Bull. Natn. Sci. Mus., Ser. A (Zool.), 5 (4), December 22, 1979

Developmental Stages of *Azygia gotoi* (Digenea, Azygiidae)[1)]

By

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(Communicated by Tadashige HABE)

A furcocystocercous cercaria, *Cercaria gotoi* ARIAKE, 1922, develops in a freshwater viviparid snail, *Cipangopaludina japonica* (v. MARTENS), formerly, *Viviparus japonicus* (v. MARTENS). Since ARIAKE (1922) described it in Sendai, Miyagi Prefecture, Japan, no investigators have added its records. In recent years, it was found in Lakes Nakatsuna and Kizaki, Omachi, Nagano Prefecture, Japan.

ITO (1964) stated that the cercaria is presumably a member of the family Azygiidae (Digenea). The present material showed that it has the Y-shaped excretory vesicle dividing between the ovary and the two testes into its two arms which are anteriorly separated. This suggested that it is a larva of *Azygia anguillae* OZAKI, 1924 (Azygiidae), because the Y-shaped excretory vesicle of this species bifurcates between the ovary and the anterior testis in the adult stage (OZAKI, 1924), a stomach parasite of the Japanese eel, *Anguilla japonica* TEMMINCK et SCHLEGEL (OZAKI, 1924; YAMAGUTI, 1934). This suggestion was confirmed by the recovery of adult flukes referable to the species in experimental feedings of the present cercariae to eels. Adult worms of the species were also obtained from naturally infected eels taken in Lake Kizaki. Since *C. gotoi* and *A. anguillae* proved to be identical, a new combination, *Azygia gotoi*, is herewith proposed according to the law of priority. This paper redescribes the parthenita, cercaria, juvenile and adult stages of the trematode, with a discussion on its life history and geographical distribution.

The author would like to express his gratitute to Mr. Takashi FUJITA, Faculty of Science, Shinshu University, Matsumoto, for his information on the cercaria and his help in collecting the snails. Thanks are also due to Mr. Kazuo OGAWA, Faculty of Agriculture, University of Tokyo, Tokyo, for supplying the eels for the experiments; Mr. Shunya KAMEGAI, Meguro Parasitological Museum, Tokyo, for the loan of Dr. Yamaguti's specimens; and Dr. WANG Waei-jioun, Institute of Hydrobiology, Academia Sinica, Wuhan, China, for sending the photocopy of TANG and TANG's paper cited.

1) Supported in part by a Grant-in-Aid for Research from the "Nagano-ken Kagaku Shinkô-kai" in 1976.

Materials and Methods

Host snails, *Ci. japonica*, naturally infected with *C. gotoi*, were collected in Lakes Nakatsuna and Kizaki during 1975 to 1977. The two lakes stand about 4 km distant from each other, both forming parts of the Nogu River, Omachi, Nagano Prefecture, Japan.

Many infected snails taken in Lake Nakatsuna on August 27, 1976, were kept in an experimental aquarium, in which cercariae emerged from them spontaneously and swam around just like mosquito larvae. Laboratory-raised killifish, *Oryzias latipes* (TEMMINCK et SCHLEGEL), when let into the aquarium, ate the swimming cercariae greedily. The cercariae ingested, after losing their tails, persisted as juvenile flukes in the intestinal canal of the killifish for some time as will be described later. The killifish harboring the flukes were fed to four eels, *An. japonica*, which had previously been free from any azygiid infections. Feeding was repeated several times during 24 days from November to December, 1976. The number of parasites given to each eel was not counted. The eels were reared at a water temperature of about 23°C in another aquarium and examined 88 or 106 days after the last feeding.

In order to ascertain whether *A. anguillae* really occurs in Lake Kizaki or not, nine eels caught there were examined; four, 480 to 670 mm in total length, on October 6, 1976; and five, 320 to 706 mm, on September 20, 1977.

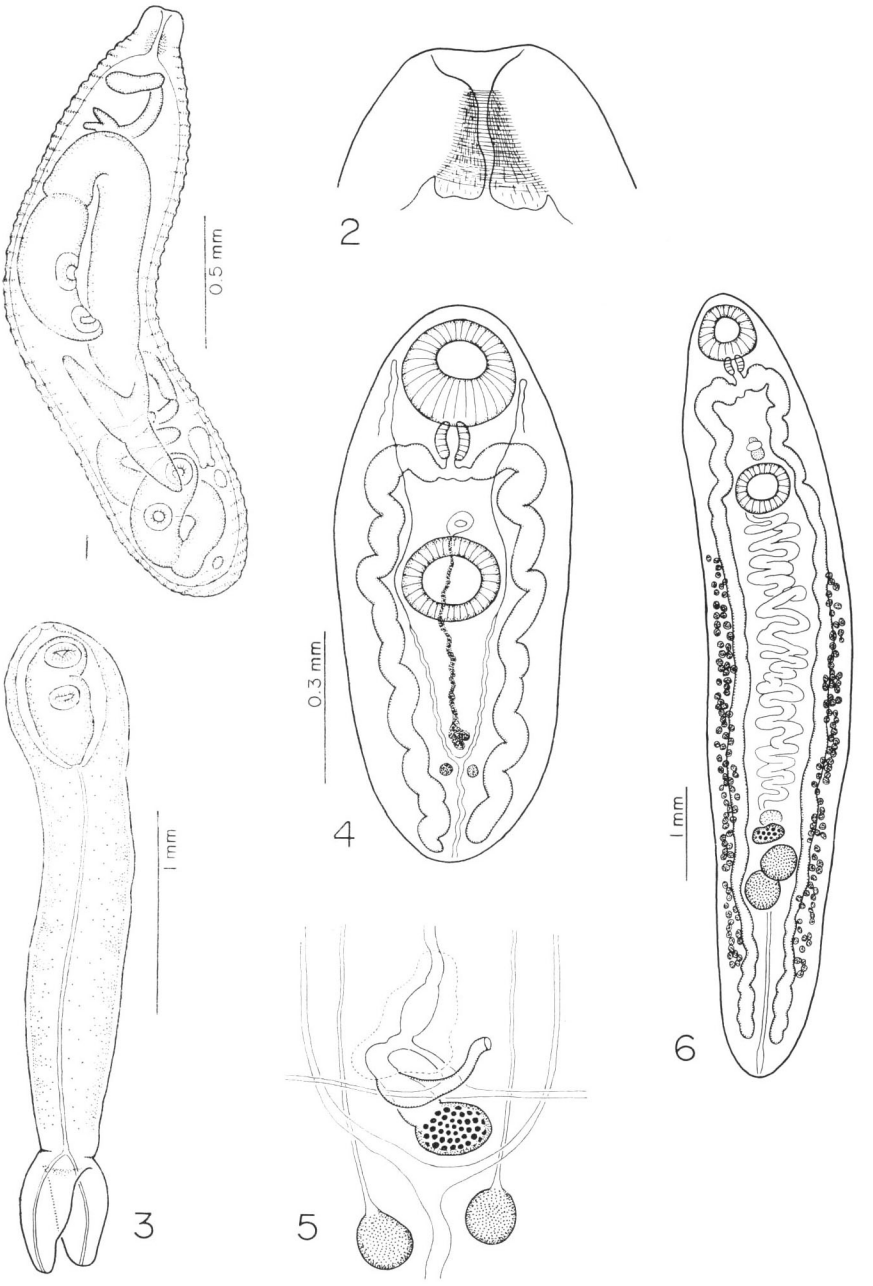
Naturally liberated, mature cercariae were fixed in hot 70% ethanol and stained with alum carmine. Specimens fixed in hot 10% formalin were measured. Parthenitae dissected out of infected snails were treated in the same way. Most of the flukes found in both experimental and wild eels were flattened, fixed in 70% ethanol or Schaudinn's solution, and stained with alum carmine or Heidenhain's iron hematoxylin. Some of adult worms of natural infection were made into serial paraffin sections which were stained with Delafield's hematoxylin and eosin. Sections were observed also of the snails' parasitized organs. Canada balsam was used as a mounting medium. Part of the parasite specimens examined are deposited in the collection of the National Science Museum, Tokyo (NSMT-Pl 1905–1936).

Azygia gotoi (ARIAKE, 1922), comb. nov.

Syns.: *Cercaria gotoi* ARIAKE, 1922; *Azygia anguillae* OZAKI, 1924.

Parthenita. The parthenita was found in 6 or 9.2% of 65 snails (21 to 45 mm in shell width) examined, collected in the east shore of Lake Kizaki on August 29, 1976.

Figs. 1–6. Developmental stages of *Azygia gotoi*, comb. nov. —— 1–2. Mature parthenitae from naturally infected *Ci. japonica* from Lake Nakatsuna; 1, entire worm; 2, anterior part of another specimen, showing the parturient canal and its muscular sphincter. —— 3–5. Spontaneously liberated, mature cercariae; 3, entire worm; 4, cercarial body or distome, slightly flattened, ventral view; 5, ovarian complex and testes, ventral view. The excretory vesicle bifurcates between the ovary and the two testes. —— 6. Adult from experimentally infected *An. japonica* examined 106 days after the last feeding, ventral view.


0.5 mm
1
2
0.3 mm
4
1 mm
6
1 mm
3
5

Data were not recorded on other snail samples examined. Larvae generally occurred in the walls of the intestine and rectum and rarely in their adjacent organs such as the mantle, the stomach and the digestive gland.

Well-developed forms sausage- to spindle-shaped, weakly annulated, white, opaque, 2.10–2.86 mm long by 0.47–0.61 mm wide, usually containing several developing cercariae and only one fully or nearly mature one (Fig. 1). Neither pharynx nor intestine seen. A short parturient canal leading from body or germinal cavity to anteriormost birth pore, proximally having a spheroidal sphincter (about 90 by 60 μm) consisting of an inner longitudinal and an outer circular muscle layer (Figs. 1–2). Excretory system not studied.

Cercaria. The cercaria was of the furcocystocercous type (Fig. 3). The cercarial body or distome was outside its tail while staying within its parthenita (Fig. 1). After leaving its snail host, it became withdrawn in a chamber in the anterior part of its tail (Fig. 3). The following description is based on naturally liberated, mature forms.

Tail large, thick, distally forked, including distome measuring 0.61–0.67 mm long by 0.29–0.38 mm wide in a depression formed in its anterior part (Fig. 3). Tail stem club-shaped, translucent owing to fine whitish granules inside, 2.73–3.26 mm long by 0.43–0.50 mm wide at mid-level. Tail furcae like a broad flattened paddle, 0.47–0.82 mm long, bearing a few pairs of small marginal papillae without sensory hairs.

Distome obovate, aspinose, non-ocellated, light flesh-colored in life, 1.16–1.34 mm long by 0.38–0.49 mm wide when moderately flattened (Figs. 4–5). Oral sucker subglobular, subterminal, opening almost ventrally, 0.20–0.24 mm long by 0.19–0.23 mm wide, having many small papillae around its orifice. Prepharynx absent. Pharynx ellipsoidal, 0.08–0.09 mm long by 0.06–0.08 mm wide. Esophagus very short, inverted T-shaped. Intestinal ceca thick, undulating, extending to posterior end of body, filled with light orange liquid. Ventral sucker spheroidal, 0.18–0.20 mm in diameter, slightly anterior to middle of body, with about 35 papillae arranged into a circle around its aperture; sucker width ratio 1: 0.86–0.93. Ovary globular, submedian, slightly posterior to middle of hindbody, about 0.03 mm in diameter. Ootype-complex in front of ovary, median, surrounded by a thin membrane. Seminal receptacle absent. Laurer's canal present. Uterus somewhat waving, median, running forward. Testes double, oval, opposite or oblique, postovarian, intercecal, about 0.02 mm in diameter. Terminal genitalia differentiated in front of ventral sucker. Vitelline glands not yet observable. Excretory vesicle Y-shaped, dividing between ovary and two testes; its arms passing forward to near anterior end of body, then turning backward, not united there; median caudal tube dividing at furcae, discharging on each furcal tip (Fig. 3). Flame cell formula highly complicated, not yet determined.

Juvenile. The cercariae eaten by the killifish became freed of their tails, which were digested rapidly, to transform into unencysted juvenile flukes in the upper part of the intestine. The flukes migrated gradually down the digestive tract, and eventually they were expelled from the fish in one month at most. During this period they

hardly grew further. A 30-day-old worm recovered, 1.59 mm long by 0.57 mm wide, was slightly larger than the above-described distome.

Adult of experimental infection. From the stomach of one eel examined 88 days after the last feeding were recovered ten still immature worms measuring 2.19–4.00 mm long by 0.61–0.77 mm wide. Of the three eels examined 106 days after the last feeding, two harbored 1 and 12 gravid flukes respectively in the stomach; the rest was negative. The smallest (4.59 mm long by 0.79 mm wide) of these 13 specimens seemed to have just matured sexually, because it contained only a few eggs in the uterus.

Azygiidae, *Azygia.* Body elongate-oblong, aspinous, flesh-colored when alive, 4.59–8.69 mm long by 0.79–1.51 mm wide, with rounded ends (Fig. 6). Oral sucker ovoid, subterminal, opening almost ventrally, 0.41–0.61 mm long by 0.43–0.61 mm wide. Prepharynx absent. Pharynx ellipsoidal, 0.14–0.21 mm long by 0.12–0.20 mm wide. Esophagus thick-walled, very short, inverted T- to Y-shaped. Intestinal ceca thick, sinuous, extending first laterally and then posteriorly to near posterior end of body. Ventral sucker spherical, lying at level of anterior one-quarter to one-third of body, 0.38–0.55 mm long by 0.40–0.58 mm wide; sucker width ratio 1: 0.78–0.95. Ovary globular to transversely reniform, pretesticular, submedian, 0.15–0.37 mm in transverse diameter. A thin-walled capsule measuring 0.13–0.28 mm in diameter surrounding distal part of oviduct, distal part of common vitelline duct, ootype-complex and proximal part of uterus, just anterior to ovary or slightly overlapping it posteriorly (Fig. 8). Laurer's canal long, winding in ovarian region, simple to open through one pore or distally biforked to open through two pores (Fig. 8). Seminal receptacle absent. Uterus preovarian, transversely folded in intercecal field of hind-body, distally forming a metraterm. Testes rounded, submedian, diagonally contiguous, about level of posterior one-quarter of body, almost equal in size, 0.26–0.41 mm in diameter. Cirrus pouch globular to ellipsoidal, thin-walled, median, just in front of ventral sucker, 0.20–0.35 mm long by 0.13–0.20 mm wide, enclosing coiled seminal vesicle and prostatic complex (Fig. 7). Pars prostatica slender, not distended nor giving off any diverticula at its base. Genital papilla small, protruding slightly from bottom of shallow spacious genital atrium, including ejaculatory duct and terminal portion of metraterm (cf. Fig. 10). Hermaphroditic duct not seen. Cirrus absent. Genital pore distensible, usually opening wide in mounted specimens, median, in front of ventral sucker. Vitelline follicles small, extracecal, distributed from a short distance (0.20–0.45 mm long) behind ventral sucker to midway or slightly farther between hind testis and posterior end of body, not reaching cecal tip levels. Excretory vesicle Y-shaped, dividing usually between ovary and anterior testis (Fig. 8) or rarely between ovary and two testes; its arms anteriorly separated; pore posteroterminal. Eggs small, operculated, light yellow, fully embryonated when laid, 63–70 by 33–38 μm in life. Miracidium in egg shell non-ciliated, having spiralling bands, five in an anterior and four in a posterior transverse row, each band bearing many short bristles; flame cells paired.

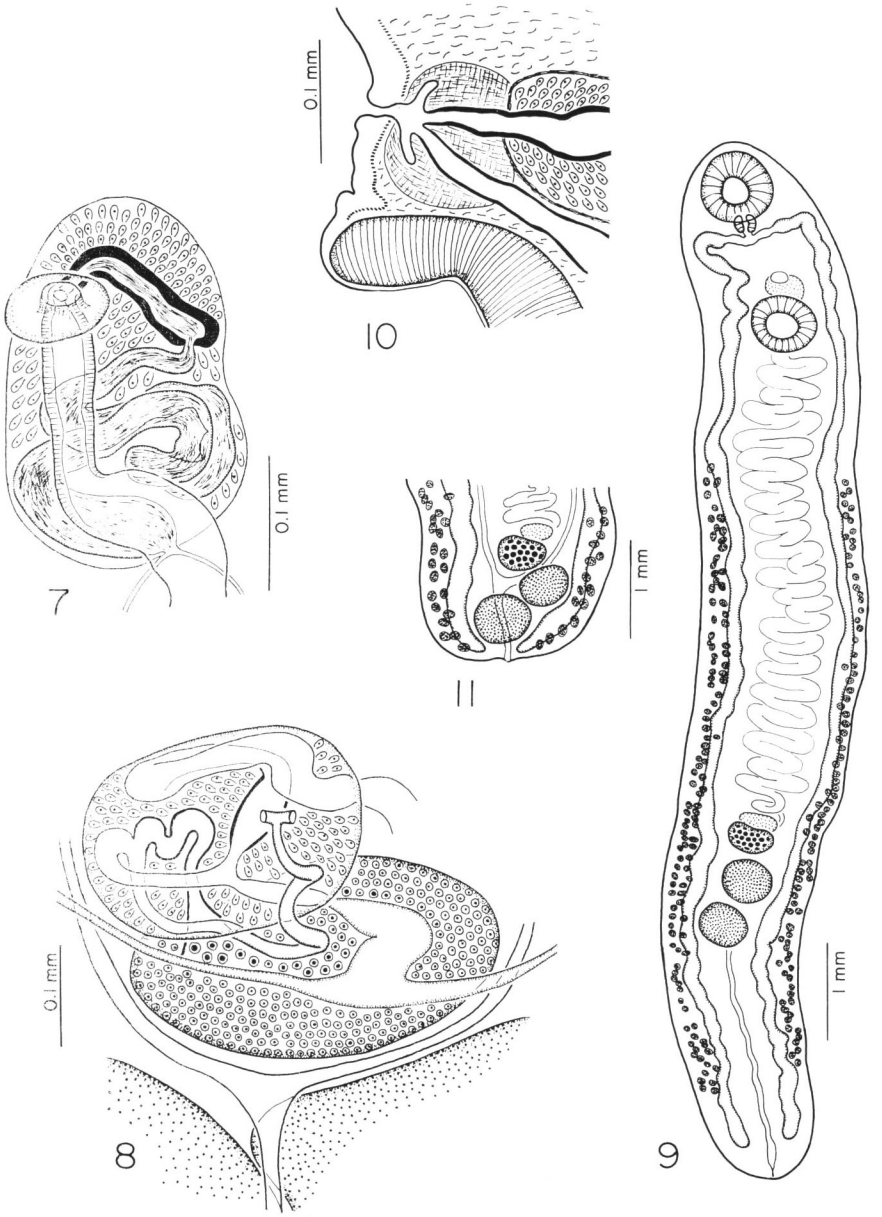
Adult of natural infection. All of the four eels examined in 1976 and three of the five examined in 1977 harbored up to 20 azygiid flukes each in the stomach; the rest were negative.[1] The flukes collected were found to belong to a single species though they were in various degrees of development from smaller immature to larger mature ones. Morphologically, they were substantially consistent with those recovered experimentally (Figs. 9–10). Oviferous ones of them measured 5.06 to 13.06 mm long by 0.92 to 2.96 mm wide. This indicates that the parasite increases twice to three times in size after attainment of sexual maturity. The Laurer's canal was distally bifid in some of the specimens examined. Rarely, the genital papilla was found projecting its point through the wide genital pore, and a very short hermaphroditic duct was seen in it. Five of larger adult worms collected measured: body 12.14–13.06 mm long by 1.84–2.96 mm wide; oral sucker 0.79–0.96 mm long by 0.80–0.96 mm wide; ventral sucker 0.63–0.84 mm long by 0.66–0.89 mm wide, with sucker width ratio being 1: 0.83–0.94; pharynx 0.20–0.28 mm long by 0.20–0.30 mm wide; ovary 0.47–0.69 mm in transverse diameter; testes 0.55–0.82 mm in diameter; and eggs 77–84 by 42–47 μm in life. Among the flukes obtained was found a morphologically abnormal adult one, 8.77 mm long by 1.84 mm wide. Not only the cecal tips and the posterior vitelline glands but also the gonads are close to the posterior end of the body without leaving any free space behind them (Fig. 11).

Discussion

The present cercaria was identified with *C. gotoi* because of its similarites in many respects: the species of the snail intermediate host; the habitat for its parthenita to develop in the host; and the shape and dimensions of the distome itself, the oral and ventral suckers and the tail. ARIAKE (1922) described the excretoty vesicle in his cercaria as bifurcating slightly posterior to the cellular mass, which he regarded as the genital primordium, lying near the middle of the hindbody. From his account and the present observations, it seems likely that in his material the mass corresponded, in fact, to the ovarian complex and that the two testes already differentiated behind it were overlooked. Comparison of the present adult flukes of both experimental and natural infection with *A. anguillae* described by OZAKI (1924) establishes their specific identity. This means that *C. gotoi* and *A. anguillae* belong to a single species. One has priority over the other. The species should, therefore, be called *Azygia gotoi*, comb. nov., in accordance with the law of priority (International Code of Zoological Nomenclature, 1961).

TANG and TANG (1964) assigned trematodes found in the stomach of *Monopterus albus* (ZUIEW) to *A. anguillae* in Fuzhou, Fujian Province, China. They obtained mature rediae and cercariae 138 days after experimentally exposing *Vivipara* [sic]

1) Other helminth parasites found were: nematodes of *Anguinicola globiceps* YAMAGUTI, 1935 (NSMT–As 1443), from the swim bladder; a cestode of the genus *Bothriocephalus* RUDOLPHI, 1808 (NSMT–Pl 1937), from the intestine; and monogenetic trematodes (NSMT–Pl 1938) from the gills.



Figs. 7–11. Developmental stages of *Azygia gotoi*, comb. nov. —— 7–8. Adults from experimentally infected *An. japonica* examined 106 days after the last feeding (continued); 7, terminal genitalia, ventral view; 8, ovarian complex and testes, dorsal view. The excretory vesicle divides between the ovary and the anterior testis. The Laurer's canal is distally biforked. —— 9–11. Adults from naturally infected *An. japonica* from Lake Kizaki; 9, entire worm, ventral view; 10, sagittal section through the genital pore; 11, morphologically abnormal specimen lacking the portion of the body posterior to the hind testis, ventral view.

quadrata BENSON [=*Sinotaia quadrata* (BENSON)] to miracidia from the trematodes. In their cercaria, according to them, the excretory vesicle bifurcates far behind the testes, namely, very near the posterior end of the distome; the median caudal tube divides at a distance about three-fifths of the tail stem; and well-developed papilla-like protuberances are present all over the surface of the tail stem and along the edges of the furcae. These observations conflict with those of the present cercaria. They need confirmation. In addition, TANG and TANG's description is not clear on the position of bifurcation of the excretory vesicle in their adult stage. It is one of the most important characters for the specific determination in the genus.

The present parthenita, at least in its fully-formed stage, resembles a sporocyst rather than a redia in lacking the pharynx and the intestine, both of which are characteristic of rediae of other trematodes. The sphincter of the parturient canal differs from the pharynx in lacking the radial muscle layer. ARIAKE (1922) called the parthenita a sporocyst. TANG and TANG (1964) observed an aggregate of the nuclei indicating the remnant of the pharynx in their experimental larva, which accordingly they termed a redia.

The killifish in the present experiments served as transport hosts to accumulate the juvenile flukes in the intestine and to bring them into the eels. ARIAKE (1922) also experimentally fed his cercariae to goldfish, carps, crucian carps, frogs and tadpoles and found tail-less worms in the intestine of these animals 20 hours to two weeks later. They passed gradually down the intestine and left the animals in time without growing at all. He inferred from these results that the cercaria might enter first the second intermediate host other than the above-mentioned animals. This inference is not accepted here. Cercariae might be able to mature sexually in eels even when ingested directly by them. However, since eels are fundamentally predatory or fish-eating fish, small fish or other animals must be involved as transport hosts in the life history of *A. gotoi* as presumed by TANG and TANG (1964). A field study on this point is making progress in Lake Kizaki.

There was found a morphologically abnormal adult in the present worms of natural infection. The cause of this abnormality is at present unknown. SIMHA and PERSHAD (1964) noted a similar abnormal specimen in their new species, *A. asiatica*, in India. Another Indian species, *A. stunkardi* RAI, 1962, is based on the trematodes that has normally the gonads in the extreme posterior region of the body as do the above-mentioned abnormal specimens and the true seminal receptacle in front of the ovary (RAI, 1964). Considering these features as being subgeneric, YAMAGUTI (1971) erected a new subgenus, *Pseudazygia*, to contain *A.* (*P.*) *stunkardi* (RAI, 1962) as the type-species. On the other hand, KAKAJI (1968) who identified his own material from India with *A. angusticauda* (STAFFORD, 1904) BHALERAO, 1942 [sic], regarded *A. asiatica* and *A. stunkardi* as synonyms of this species. According to him, the species shows great morphological variations. The seminal receptacle is said to be absent or present, and its measurements are given in his paper. The organ is absent in all other members of the genus. Moreover, *A. angusticauda* is originally a parasite of Canadian fresh-

water fishes (STAFFORD, 1904). At any rate, critical restudies of these three Indian species are desirable.

Azygia gotoi lives in the stomach of *An. japonica* in Japan. The cercarial stage or *C. gotoi* has been reported from *Ci. japonica* in Sendai, Miyagi Prefecture (ARIAKE, 1922); and in Nakatsuna and Kizaki, Nagano Prefecture (the present paper). The adult stage or *A. anguillae* has been recorded from the eel: near Tokyo (OZAKI, 1924); in Kasumiga-ura (Tsuchiura), Ibaragi Prefecture (YAMAGUTI, 1934; specimen, Meguro Parasitological Museum, Tokyo, MPM Coll. No. 22355); in Lake Kizaki (the present paper); and in Lake Suwa, Nagano Prefecture (the author's unpublished data). In the last locality, an immature *A. gotoi* was found in the stomach of *Parasilurus asotus* (LINNÉ), and a furcocystocercous cercaria similar to *C. gotoi* in *S. quadrata historica* (GOULD). These unpublished data will be presented in detail elsewhere. The author (unpublished data) obtained a mature fluke (NSMT–Pl 1939), probably *A. gotoi*, from the esophagus of preserved visceral organs[2] for four eels taken in Lake Hira-numa, Aomori Prefecture, in December, 1975, sent from Dr. Kouhei YAMAUCHI, Faculty of Fisheries, Hokkaido University, Hakodate, whom he thanks for it. YAMAGUTI's unidentified adult specimen (MPM Coll. No. 22354, unpublished) found in the eel from Lake Biwa, Shiga Prefecture, on July 1, 1939, is most likely *A. gotoi* (the author's identification).

The trematode also ranges in China, where it parasitizes the stomach of *M. albus*. As cited above, TANG and TANG (1964) studied partly its life history in Fujian Province. The Institute of Hydrobiology, Hubei Province (1973), recorded it in Hubei Province. The adult fluke from this locality has the Y-shaped excretory vesicle divided anterior to the testes. The vitelline glands extend as far backward as the cecal tips. The egg size, 0.30 by 0.14 mm, is apparently given in error.

Summary

Furcocystocercous cercariae, *Cercaria gotoi* ARIAKE, 1922, were found in freshwater viviparid snails, *Cipangopaludina japonica*, taken in Lakes Nakatsuna and Kizaki, Omachi, Nagano Prefecture, Japan. Experiments were carried out to feed them to eels, *Anguilla japonica*, using killifish, *Oryzias latipes*, as transport hosts. Gravid flukes to be identified with *Azygia anguillae* OZAKI, 1924 (Azygiidae), were recovered from the stomach of the eels examined 106 days after the last feeding. Worms of this species were also obtained from naturally infected eels from Lake Kizaki. It is concluded that *C. gotoi* is a larva of *A. anguillae*. A new combination, *Azygia gotoi*, is proposed for *A. anguillae* according to the law of priority. The parthenita, cercaria, juvenile and adult stages of the trematode are redescribed. The life history and geographical distribution are briefly discussed.

2) *Anguinicola globiceps* (NSMT–As 1445) was found in the swim bladder.

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Addenda

1) Recently, the parasite cellection of the late Professor Yoshimasa OZAKI has been transferred from his bereaved family to the Meguro Parasitological Museum, Tokyo. The author was unable to find the type-specimen of *A. anguillae* in it.

2) It was found that in the east shore of Lake Kizaki, *Rhinogobius brunneus* (TEMMINCK et SCHLEGEL) harbored juvenile *A. gotoi* in the digestive tract. This fish must be one of natural transport hosts for the parasite in the lake.

(October 20, 1979)