: 7 ungravid specimens simultaneously obtained with the type materials (nos. 285-291).

Type depository: Holotype, 22 paratypes (nos. 261-282), and other ungravid specimens are preserved in the Department of Parasitology, Kurume University School of Medicine. Two paratypes (nos. 283 and 284) are in the collection of Meguro Parasitological Museum, Meguro, Tokyo.

The genus *Neodiplostomum* contains 42 species (Dubois, 1970), which are separated in 2 subgenera, *Neodiplostomum* and *Conodiplostomum*, as a result of synonymizations of various species. Out of them, there have been recorded 4 species by Yamaguti (1933, 1935, 1939) from Japan. The Japanese 5 species, including the present new subspecies, are distinguishable from one another by the following key.

Key to the Japanese species of the genus
Neodiplostomum Railliet

(All of the Japanese species belong
to the subgenus *Neodiplostomum*)

1. Anterior margin of vitellaria at level of acetabulum. 2
 - Anterior margin of vitellaria distributed near cecal bifurcation or more anteriorly. 3
2. Body slender, 2.2-2.8 mm long. Tribocytic organ relatively large, about one-third as long as forebody.
 - *N. (N.) strigis* Yamaguti, 1939
 - Body not slender, 1.24-1.57 mm long. Tribocytic organ about one-fourth as long as forebody
 - *N. (N.) attenuatum micropharyngeum*
Dubois et Richard, 1964¹⁾
3. Tribocytic organ relatively small, about

1) *N. attenuatum* Yamaguti, 1935, nec Linstow, 1906.

- one-fourth as long as forebody, clearly separated from acetabulum.
- *N. (N.) japonicum* Dubois, 1970²⁾
- Tribocytic organ large, about one-third as long as forebody, just posterior to or often overlapping acetabulum. 4
- 4. Acetabulum circular, about one-fifth as broad as tribocytic organ.
- *N. (N.) lineatum* Dubois, 1970³⁾
- Acetabulum transversely elongated, elliptical, about one-fourth as broad as tribocytic organ.
- *N. (N.) brachypteris nipponense*
subsp. nov.

Summary

A new subspecies of a diplostomid trematode is described as *Neodiplostomum (Neodiplostomum) brachypteris nipponense* subsp. nov. The metacercaria of the present new subspecies is commonly found in liver of *Rana nigromaculata* Hallowell from Kumamoto City. The adult has been obtained from newborn chickens (White Leghorn) experimentally infected with the metacercariae. Adults are parasitic in the duodenum.

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複口吸虫科 (新称) の一新亜種 *Neodiplostomum* (*Neodiplostomum*)

brachypteris nipponense の記載とトノサマガエルに

見出されるその被囊幼虫について

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熊本市内江津湖周辺で採取された若干のトノサマガエル成体の肝臓に多数の被囊幼虫が寄生しているのを見出したので、これを初生雛 (白色レグホン) に与えたところ、低率ながら十二指腸より成虫 33 隻を回収することができた。圧平染色標本として調べた結果、インドより記載された *Neodiplostomum* (*Neodiplostomum*) *brachypteris* Chatterji, 1942 に最もよく似るが、後体部はより大きくて前体部とほぼ同長、卵黄巣の前縁は咽頭に達するなどの点で、別亜種とするのが適当と認められたので、標記の通り命名記載した。この属はすでに本邦から 4 種記録されているので、新亜種を含めた 5 種につい

での検索表を作成、附記した。なお、トノサマガエルの肝臓に寄生する被囊幼虫の記録は本邦からはこれが最初である。属の和名としては前体部が円形、扁平なものが多し所から、扇状吸虫 (新称) を与え、本新亜種はその産地に因んでクマモトセンジョウキウチュウと呼びたい。また同属の他の既知 4 種 *N. strigis*, *N. attenuatum micropharyngeum*, *N. japonicum*, *N. lineatum* はそれぞれフクロウセンジョウキウチュウ、ノスリセンジョウキウチュウ、ニホンセンジョウキウチュウ、トビセンジョウキウチュウ (いずれも新称) と呼ぶことを提唱する。