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Digeneans Parasitic in Freshwater Fishes (Osteichthyes) of Japan. III. Azygiidae and Bucephalidae

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Abstract Digeneans (Trematoda) parasitic in freshwater fishes of Japan are reviewed: *Azygia gotoi* (Ariake, 1922), *Azygia perryii* Fujita, 1918 and *Azygia rhinogobii* Shimazu, 2007 (Azygioidea, Azygiidae) and *Parabucephalopsis parasiluri* Wang, 1985 and *Prosorhynchoides ozakii* (Nagaty, 1937) (Bucephaloidea, Bucephalidae). Each species is described and figured with a summarized life cycle where known. Keys to the Japanese species of the two families are presented. It is thought that the two bucephalid species were introduced artificially and accidentally, together with their common first intermediate host *Limnoperna fortunei* (Dunker, 1857) (Bivalvia, Mytilidae), from China into the Uji River (Yodo River system) in Kyoto Prefecture in the late 1990s. The generic name *Parabucephalopsis* Tang and Tang, 1963 and the species name *Parabucephalopsis prosthorchis* Tang and Tang, 1963 are unavailable because they were unpublished.
Key words: Digeneans, *Azygia*, *Parabucephalopsis*, *Prosorhynchoides*, freshwater fishes, Japan, review.

Introduction

This is the third paper of a series that reviews adult digeneans (Trematoda) parasitic in freshwater fishes (Osteichthyes) of Japan. This contribution deals with the family Azygiidae Lühe, 1909 of the superfamily Azygioidea Lühe, 1909 *sensu* Gibson (2002) and the family Bucephalidae Poche, 1907 of the superfamily Bucephaloidea Poche, 1907 *sensu* Overstreet and Curran (2002). The Introduction and Materials and Methods for the review were given in the first paper (Shimazu, 2013).

Abbreviations used in the figures. c, compartment; ci, cirrus; cp, cirrus pouch; csd, common sperm duct; cvd, common vitelline duct; Dm, Drüsenmagen; e, esophagus; egg, egg; ep, excretory pore; ev, excretory vesicle; ga, genital atrium; gp, genital pore; hd, hermaphroditic duct; ic, intestinal cecum; Lc, Laurer's canal; m, metraterm; Mg, Mehlis' gland; mo, mouth; o, ovary; od, oviduct; op, ootype pouch; os, oral sucker; ot, ootype; p, pharynx; pc, prostatic cells; pp, pars prostatica; ps, prostatic sac; r, rhynchus; sd, sperm duct; so, sinus organ; sp, sphincter; sv, seminal vesicle; t, testis; tnc, transverse nerve commissure; u, uterus; vd, vitelline duct; vf, vitelline follicles; vs, ventral sucker.

Superfamily Azygioidea Lühe, 1909

Family Azygiidae Lühe, 1909

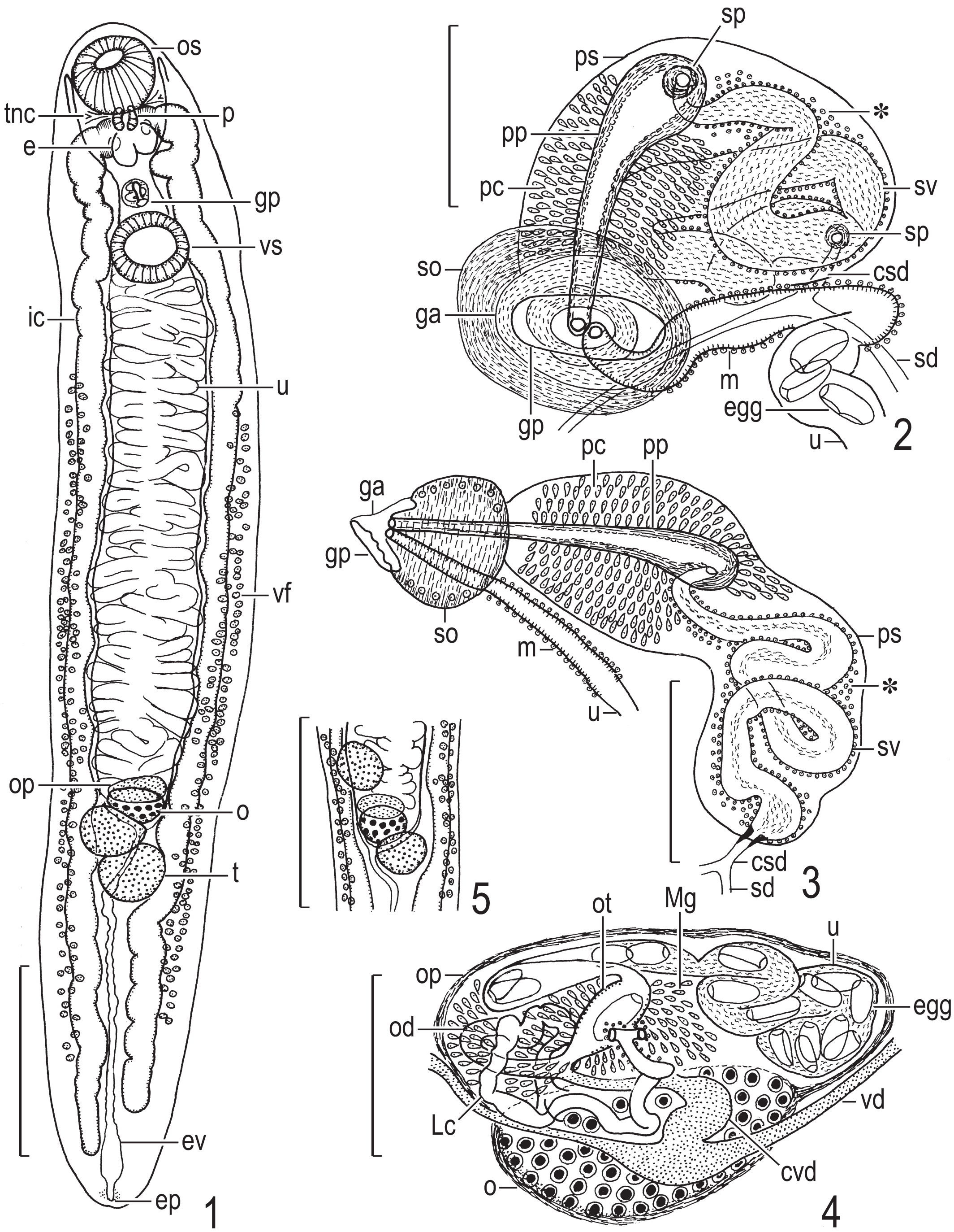
Genus *Azygia* Looss, 1899

Azygia gotoi (Ariake, 1922)

(Figs. 1–10)

Cercaria gotoi Ariake, 1922: 236–238, figs. 2–5, tables 1–2; Shimazu, 1979: 228, figs. 3–5.
Azygia anguillae Ozaki, 1924: 426–430, plate figs. 1–3, text fig. 2; Yamaguti, 1934: 360; Shimazu, 2007: 10–11, figs. 14–15.
Azygia gotoi: Shimazu, 1979: 229–230, figs. 6–11; Shimazu *et al.*, 2011: 3, 5–7, figs. 2–5.

Host in Japan. Anguilla japonica Temminck



Figs. 1–5. *Azygia gotoi*, adults found in stomach of *Anguilla japonica.* — 1, NSMT-Pl 1926, entire body, uterine eggs omitted, ventral view; 2, NSMT-Pl 1929, terminal genitalia, small (gland (?)) cells (∗) surrounding seminal vesicle, ventral view; 3, NSMT-Pl 5358, strongly flattened, terminal genitalia, small (gland (?)) cells (∗) surrounding seminal vesicle, ventrolateral view; 4, NSMT-Pl 1924, ovarian complex, dorsal view; 5, MPM Coll. No. 30210, showing anterior testis located preovarian abnormally, ventral view. Scale bars: 2 mm in Figs. 1 and 5; 0.2 mm in Figs. 2–4.

and Schlegel, 1846 (Anguillidae) (type host) (Ozaki, 1924; Yamaguti, 1934; Shimazu, 1979, 2007; Iwashita *et al.*, 2003; Shimazu *et al.*, 2011).

Sites of infection. Primarily stomach, and esophagus (presumably post-mortem migration from stomach).

Geographical distribution. (1) Aomori Prefecture: Hiranuma, Rokkasho Village (Shimazu, 1979; this paper). (2) Ibaraki Prefecture: Lake Kasumigaura at Tsuchiura City (Yamaguti, 1934; Shimazu, 1979). (3) Chiba Prefecture: Tone River at Tonosho Town (Iwashita *et al.*, 2003). (4) Tokyo: in the vicinity of Tokyo (type locality) (Ozaki, 1924). (5) Nagano Prefecture: Lakes Nakatsuna and Kizaki in Oomachi City and Lake Suwa at Suwa City (Shimazu, 1979, 2007) and probably Lake Nojiri in Shinano Town (Shimazu, 2007). (6) Shiga Prefecture: Lake Biwa basin (Lake Biwa and Uso River) (Shimazu, 1979; Shimazu *et al.*, 2011).

In China (*e.g.* Tang and Tang, 1964a; Institute of Hydrobiology, Hubei Province, China (chief ed.), 1973; Wang, 1991).

Material examined. (1) 11 specimens (Ozaki's Collection, MPM Coll. No. 30210, most likely referring to syntypes) of *Azygia anguillae*, 1 immature, 6 adult, whole-mounted, 4 adult, serially sectioned, ex stomach of *Anguilla japonica*, other data not given (Shimazu, 1995b). (2) 1 (Yamaguti's Collection, MPM Coll. No. 22355) of *A. gotoi*, adult, whole-mounted, ex stomach of *An. japonica*, Lake Kasumigaura, 16 April 1929 (Yamaguti, 1934; Shimazu, 1979). (3) 1 (NSMT-Pl 1939) of *A. gotoi*, adult, whole-mounted, ex esophagus (presumably post-mortem migration from stomach) of *An. japonica*, Hiranuma [not Lake Hira-numa], Rokkasho Village, 14 December 1975 (Shimazu, 1979). (4) 44 (NSMT-Pl 1922–1924) of *A. gotoi*, immature, adult, flattened, whole-mounted, ex stomach of *An. japonica* (experimental infection, 15–20 and 106 days after the last feeding), 28 October 1975, 22 March 1977 (Shimazu, 1979, 2007). (5) 70 (NSMT-Pl 1925–1936, 5357–5359) of *A. gotoi*, adult, whole-mounted, serially sectioned, ex stomach of *An. japonica*, Lake Kizaki, 6 October 1976, 20 September 1977, 8 September 1981 (Shimazu, 1979, 2007). (6) 48 (NSMT-Pl 5360–5361) of *A. gotoi*, adult, whole-mounted, serially sectioned, ex stomach of *An. japonica*, Lake Suwa, 10 September 1976, 9 July 1994 (Shimazu, 1979, 2007). (7) 1 (Yamaguti's Collection, MPM Coll. No. 22354) of *A. gotoi*, adult, whole-mounted, ex stomach of *An. japonica*, Lake Biwa, 1 July 1939 (Shimazu, 1979; Shimazu *et al.*, 2011). (8) 68 (NSMT-Pl 5739, Urabe's personal collection) of *A. gotoi*, immature, adult, whole-mounted, ex stomach of *An. japonica*, Uso River, 16 May 2006 (Shimazu *et al.*, 2011).

Description. 1) Based on large fully mature adult specimens (NSMT-Pl 1925–1936, 5357–5361), 10 measured (Figs. 1–4). Body elongate, with round ends, anoculate, fairly large, 11.03–13.17 by 1.98–2.81; forebody 1.90–2.62 long, occupying 16–23% of body length. Tegument smooth. Oral sucker subglobular, anteroventral, 0.70–0.98 by 0.79–0.98. Prepharynx absent. Pharynx elliptical, 0.16–0.32 by 0.17–0.28. Esophagus inverted Y- to T-shaped, short, 0.16–0.47 long, surrounded by small gland cells, with sphincter at each end of arms. Drüsenmagen globular, small, between esophageal arm and intestinal cecum. Intestinal ceca ending blindly at short distance from posterior extremity of body. Ventral sucker subglobular, 0.63–0.82 by 0.69–0.89, slightly anterior to junction of anterior and second fifths of body; sucker width ratio 1: 0.9–1.0. Distance from anterior extremity of body to ovary 6.27–10.87, occupying 55–82% of body length. Testes two, at about posterior third of hindbody, almost globular, slightly diagonal, contiguous; anterior (usually right or sometimes left) testis 0.47–0.79 by 0.43–0.68, posterior testis 0.47–0.79 by 0.47–0.74. Sperm ducts long; common sperm duct short. Prostatic sac (cirrus pouch in Shimazu, 1979), usually globular, thin-walled, small, 0.32–0.40 by 0.32–0.43, median, immediately anterior to ventral sucker, including seminal vesicle and prostatic complex. Seminal vesicle long, fairly thick-walled, thin, convoluted, surrounded by small (gland (?)) cells (Figs. 2–3, ＊), leading to pars prostatica at lateral side near its proximal end, with well-developed sphincter around opening into pars prostatica. Pars prostatica club-shaped, thick-walled, with no compartments at proximal base; prostatic cells well developed. Ejaculatory duct running in sinus organ, opening side by side with metraterm into small common depression (rather than pore) on tip of cone-like small protrusion of sinus organ in center of bottom of genital atrium; hermaphroditic duct absent. Sinus organ (genital papilla in Shimazu, 1979) elliptical, small. Genital atrium large. Genital pore large, slightly anterior to ventral sucker. Ovary transversely reniform, 0.51–0.74 by 0.32–0.49, immediately pretesticular. Seminal receptacle absent. Ootype pouch (terminology of Shimazu *et al.*, 2011; capsule in Shimazu, 1979) fairly thick-walled, 0.41–0.55 by 0.25–0.32, anterodorsal to ovary, enveloping distal part of oviduct, distal part of common vitelline duct, proximal part of Laurer's canal, ovovitelline duct, ootype complex and proximal coils of uterus. Laurer's canal long, opening dorsally through either one or two pores, surrounded by small gland cells. Ootype vesicular, large; Mehlis' gland well developed. Uterus much folded transversely from ootype pouch to prostatic sac in intercecal field of body; metraterm well developed, almost as long as prostatic sac (Fig. 2), entering sinus organ, surrounded by small gland cells; uterine seminal receptacle present in proximal coils of uterus. Eggs numerous, elongate-oval to oboval, operculate, yellow, fully embryonated, 71–87 by 36–44 μm. Vitellaria follicular; follicles lateral to and slightly overlapping intestinal cecum on either side of hindbody. Distance from midlevel of ventral sucker to anterior commencement of vitelline follicles (at significant distance posterior to ventral sucker) 0.95–2.70, occupying 10–25% of hindbody length; distance from midlevel of ventral sucker to posterior end of vitelline follicles (usually at significant distance anterior to or rarely at cecal ends) 7.30–10.31, occupying 81–98% of hindbody length. Excretory vesicle Y-shaped, bifurcating between anterior testis and ovary; arms extending forward to midlevel of oral sucker, turning backward beside oral sucker, separate there (Fig. 1; see also Fig. 9); excretory pore posterodorsal.

2) Ozaki's adult specimens (MPM Coll. No. 30210), fully gravid, very similar in morphology and measurements to the above description; anterior testis abnormally preovarian (Fig. 5) in one of them.

Remarks. Ozaki (1924) described this species on the basis of both whole-mounted and serially sectioned adult specimens found in the stomach of *Anguilla japonica* collected near Tokyo. He did not designate a holotype. Nobody has previously designated a neotype for the species.

Ozaki's 11 specimens (MPM Coll. No. 30210) of *A. anguillae* have been deposited in the Meguro Parasitological Museum, Tokyo (Shimazu, 1995b). On one of the three labels left on these slides, the letters "Y, OZAKI." and "No:" were clearly printed black, but handwritten letters had faded away leaving faint ink stains. The faded letters could be deciphered as "(6)" and "stomach of A. japonica" by manipulating a digital image taken with a KEYENCE VHX-2000 Digital microscope (×100) (KEYENCE Corporation, Osaka) using the photo manipulation software Adobe Photoshop CC™ (Adobe Systems Incorporated, Tokyo) in the present study. Ozaki's specimens are most likely syntypes of *A. anguillae* in accordance with Article 73.2 of the International Code of Zoological Nomenclature (International Commission on Zoological Nomenclature (ICZN), 1999). There is no conclusive evidence to support this conclusion at present.

Ariake (1922) described a large furcocystocercous cercaria as *Cercaria gotoi* Ariake, 1922 from the snail *Cipangopaludina japonica* (von Martens, 1860) (Gastropoda, Viviparidae) (Japanese name: Oo-tanishi) in a pond in Sendai City, Miyagi Prefecture. Shimazu (1979) obtained cercariae of *C. gotoi* from *Ci. japonica* collected in Lakes Nakatsuna and Kizaki in Oomachi City. He experimentally demonstrated that *C. gotoi* was the cercaria of *A. anguillae* and proposed a new combination, *Azygia gotoi* (Ariake, 1922), for *A. anguillae.* Shimazu (2007) erroneously used the species name *A. anguillae* again, but Shimazu *et al.* (2011) recognized that the valid species name of the taxon was *A. gotoi* in accordance with Articles 23.3.2.2, 23.7.1 and 67.14, Example of the Code (ICZN, 1999).

Tang and Tang (1964a) claimed that they experimentally obtained cercariae of *Azygia anguillae* from *Sinotaia quadrata* (Benson) (syn. *Viviparus quadrata* Benson) in Fuzhou, Fujian Province, China. The cercariae obtained were slightly different in morphology from *Cercaria gotoi* (Shimazu, 1979).

The seminal vesicle was surrounded by small (gland (?)) cells (Figs. 2–3, ∗) in *A. gotoi* and the next two species of *Azygia.* Ozaki (1924, fig. 2) and Shimazu *et al.* (2011, fig. 4) suggested the presence of such small cells in *A. gotoi.* The small cells were misinterpreted by Shimazu (1979, fig. 7) as part of the prostatic cells, but they are obviously independent of the prostatic cells (Fig. 3). The histological structure of the wall of the seminal vesicle needs further investigating.

Laurer's canal opens dorsally through either one pore or two pores in *A. gotoi* and *Azygia rhinogobii* Shimazu, 2007 (this paper). Looss (1894, fig. 69) described two pores of Laurer's canal in *Distomum tereticolle* (Rudolphi, 1802), now *Azygia lucii* (Müller, 1776), and considered the presence of the two pores to be abnormal.

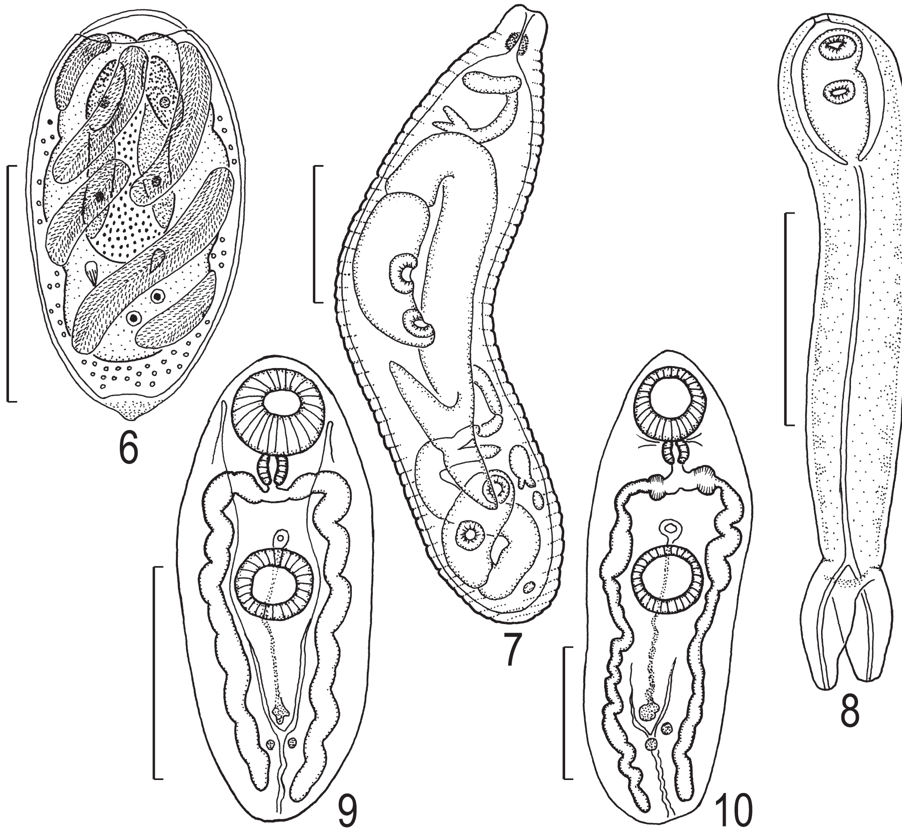
Life cycle. Eggs became fully embryonated in the uterus of adults. They were 77–84 by 42–47 μm in life. Miracidia had five and four clockwise semispiral plates (probably epidermal cells) bearing minute bristles in the anterior and posterior transverse rows, respectively, and a pair of flame cells (Fig. 6). They did not hatch in the water (my unpublished data).

Shimazu (1979) studied the life cycle of *Azygia gotoi* in the field (Lakes Nakatsuna, Kizaki and Suwa) and laboratory (see also Shimazu, 2007). A natural intermediate host was *Cipangopaludina japonica.* Large furcocystocercous cercariae (*Cercaria gotoi*) (Figs. 8–9) were produced in sausage- to spindle-shaped [daughter (?)] rediae (parthenitae in Shimazu, 1979; NSMT-Pl 1905–1919, 5371–5373; Fig. 7) in the snail intermediate host. Neither a sporocyst nor a mother redia has yet been found. Presumably, when mature cercariae emerge into the mantle cavity to leave the snail host, the body proper (distome in Shimazu, 1979) becomes withdrawn in an inverted chamber in the base of the tail (Fig. 8). The tail containing the body proper swam around like a mosquito larva in the water as was showed by Ariake (1922).

Oryzias latipes (Temminck and Schlegel, 1846) (*sensu lato*) (Adrianichthyidae) was used as an experimental transport host. Free-swimming cercariae were quickly eaten by the fish. In the fish they underwent metamorphosis by losing the tail to become unencysted small immature worms or juveniles (NSMT-Pl 1920–1921, 5370; Fig. 9) in the upper part of the intestine of the fish. When cercariae were fed to *An. japonica* with a stomach tube, juveniles were recovered from the stomach 1–5 and 15–20 days after feeding (unpublished, 1975, NSMT-Pl 5368–5369). Ariake (1922) also obtained juveniles from the intestine of carp, crucian carp, frogs and tadpoles, to which he had fed cercariae 20 hours to 2 weeks before. Small juveniles were found in the stomach of *Rhinogobius brunneus* (Temminck and Schlegel, 1845) (Gobiidae), correctly *Rhinogobius* sp. OR (most likely referring to *Rhinogobius kurodai* (Tanaka, 1908)), from Lake Kizaki (Shimazu, 1979; NSMT-Pl 5362–5363; Fig. 10).

A natural and experimental final host was *Anguilla japonica.* The juveniles from *Oryzias latipes* were fed to the fish, and gravid adults were recovered from the stomach of the fish 106 days after feeding. In China, final hosts are *An. japonica* and *Monopterus albus* (Zuiew, 1793) (Synbranchidae) (*e.g.* Tang and Tang, 1964a; Institute of Hydrobiology, Hubei province, China (chief editor), 1973; Wang, 1991).

Some terms concerning the germinal sacs, developmental stages, life cycle and hosts in the



Figs. 6–10. *Azygia gotoi* (continued), life cycle. — 6, fully embryonated egg, containing fully developed miracidium; 7, [daughter (?)] sporocyst, containing developing cercariae, natural infection in *Cipangopaludina japonica*; 8, cercaria, spontaneously shed from *Ci. japonica*; 9, cercarial body proper, flattened, entire body, ventral view; 10, possible juvenile (NSMT-Pl 5363), natural infection in stomach of *Rhinogobius* sp. (most likely referring to *R. kurodai*), entire body, ventral view. Figs. 7–9 redrawn from Shimazu (1979), slightly altered. Scale bars: 1 mm in Fig. 8; 0.5 mm in Figs. 7, 9 and 10; 0.05 mm in Fig. 6.

Azygiidae are mentioned here (see also Stunkard, 1956; Yamaguti, 1975). The snail host is termed an intermediate host, not a first intermediate host by Shimazu *et al.* (2011). The germinal sac that produces cercariae is termed a redia in the daughter generation (?) (a parthenita in Shimazu, 1979), not a sporocyst by Shimazu *et al.* (2011). In the redia in *A. gotoi*, neither the pharynx nor the intestine is seen; a short parturient canal leads from the body cavity to the birth pore at the anterior tip of the body; the canal has a spheroidal sphincter consisting of an inner longitudinal [not radial] and an outer circular muscle layer; and the sphincter does not appear to be a vestigial pharynx (Shimazu, 1979). *Oryzias latipes* is termed a transport or paratenic host (a transport host in Shimazu, 1979), not a second intermediate host by Shimazu *et al.* (2011). Small immature worms after metamorphosis by losing the tail in *O. latipes* are termed juveniles, not metacercariae in Shimazu *et al.* (2011).

Cercariae have fairly well developed gonads (Shimazu, 1979, figs. 4–5; Fig. 9) and are evidently infective to *An. japonica* as mentioned above, though it is unknown at present whether juveniles attain sexual maturity in *An. japonica.* Since *An. japonica* is predatory, it seems improbable that *An. japonica* becomes naturally infected with *A. gotoi* by ingesting cercariae swimming in the water (Shimazu, 1979; Shimazu *et al.*, 2011).

It is thus considered to be essential that small fish are involved as transport or paratenic hosts in the life cycle of *A. gotoi* (Tang and Tang, 1964a; Shimazu 1979; Shimazu *et al.*, 2011). In this case *A. gotoi* has a three-host life cycle.

Similar juveniles were also found in the stomach of *Silurus asotus* Linnaeus, 1758 (Siluridae) from Lake Kizaki (NSMT-Pl 5364–5366) and Lake Suwa (unpublished, 1975, NSMT-Pl 4014), *Micropterus salmoides* (Lacepède, 1802) (Centrarchidae) from Lake Kizaki (unpublished, 1989, NSMT-Pl 4025) and *Micropterus dolomieu* (Lacepède, 1802) from Lake Nojiri (NSMT-Pl 5367) (Shimazu, 2007). It is considered that these fishes are paratenic hosts, which acquire juveniles by ingesting small transport hosts but perhaps are not eaten by *An. japonica.*

Azygia perryii Fujita, 1918

(Figs. 11–14)

Azygia perryii Fujita, 1918: 270–273, pl. 6, figs. 1–6, 1 text table (p. 270); Manter, 1926: 202–203, 1 text table (p. 202); Shimazu, 1981: 15, figs. 1–4.

Azygia lucii (not of (Müller, 1776) Lühe, 1909): Seki, 1975a: 7, 9–10, fig. 1.

Hosts in Japan. Parahucho perryi (Brevoort, 1956) (Salmonidae) (type host) and *Salvelinus leucomaenis leucomaenis* (Pallas, 1814) (Salmonidae) (Fujita, 1918; Seki, 1975a, b; Shimazu, 1981; this paper).

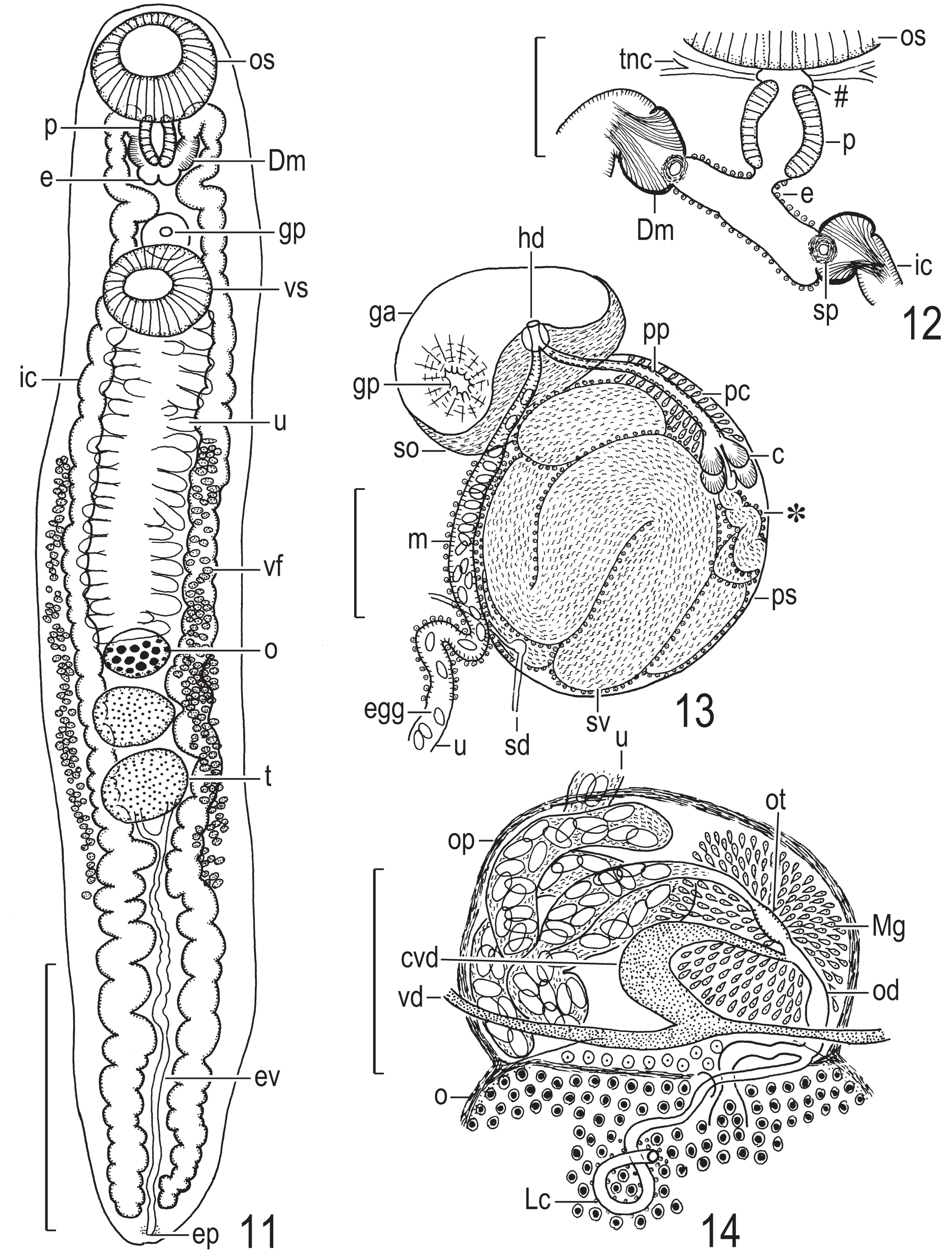
Sites of infection. Primarily stomach; and esophagus, buccal cavity, branchial cavity, skin and fins (post-mortem migration from stomach).

Geographical distribution. Eastern Hokkaido: (1) Lake Kussharo (type locality) in Teshikaga Town; (2) Lake Panketo in Akan-cho, Kushiro City; (3) Lake Shikaribetsu at Shikaoi Town; (4) Ashibetsu, Settsuri and Hororo in Tsurui Village; (5) Kottaro, Numaoro and Kushiro rivers in Shibecha Town; and (6) Bekanbeushi and Toraibetsu rivers in Akkeshi Town (Fujita, 1918; Seki, 1975a, b; Shimazu, 1981, 1994; this paper).

In Russia: Sakhalin Island (*e.g.* Bykhovskaya-Pavlovskaya, 1962).

Material examined. (1) 5 specimens (Fujita's Collection, HUNHM 48666, syntypes) of *Azygia perryii*, adult, unflattened, whole-mounted, on *Parahucho perryi* (Brevoort, 1856) (syn. *Hucho perryi* (Brevoort, 1856)), Lake Kussharo, September 1915 (Fujita, 1918). (2) 14 (NSMT-Pl 2177–2179) of *A. perryii*, adult, flattened, whole-mounted, ex stomach and buccal cavity and on gills of *P. perryi* [frozen on 24 April 1977], Numaoro [not Numahoro] River (Shimazu, 1981). (3) 81 (NSMT-Pl 2180–2182) of *A. perryii*, 18 immature, 56 adult, flattened or unflattened, whole-mounted, 7 adult, serially sectioned, ex stomach and buccal cavity of *P. perryi* (fresh), Ashibetsu River, 10 May 1977 (Shimazu, 1981). (4) 10 (NSMT-Pl 2183–2185) of *A. perryii*, adult, flattened, whole-mounted, ex stomach and buccal cavity of *P. perryi* [frozen on 30 May 1976], Toraibetsu River (Shimazu, 1981). (5) 2 (NSMT-Pl 2186) of *A. perryii*, adult, flattened, whole-mounted, ex buccal cavity of *Salvelinus leucomaenis leucomaenis* (syn. *S. leucomaenis*), Ashibetsu River, 10 May 1977 (Shimazu, 1981).

Description. 1) Based on Fujita's 5 adult specimens (Fig. 11). Similar to *Azygia gotoi* (this paper) in general morphology. Body medium, 5.20–9.36 by 1.12–1.47; forebody 1.60–2.16 long, occupying 23–30% of body length. Oral sucker 0.60–0.85 by 0.68–0.92. Pharynx 0.27–0.33 by 0.21–0.28; ratio of width to length 1: 1.0–1.3; ratio of pharynx length to oral sucker length 1: 2.1–2.8. Intestinal ceca proximally extending forward laterally to oral sucker, turning backward there, running zigzag, ending at short distance from posterior extremity of body. Ventral sucker 0.53–0.72 by 0.59–0.81; sucker width ratio 1: 0.8–0.9. Distance from anterior extremity of body to ovary 2.80–4.80, occupying 51–55% of body length. Testes almost tandem, contiguous or a little separate; anterior testis 0.47–0.66 by 0.26–0.47, posterior testis 0.41–0.66 by 0.34–0.56. Seminal vesicle long, thick, convoluted, leading to proximal end of pars prostatica, surrounded by small cells (Fig. 13, *). Pars prostatica with 4–6 compartments. Prostatic sac (cirrus pouch in Shimazu, 1981) round, anterodorsal to ventral sucker. Sinus organ (geni-



Figs. 11–14. *Azygia perryii*, adults found in stomach of *Hucho perryi.* — 11, syntype (HUNHM 48666), unflattened, entire body, uterine eggs omitted, ventral view; 12, NSMT-Pl 2180, strongly flattened, prepharynx-like anteriormost part (#) of digestive tract, ventral view; 13, NSMT-Pl 2181, flattened, terminal genitalia, short everted hermaphroditic duct (hd) seen, small (gland (?)) cells (∗) surrounding seminal vesicle, ventral view; 14, NSMT-Pl 2182, flattened, ovarian complex, dorsal view. Scale bars: 2 mm in Fig. 11; 0.5 mm in Fig. 12; 0.3 mm in Figs. 13–14.

tal cone in Shimazu, 1981) conical, low, wide at base, immediately anterior to prostatic sac. Genital atrium spacious, sometimes enlarged by presence of eggs, 0.22–0.34 by 0.20–0.37. Genital pore usually on ventral wall of genital atrium. Ovary 0.29–0.50 by 0.19–0.37, about equatorial (slightly pre-equatorial in hindbody), a little pretesticular. Laurer's canal opening dorsally through one pore. Ootype pouch (capsule in Shimazu, 1981) 0.19–0.44 by 0.12–0.44. Uterus slightly overlapping intestinal ceca; metraterm well developed, almost as long as prostatic sac; uterine seminal receptacle present. Eggs 45–51 by 29–35 μm. Distance from midlevel of ventral sucker to anterior commencement of vitelline follicles (at significant distance from ventral sucker) 0.16–1.12, occupying 3–15% of hindbody length; distance from midlevel of ventral sucker to posterior end of vitelline follicles (slightly posterior to posterior testis) 2.24–5.04, occupying 62–74% of hindbody length. Excretory vesicle bifurcating posterior to posterior testis.

2) Based on fully mature adult specimens (NSMT-Pl 2180, 2182); 5 unflattened ones of them measured (Figs. 12–14). Body 8.01–13.57 by 1.62–2.14; forebody 1.90–2.70 long, occupying 17–24% of body length. Oral sucker 0.87–1.24 by 1.05–1.44. Anteriormost part of digestive tract rarely seen like prepharynx in strongly flattened specimens (Fig. 12, #). Pharynx 0.40–0.51 by 0.36–0.40; ratio of width to length 1: 1.1–1.3; ratio of pharynx length to oral sucker length 1: 2.0–2.5. Intestinal ceca not proximally extending forward. Ventral sucker 0.76–0.90 by 0.90–1.27; sucker width ratio 1: 0.8–0.9. Distance from anterior end of body to ovary 4.20–6.66, occupying 49–52% of body length. Testes tandem or slightly oblique; anterior testis 0.52–0.87 by 0.27–0.71, posterior one 0.57–0.81 by 0.36–0.71. Ejaculatory duct possibly joining to metraterm to form very short eversible hermaphroditic duct opening on tip of sinus organ (Fig. 13). Prostatic sac obscured by eggs in genital atrium. Genital atrium 0.35–0.62 by 0.43–0.76. Ovary 0.44–0.52 by 0.25–0.47, about equatorial, a little pretesticular. Ootype pouch 0.47–0.71 by 0.24–0.40. Eggs 48–55 by 27–32 μm. Distance from midlevel of ventral sucker to anterior commencement of vitelline follicles 0.47–0.63, occupying 4–10% of hindbody length; distance from midlevel of ventral sucker to posterior end of vitelline follicles 3.81–7.30, occupying 55–67% of hindbody length. Excretory vesicle bifurcating posterior to posterior testis.

In specimens flattened when alive, body larger, measuring up to 28 by 2. In specimens from frozen fish, body much larger, measuring up to 40 by 3.3; and uterine eggs 50–57 by 28–32 μm in formalin and 42–51 by 25–28 μm in balsam (Shimazu, 1981).

Remarks. Fujita (1918) described this species based on formalin-preserved specimens, which had been found in the buccal cavity, on the gills, on either side of the opercula and on the pectoral fins of *Parahucho perryi* (syn. *Hucho perryi*) caught in Lake Kussharo. He did not designate a holotype. Nobody has previously designated a neotype for the species.

Fujita's Collection has been deposited in Botanic Garden, Field Science Center for Northern Biosphere, Hokkaido University (formerly The Hokkaido University Natural History Museum), Sapporo. The collection included 16 unflattened specimens of *Azygia perryii* (HUNHM 48666) in a vial labeled "IV-1 *Azygia perryi* [*sic*] Fujita [Ito] [Kushiro] [Nijibetsu])" and "[Parasites attached onto Oberabe (Ito) from Lake Kussharo, September 1915, Nijibetsu Branch]." These specimens are regarded as syntypes of *A. perryii* (Article 73.2 of the Code) (ICZN, 1999). Five of them were stained with Semichon's carmine and mounted in Canada balsam for the present study.

Manter (1926) briefly described two "type specimens" [syntypes] of *A. perryii* and gave an ovoid pharynx as 0.317 by 0.28 mm (ratio of width to length 1: 1.1, my calculation) in one 8.6 mm long and an elongate pharynx as 0.24 by 0.11 mm (ratio 1: 2.2, my calculation) in the other 5.6 mm long. Such an elongate pharynx as the latter was never observed in any of the present specimens (see also Shimazu, 1981). When I reexamined the two syntypes (USNM Helm. Coll. No. 50017, or now USNPC No. 050017.00, "cotypes or syntypes" in vial) in the US National Parasite Collection (USNPC), Agricultural Research Service, USDA, Beltsville, Maryland, USA, they had been tinged so dark gray that they were useless for observation of the internal organs and confirmation of Manter's description (Shimazu, 1981).

Shimazu's (1981) specimens (NSMT-Pl 2177–2186) of *A. perryii* were from *P. perryi* and *S. leucomaenis leucomaenis* in eastern Hokkaido. Fujita's and Shimazu's unflattened specimens agree substantially in morphology and measurements with Fujita's (1918) original description and figure (fig. 1) for *A. perryii*, though eggs in balsam were slightly smaller, 45–51 by 29–35 μm (Fujita's) and 48–55 by 27–32 μm (Shimazu's) instead of 58 by 33 μm (Fijita, 1918). Shimazu (1981) said that the hermaphroditic duct was almost lacking. However, it appeared that a very short hermaphroditic duct was present and everted (Fig. 13, hd) in the specimen figured by Shimazu (1981, fig. 3).

In addition to the present material examined, many specimens of *A. perryii* were found in the stomach and buccal cavity and on the gills of frozen fish on 10 May 1977: *P. perryi* from the Kottaro River (frozen on 15 February 1976), Settsuri River (frozen on 10 April 1976), Hororo River (frozen on 17 June 1969), Kushiro River (frozen on 24 April 1966) and Bekanbeushi River (frozen on 1 December 1968). These specimens had been flattened and fixed in 70% ethanol. Unfortunately, while being stained with alum carmine, most of them melted into shapeless masses to become useless for morphological observation (Shimazu, 1981).

Seki (1975a, b) identified adult specimens found in the stomach, esophagus and buccal cavity of *Salvelinus leucomaenis leucomaenis* from Lake Panketo as *Azygia lucii* (Müller, 1776). Shimazu (1981) demonstrated that Seki's material belonged to *A. perryii* and that *A. perryii* was readily distinguished from *A. lucii* by the shape of the pharynx: elliptical, with the ratio of width to length of 1: 1.04–1.35, instead of elongate, with the ratio of 1: 1.4–2.1 (Odening, 1976).

Seki's collection also included three immature specimens (No. 374, unidentified, unpublished) of *A. perryii* found in *Oncorhynchus mykiss* (Walbaum 1792) (syn. *Salmo gairdneri irideus* Gibbons, 1855) (Salmonidae) from Lake Shikaribetsu on 20 June 1974 (Shimazu, 1990, 1994). Evidently, *A. perryii* also occurs in Lake Shikaribetsu, though no adults have yet been obtained there.

In Japan, the geographical distribution of *A. perryii* is confined to eastern Hokkaido (Fujita, 1918; Seki, 1975a, b; Shimazu, 1981, 1990, 1994). Bykhovskaya-Pavlovskaya (1962) briefly described and figured *A. perryii* found in the stomach and buccal cavity of *P. perryi* on Sakhalin Island, Russia. Later, Bykhovskaya-Pavlovskaya and Kulakova (1987) synonymized *A. perryii* with *Azygia robusta* Odhner, 1911 from fishes of several species including *Hucho taimen* (Pallas, 1773) from eastern Russia. However, it seems to me that *A. perryii* differs from *A. robusta* in having a higher sucker width ratio, 1: 0.8 (my calculation from Bykhovskaya-Pavlovskaya, 1962), 1: 0.81–0.89 (Shimazu, 1981) and 1: 0.8–0.9 (this paper), instead of 1: 0.7 (my calculation from Odhner, 1911) and 1: 0.7 (my calculation from Bykhovskaya-Pavlovskaya, 1962); and larger eggs, 49–55 by 24–270 [*sic*, most likely 27] μm (Bykhovskaya-Pavlovskaya, 1962), 50–57 by 28–32 μm (Shimazu, 1981) and 45–55 by 27–35 μm (this paper), instead of 40–49 by 20–24 μm (Layman, 1933) and 43–47 by 21–23 μm (Bykhovskaya-Pavlovskaya, 1962). Further morphological and molecular studies are required concerning species difference between the two species. I treat *A. perryii* as a valid species for the time being.

Specimens of *A. perryii* were recorded from the stomach and sometimes from other sites of *P. perryi* and *S. leucomaenis leucomaenis*: the esophagus, buccal and branchial cavities and even the outer surface of the body (Fujita, 1918; Seki, 1975a, b; Shimazu, 1981). The primary site of infection is the stomach. As was noted by Ward (1910) and Manter (1926) in *Azygia sebago* Ward, 1910, soon after the death of the hosts, worms rapidly migrate actively from the stomach up the esophagus to move into the buccal and branchial cavities and even to the exterior to crawl on the skin (Shimazu, 1981).

The seminal vesicle in *A. perryii* was also surrounded by small cells (Shimazu, 1981, fig. 2; this paper, Fig. 13, ∗) as in *A. gotoi.*

Life cycle. Not known. Since the final hosts *P. perryi* and *S. leucomaenis leucomaenis* are predatory, some transport hosts may be involved between the snail intermediate host and them.

Azygia rhinogobii Shimazu, 2007

(Figs. 15–19)

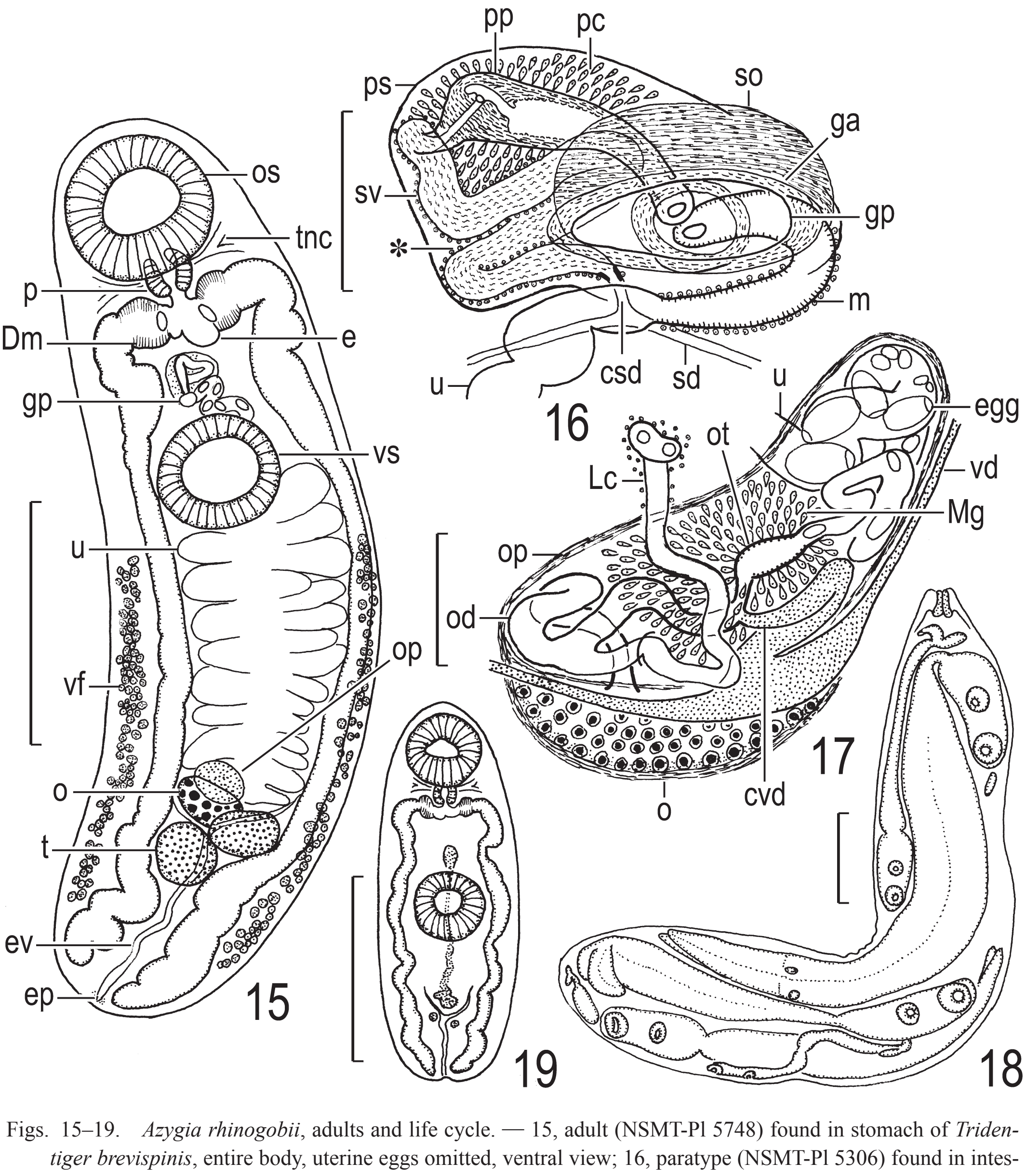
Azygia rhinogobii Shimazu, 2007: 6–7, figs. 7–10.

Hosts in Japan. Rhinogobius sp. OR (type host), *Gymnogobius urotaenia* (Hilgendorf, 1879) and *Tridentiger brevispinis* Katsuyama, Arai and Nakamura, 1972 (Gobiidae); and *Tribolodon hakonensis* (Günther, 1877) (Cyprinidae) (probably accidental host) (Shimazu, 2007; this paper).

Sites of infection. Primarily stomach, and intestine (probably accidental).

Geographical distribution. (1) Ibaraki Prefecture: Lake Kasumigaura at Tamatsukuri-Ko, Namegata City, and possibly Sakamoto, Minaminemoto, Kasumigaura City; Lake Kitaura at Yamada, Namegata City, and at Egawa, Hokota City; Gantsu River at Yabata, Namegata City; Ichinose River at Ichinose at Kasumigaura City; Kajinashi River at Tamatsukuri-Otsu, Namegata City; and irrigation canals at Oou, Itako City (Shimazu, 2007; this paper). (2) Nagano Prefecture: Lake Suwa (type locality) at Suwa City and possibly Lake Kizaki in Oomachi City (Shimazu, 2007; this paper).

Material examined. (1) Specimens of *Azygia rhinogobii*, flattened, whole-mounted, ex stomach of *Rhinogobius* sp. [*Rhinogobius* sp. OR, most likely referring to *R. kurodai* (Tanaka, 1908)], Lake Suwa: 1 (NSMT-Pl 5300, holotype), adult, 20 November 1993; 1 (NSMT-Pl 5301, paratype), adult, 30 October 1993; 10 (NSMT-Pl 5301–5305), immature, 24 September 1992, 23 October 1992, 2 and 30 October 1993, 2 August 1996 (Shimazu, 2007); and 5 (NSMT-Pl 5744), immature, 18 November 2008. (2) 1 (NSMT-Pl 5306, paratype) of *A. rhinogobii*, flattened, whole-mounted, ex intestine of *Tribolodon hakonensis*, Lake Suwa, 5 October 1991 (Shimazu, 2007). (3) 4 (NSMT-Pl 5307–5309) of *A. rhinogobii*, immature, flattened, whole-mounted, ex stomach of *Gymnogobius urotaenia*, Lake Suwa, 16 and 30 October 1993, 20 November 1993 (Shimazu, 2007). (4) 4 (NSMT-Pl 5745), immature, adult, flattened, whole-mounted, ex stomach of *G. urotaenia*, Lake Suwa, 18 November 2008. (5) 6 (NSMT-Pl 5746), immature, adult, flattened, whole-mounted, ex stomach of *Tridentiger brevispinis*, Lake Suwa, 18 November 2008. (6) Specimens of *A. rhinogobii*, flattened, whole-mounted, ex stomach of *Rhinogobius* sp. [*Rhinogobius* sp. OR, most likely referring to *Rhinogoius kurodai*], Ibaraki Prefecture: 4 (NSMT-Pl 5317), immature, irrigation canals, Oou, Itako Town, now Itako City, 8 and 11 August 1994; 3 (NSMT-Pl 5318), immature, Gantsu River, Yabata, Aso Town, now Namegata City, 9 August 1994; 15 (NSMT-Pl 5319), immature, Lake Kitaura, Kitaura Village, now Yamada, Namegata City, 10 August 1994 (Shimazu, 2007). (7) Specimens of *A. rhinogobii*, flattened, whole-mounted, ex stomach of *G. urotaenia*, Ibaraki Prefecture: 1 (NSMT-Pl 5320), immature, irrigation canals, Oou, 10 August 1994; and 2 (NSMT-Pl 5321), immature, Gantsu River, 9 August 1994 (Shimazu, 2007). (8) Specimens of *A. rhinogobii*, flattened, whole-mounted, ex stomach of *T. brevispinis*, Ibaraki Prefecture: 18 (NSMT-Pl 5314), immature, Lake Kitaura, Kitaura Village, 6 and 10 August 1994; 14 (NSMT-Pl 5315), immature, Gantsu River, 9 August 1994; and 4 (NSMT-Pl 5316) of "*Azygia anguillae* (?)," immature, adult, Lake Kasumigaura, Tamatsukuri Town, now Tamatsukuri-Ko, Namegata City,



Figs. 15–19. *Azygia rhinogobii*, adults and life cycle. — 15, adult (NSMT-Pl 5748) found in stomach of *Tridentiger brevispinis*, entire body, uterine eggs omitted, ventral view; 16, paratype (NSMT-Pl 5306) found in intestine of *Tribolodon hakonensis*, terminal genitalia, small (gland (?)) cells (∗) surrounding seminal vesicle, ventral view; 17, paratype (NSMT-Pl 5306), ovarian complex, dorsal view; 18, possible daughter sporocyst (NSMT-Pl 5312) found in *Sinotaia quadrata histrica*, containing developing cercariae; 19, possible cercaria (NSMT-Pl 5311), body proper, flattened, entire body, ventral view. Scale bars: 1 mm in Fig. 15; 0.5 mm in Figs. 18–19: 0.1 mm in Figs. 16–17.

2 November 1994, 5 April 1996 (Shimazu, 2007). (9) Specimens, unflattened, whole-mounted, ex stomach of *T. brevispinis*, Ibaraki Prefecture: 9 (NSMT-Pl 5747), immature, adult, Lake Kitaura, Egawa, Hokota City, 1 and 27 October 1998; 8 (NSMT-Pl 5748), adult, Kajinashi River, Tamatsukuri-Otsu, Namegata City, 14 and 21 October 2008; and 1 (NSMT-Pl 5749), immature, Ichinose River, Ichinose, Kasumigaura City, 14 October 2008.

Description. Based on 10 adult specimens including type specimens (Figs. 15–17). Similar to *A. gotoi* (this paper) in general morphology. Body fairly small, 2.30–3.97 by 0.84–1.27 (holotype 2.42 by 0.87); forebody 0.79–1.51 long, occupying 32–51% of body length. Oral sucker 0.40–0.60 in diameter. Pharynx 0.13–0.19 by 0.11–0.19. Esophagus 0.13–0.25 long. Intestinal ceca slightly undulating, ending near posterior extremity of body. Ventral sucker 0.37–0.47 by 0.40–0.51, pre-equatorial; sucker width ratio 1: 0.8–1.0. Distance from anterior extremity of body to ovary 1.82–2.85, occupying 72–87% of body length (distance from midlevel of ventral sucker to ovary 0.65–1.55, occupying 51–73% of hindbody length). Testes diagonal, contiguous; anterior (right or left) testis lateral or posterolateral to ovary, 0.20–0.32 by 0.09–0.22; posterior testis 0.19–0.32 by 0.11–0.25. Prostatic sac between esophagus and ventral sucker, 0.16–0.23 by 0.16–0.25. Seminal vesicle long, thin, convoluted, opening into pars prostatica at lateral side near its proximal end, surrounded by small cells (Fig. 16, ＊). Pars prostatica with no compartments. Hermaphroditic duct absent. Sinus organ protruded like cone in center of genital atrium. Genital atrium large, shallow. Genital pore slightly anterior to ventral sucker. Ovary 0.16–0.28 by 0.12–0.22, at about junction of fourth and posterior fifths of body (at junction of third and posterior fourths of hindbody). Laurer's canal opening dorsally through one or two pores. Ootype pouch (capsule in Shimazu, 2007) 0.16–0.25 by 0.13–0.22, anterodorsal to ovary. Uterus much folded transversely; metraterm well developed, shorter than prostatic sac; uterine seminal receptacle sometimes present. Eggs 37–64 by 19–38 μm in type specimens, 60–75 by 36–43 μm in new specimens. Distance from midlevel of ventral sucker to anterior commencement of vitelline follicles (usually slightly posterior to ventral sucker or rarely lateral to it) 0.09–0.54, occupying 4–26% of hindbody length; distance from midlevel of ventral sucker to posterior end of vitelline follicles (at posterior ends of intestinal ceca or slightly anterior to them) 1.20–2.47, occupying 79–97% of hindbody length. Excretory vesicle bifurcating between anterior testis and ovary.

Remarks. Shimazu (2007) described this species on the basis of the type specimens. The specific name "*rhynogobii*" sometimes appears in Shimazu's (2007) paper, but this is a misspelling of "*rhinogobii*." All the present new specimens are identified *A. rhinogobii.*

The egg size in the type specimens (37–64 by 19–38 μm) varied more than that in the present new specimens (60–75 by 36–43 μm). This may be due to the fact that the eggs measured included somewhat malformed ones in the holotype, obviously a barely matured adult (Shimazu, 2007, fig. 7). I consider that the latter size (60–75 by 36–43 μm) is normal in balsam. Shimazu (2007, figs. 8–10) figured one (NSMT-Pl 5306) of the two paratypes with better-formed eggs. Reexamination of this figured paratype has shown that: (1) the seminal vesicle is surrounded by small cells (Fig. 16, ＊), which Shimazu (2007, fig. 9) mistook for part of the prostatic cells; (2) the sinus organ is larger (Fig. 16) than figured (fig. 9); (3) the proximal coils of the uterus in the ootype pouch is more convoluted (Fig. 17) than figured (fig. 10); and (4) Laurer's canal opens through two pores (Fig. 17) instead of one pore (fig. 10).

Although Shimazu (2007) distinguished *A. rhinogobii* from *Azygia angusticauda* (Stafford, 1904) of North America, *A. rhinogobii* more closely resembles two Chinese species, *Azygia sangangensis* Wang, 1981 and *Azygia sinipercae* Wang and Pan, 1973, in the posterior position of the gonads in the adult stage. *Azygia rhinogobii* differs from *A. sangangensis* as described by Wang (1981) from the intestine of *Pseudogastromyzon zebroidus* (Nichols) (Balitoridae) of Chong'an, Sangang, Fujian Province, in that the sucker width ratio is higher, 1: 0.8–1.0 instead of 1 : 0.8 (my calculation); the testes are diagonal instead of tandem; the ovary is located at about junction of the fourth and posterior fifths of the body instead of at about the junction of the fifth and posterior sixths of the body; eggs are larger in width, 60–75 by 36–43 μm instead of 60–72 by 32–35 μm; and possibly the host fish are gobiids instead of a balitorid. *Azygia rhinogobii* is different from *A. sinipercae* as described by Wang and Pan (1973) from the stomach of *Siniperca chuatsi* (Basilewsky) (Percichthyidae) of Chenpu and Lake Shatao, Hubei Province, in that the body is smaller and broader, 2.30–3.97 by 0.84–1.27 instead of 5.4–6.1 by 0.9–1.2; the gonads are located more anterior, the distance from the midlevel of the ventral sucker to the ovary occupying 51–73% instead of 72% (my calculation from Wang and Pan (1973, pl. 104, fig. 17)) of the hindbody; and eggs are larger, 60–75 by 36–43 μm instead of 32–51 by 20–31 μm (Wang, 1991).

Shimazu (2007) considered that the three immature and one mature specimens (NSMT-Pl 5316) found in the stomach of *T. brevispinis* from Lake Kasumigaura were more similar to *A. gotoi* (syn. *A. anguillae*) than to *A. rhinogobii.* However, I here regard them as *A. rhinogobii*, because fully matured adult specimens of *A. rhinogobii* were obtained from *T. brevispinis* (this paper) and because mature worms of *A. gotoi* have not yet been recorded from any species of gobiids (this paper).

Ozaki's Collection included 1 immature specimen of *Azygia* (MPM Coll. No. 30208, labeled "*Genarchopsis Tridentiger ob.* [Goro] [Kasumigaura] [Sakamoto]") (Shimazu, 1995a). This specimen is identified as *A. rhinogobii* [not *A. gotoi*]. The host fish "*Tridentiger ob.* [Goro]" should be "Numachichibu," or *Tridentiger brevispinis*, in Lake Kasumigaura (Shimazu, 1995a). "[Sakamoto]" is possibly the locality name, or Sakamoto, Minaminemoto, Dejima Village, now in Kasumigaura City.

In Lake Kizaki, 11 immature specimens (NSMT-Pl 4026) were found in the stomach of *Micropterus salmoides* on 11 September 1989 (my unpublished data). At least two of them may be assigned to *A. rhinogobii*, because the vitelline follicles were considerably well developed in them. Shimazu (2007) suggested that *A. gotoi* could be morphologically distinguished from *A. rhinogobii* even in juveniles. However, it is not easy to identify them with certainty. The species identification requires experimental confirmation (Shimazu, 2007) or molecular determination.

Life cycle. Not known. It is possible that an unidentified cercaria of the furcocystocercous type may be the larva of *A. rhinogobii.* This cercaria was found in a viviparid snail, *Sinotaia quadrata histrica* (Gould, 1859) (Japanese name: Hime-tanishi), from Lake Suwa (Shimazu, 1979; 2007, figs. 12–13; rediae and cercariae obtained in 1973–1975, NSMT-Pl 5310–5313) (Figs. 18–19).

Rhinogobius sp. OR (most likely referring to *R. kurodai*), *Gymnogobius urotaenia* and *Tridentiger brevispinis* serve as final hosts for *A. rhinogobii* in both Ibaraki Prefecture and Lake Suwa (this paper). *Tribolodon hakonensis* was considered to be an accidental host that had ingested a gobiid harboring the adult specimen (Shimazu, 2007).

Azygia rhinogobii may be completed its life cycle without involving no transport hosts on the occasion when small fish of *Rhinogobius* sp. OR, *G. urotaenia* and *T. brevispinis* ingest cercariae. In this case *A. rhinogobii* has a two-host life cycle.

Key to the species of *Azygia* in this paper

1.1. Ovary about equatorial; pars prostatica with posterior compartments; excretory vesicle bifurcating posterior to testes . . . . *A. perryii*

1.2. Ovary postequatorial; pars prostatica with no posterior compartments; excretory vesicle bifurcating between anterior testis and ovary 2

2.1. Body fairly large (11.03–13.17 long); ventral sucker at about junction of anterior and second fifths of body . . . . *A. gotoi*

2.2. Body fairly small (2.30–3.97 long); ventral sucker at about junction of second and middle fifths of body . . . . *A. rhinogobii*

Superfamily Bucephaloidea Poche, 1907

Family Bucephalidae Poche, 1907

Genus ***Parabucephalopsis*** Tang and Tang, 1963

Parabucephalopsis parasiluri Wang, 1985

(Figs. 20–22)

Parabucephalopsis parasiluri Wang, 1985: 76, 83, fig. 10; Urabe, Ogawa, Nakatsugawa, Nakai *et al.*, 2007: 270, fig. 1A–C; Shimazu *et al.*, 2011: 86–88, figs. 119–121.

Hosts in Japan. Silurus biwaensis (Tomoda, 1961) (Siluridae) and *Silurus lithophilus* (Tomoda, 1961) (Urabe, Ogawa, Nakatsugawa and Wang, 2007; Urabe, Ogawa, Nakatsugawa, Nakai *et al.*, 2007; Urabe *et al.*, 2008; Shimazu *et al.*, 2011; Baba, 2013).

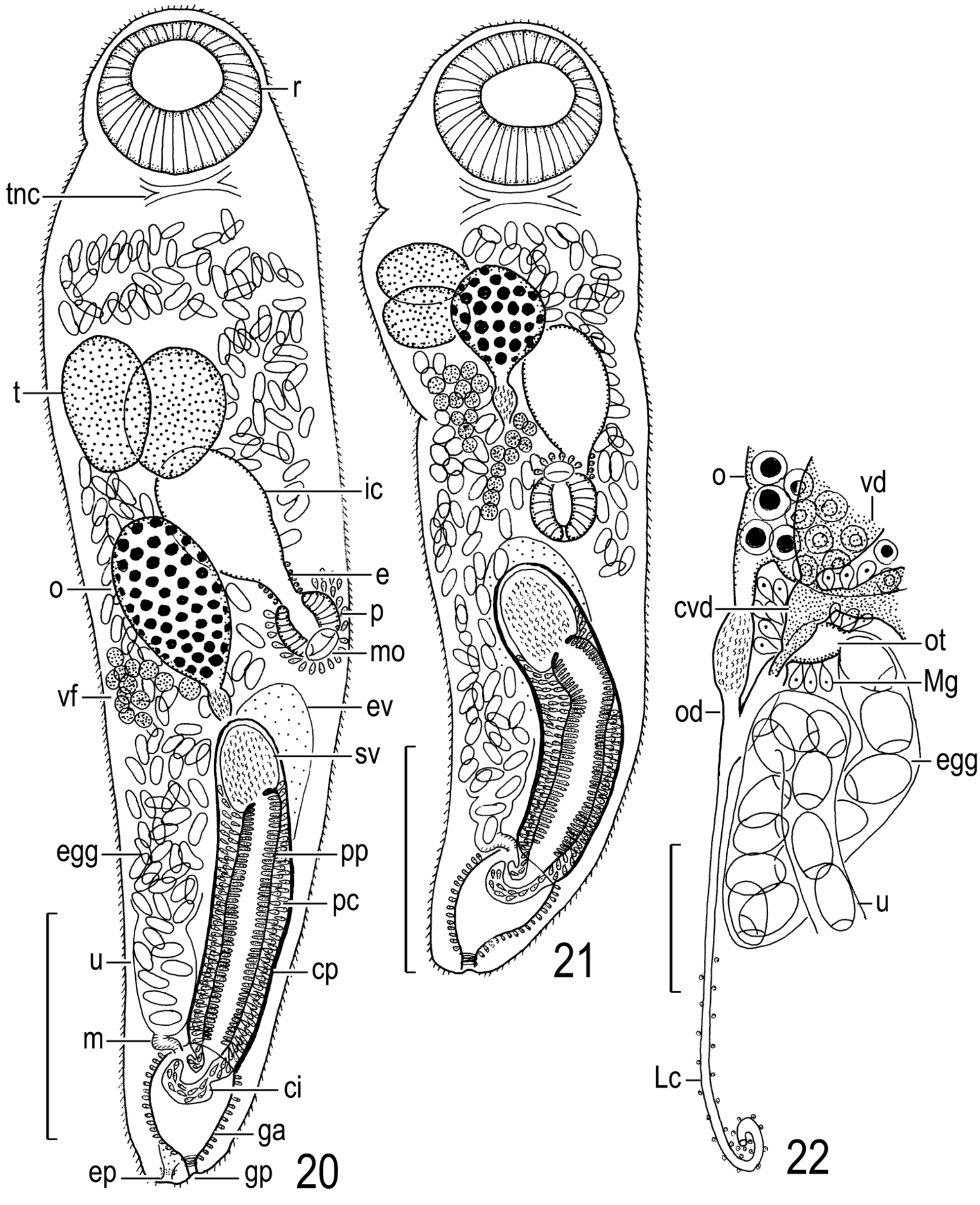
Sites of infection. Usually rectum and rarely intestine.

Geographical distribution. (1) Shiga Prefecture: Lake Biwa at Kinoshita-cho, Otsu City; and Seta River at Ishiyamanango-cho, Otsu City (Urabe *et al.*, 2008; Baba and Urabe, 2011a; Shimazu *et al.*, 2011). (2) Kyoto Prefecture: Lake Hoo (Amagase Dam Reservoir) at Uji City and Uji River at Uji City (Urabe, Ogawa, Nakatsugawa and Wang, 2007; Urabe, Ogawa, Nakatsugawa, Nakai *et al.*, 2007; Baba, 2013). (3) Osaka Prefecture: Yodo River at Hirakata City (Baba, 2013).

In China (*e.g.* Wang, 1985; Jiang, 1991; Wang and Wang, 1998).

Material examined. (1) 5 specimens (NSMT-Pl 5565) of *Parabucephalopsis parasiluri*, adult, flattened, whole-mounted, ex intestine (mainly rectum) of *Silurus biwaensis*, Uji River, 14 November 2006 (Urabe, Ogawa, Nakatsugawa, Nakai *et al.*, 2007). (2) 46 (NSMT-Pl 5743) of *Pa. parasiluri*, immature, adult, flattened, whole-mounted, ex rectum of *S. biwaensis*, Seta River, 1 June 2007 (Urabe *et al.*, 2008; Shimazu *et al.*, 2011).

Description. Based on 17 adult specimens; 6 measured (Figs. 20–22). Body elongate-ovate, rounded anteriorly, slightly attenuated posteriorly, anoculate, very small, 0.58–1.06 by 0.17–0.27. Tegument spinose throughout body; spines thick in anterior part of body, becoming gradually thinner posteriorly. Oral and ventral suckers absent. Anterior holdfast organ rhynchus, simple, sucker-like, round, 0.10–0.15 by 0.09–0.16, anteroventral. Mouth ventral, opening on tip of pharynx, median (Fig. 21) (specimen (Fig. 20) mounted laterally), surrounded by large gland cells. Prepharynx not seen. Pharynx pyriform to globular, usually directed anteriorly but rarely posteriorly, 0.04–0.07 by 0.03–0.07, median or submedian, slightly postequatorial; sphincter may be present between mouth aperture and pharynx; distance from anterior end of body to pharynx 0.47–0.62, occupying 55–67% of body length. Esophagus directed anteriorly, 0.04–0.06 long, surrounded by small gland cells. Intestinal cecum single, directed anteriorly, globular to elliptical, 0.07–0.13 by 0.05–0.08. Testes two, elliptical, symmetrical to almost tandem, slightly overlapping each other, dextrally lateral or submedian, far posterior to rhynchus; anterior or right testis 0.08–0.12 by 0.05–0.11, posterior or left testis 0.06–0.12 by 0.05–0.08. Sperm ducts short; common sperm duct long. Cirrus pouch tubular, slightly curved, thick-walled, muscular, 0.19–0.29 by 0.05–0.08, median to sinistrally submedian, proximal end slightly posterior to pharynx. Seminal vesicle elliptical, 0.04–0.09 by 0.03–0.09. Pars prostatica straight or curved, two- to three-fourths of cirrus pouch length; prostatic cells well developed. Ejaculatory duct short. Cirrus (or genital lobe) projecting into genital atrium in form of crooked lobe, 0.03–0.05 long. Genital atrium deep, with sphincter at aperture. Genital pore almost median, nearly posteroterminal. Ovary single, obovate, 0.09–0.14 by 0.06–0.09, post-testicular, dextrally submedian



Figs. 20–22. *Parabucephalopsis parasiluri*, adults (NSMT-Pl 5565) found in rectum of *Silurus biwaensis*. — 20, entire body, ventral view; 21, entire body, ventral view; 22, ovarian complex, dorsal view. Scale bars: 0.2 mm in Figs. 20–21; 0.1 mm in Fig. 22.

or median, antero- to postero-lateral to cecum. Ovarian complex median, postovarian. Oviduct forming dilatation to store sperm in it before giving off Laurer's canal. Seminal receptacle absent. Laurer's canal long, running backward, opening dorsally near lateral margin at level of cirrus on right side of body. Ootype complex dextrally submedian. Uterus winding, extending forward between rhynchus and testes, folded on either side of intestinal cecum and dextrally to cirrus pouch; metraterm short, flowing into genital atrium at lateral side of its base, facing cirrus; uterine seminal receptacle sometimes weakly developed. Eggs numerous, elliptical, sometimes curved, thin-shelled, operculate, light brown, 27–35 by 13–17 μm (collapsed), embryonated. Vitellaria follicular; follicles globular, small, 10–14 each forming two compact clusters, oblique to nearly symmetrical, close to each other, level with ovary or posterior to it. Excretory vesicle I-shaped, thick, extending forward slightly beyond cirrus pouch; excretory pore posteroterminal.

Remarks. Wang (1985) described this species on the basis of adult specimens found in the intestine of *Silurus asotus* (syn. *Parasilurus asotus*) from Fuzhou, Fujian Province, China. The present specimens were identified as *P. parasiluri* by Urabe, Ogawa, Nakatsugawa, Nakai *et al.* (2007) and Urabe *et al.* (2008). Shimazu *et al.* (2011) described the specimens (NSMT-Pl 5743).

Metacercariae were found encysted in the body muscles, fins, skin and eyes of second intermediate hosts, chiefly cyprinids. Heavy infection of metacercariae in fish causes inactive swimming and hemorrhage of fish on the fins, skin and eyes, and eventually death (Ogawa *et al.*, 2004). Baba (2013) carried out an extensive study of *Pa. parasiluri* to predict the possibility of the outbreak of *Pa. parasiluri* of second intermediate host fishes in Lake Biwa and investigate the means to prevent its occurring.

Life cycle. A natural first intermediate host is *Limnoperna fortunei* (Dunker, 1857) (Bivalvia, Mytilidae) (Japanese name: Kawa-hibarigai), in which cercariae are produced in sporocysts (in one generation (?)). Sporocysts and cercariae were recorded from the Seta, Uji and Yodo rivers (Urabe *et al.*, 2001; Urabe *et al.*, 2008; Baba and Urabe, 2011a; Baba *et al.*, 2012; Baba, 2013) and Lake Biwa at Otsu City, Shiga Prefecture (Baba and Urabe, 2011a; Baba, 2013). Sporocysts were molecularly identified to species. Neither sporocysts nor cercariae have yet been described. Cercariae in Urabe *et al.* (2001, fig. 3) and Baba and Urabe (2011b, fig. 5) are likely to belong to *Pa. parasiluri.*

Natural second intermediate hosts are freshwater fishes of many species (chiefly cyprinids), in the caudal fin of which metacercariae encyst most heavily (Ogawa *et al.*, 2004; Baba and Urabe, 2011b; Baba, 2013). Metacercaria A of Ogawa *et al.* (2004, fig. [3a], fig. 4a–c) refers to *Pa. parasiluri.* Metacercariae have not yet been fully described.

Natural final hosts, in the rectum of which adults develop, are *Silurus biwaensis* in the Seta, Uji and Yodo rivers (Urabe, Ogawa, Nakatsugawa and Wang, 2007; Urabe, Ogawa, Nakatsugawa, Nakai *et al.*, 2007; Urabe *et al.*, 2008; Shimazu *et al.*, 2011; Baba, 2013; this paper) and S. *lithophilus* in the Seta River (Baba, 2013). Immature worms were also recorded from *Micropterus salmoides* (Urabe *et al.*, 2001).

In China, natural final hosts are *Silurus asotus*, *Silurus meridionalis* Chen, 1977, *Liobagrus marginatus* (Günther, 1892) (Amblycipitidae) and *Sinilabeo rendahli* (Kimura, 1934) (Cyprinidae) (Wang and Wang, 1998). The entire life cycle is unknown.

Genus ***Prosorhynchoides*** Dollfus, 1929

Prosorhynchoides ozakii (Nagaty, 1937)

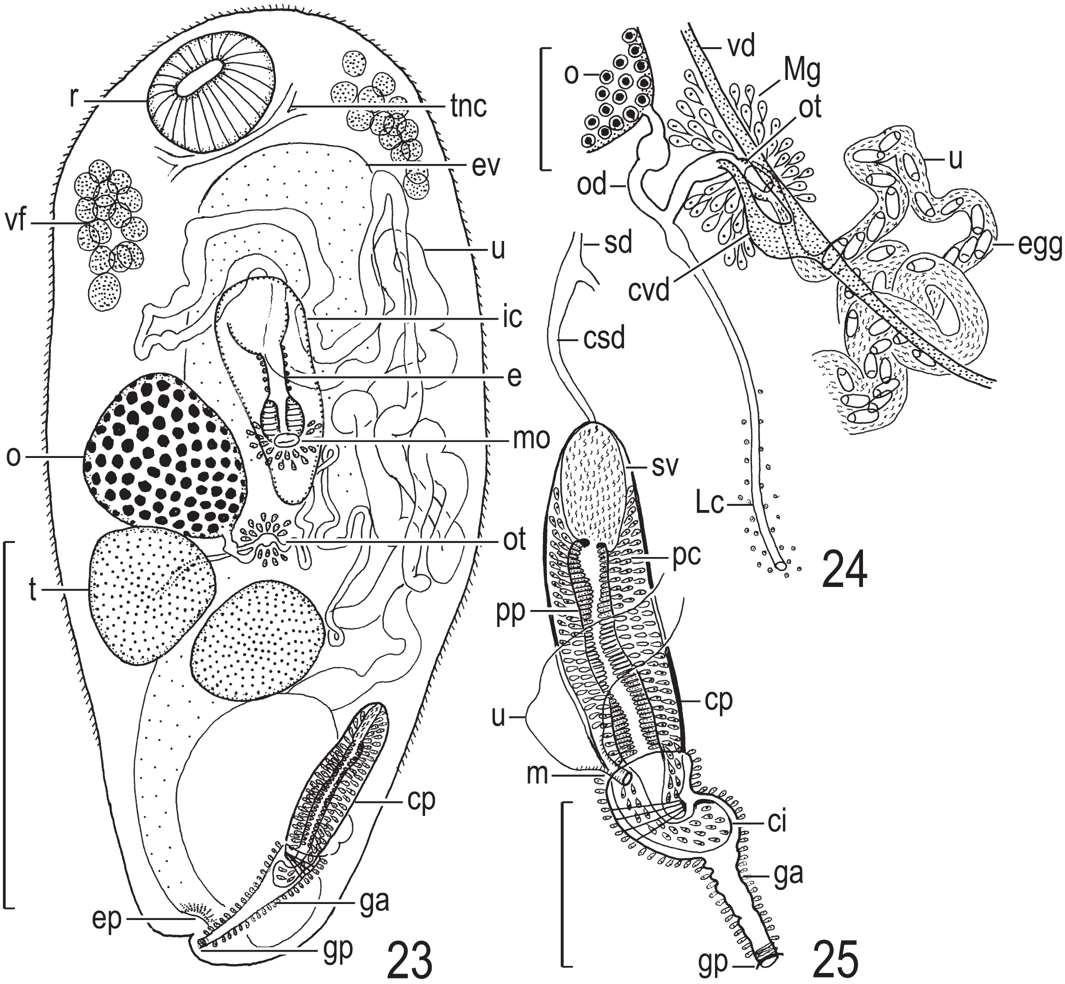
(Figs. 23–25)

Bucephalopsis ovatus Ozaki, 1928 [*sic*, should be *ovata*] (junior secondary homonym of *Bucephalopsis ovatus* (Linton, 1900) [*sic*]): 56–58, fig. 27.
Bucephalopsis ozakii Nagaty, 1937: 37.
Prosorhynchoides ozakii: Margolis and Arthur, 1979: 58; Urabe, Ogawa, Nakatsugawa, Nakai *et al.*, 2007: 68.
Bucephalopsis basargini (not of Layman, 1930): Urabe, 2006: 366; Urabe, Ogawa, Nakatsugawa and Wang, 2007: 271–272, fig. 2A–D.

Hosts in Japan. Silurus biwaensis, *S. asotus* and *S. lithophilus* (Urabe, Ogawa, Nakatsugawa and Wang, 2007; Urabe, Ogawa, Nakatsugawa, Nakai *et al.*, 2007; Baba and Urabe, 2011a; Baba, 2013).

Sites of infection. Usually intestine and rarely rectum.

Geographical distribution. (1) Kyoto Prefecture: Lake Hoo (Amagase Dam Reservoir) at Uji City and Uji River at Uji City (Urabe, Ogawa, Nakatsugawa, Nakai *et al.*, 2007; Baba and Urabe, 2011a; Baba, 2013). (2) Osaka Prefecture:



Figs. 23–25. *Prosorhynchoides ozakii*, adults (NSMT-Pl 5567) found in rectum of *Silurus biwaensis*. — 23, entire body, uterine eggs omitted, ventral view; 24, ovarian complex, dorsal view; 25, terminal genitalia, dorsal view. Scale bars: 1 mm in Fig. 23; 0.2 mm in Fig. 25; 0.1 mm in Fig. 24.

Yodo River at Hirakata City (Baba, 2013).

In Korea (*e.g.* Ozaki, 1928); China (*e.g.* Jiang, 1991; Wang and Wang, 1998); and Vietnam (*e.g.* Moravec and Sey, 1989).

Material examined. 11 specimens (NSMT-Pl 5566–5567) of *Prosorhynchoides ozakii*, adult, flattened, whole-mounted, ex chiefly intestine of *Silurus biwaensis*, Uji River, 22 May 2005 (Urabe, Ogawa, Nakatsugawa, Nakai *et al.*, 2007).

Description. Based on 11 adult specimens (Figs. 23–25). Similar to *Parabucephalopsis parasiluri* (this paper) in general morphology. Body broad-ovate, rounded anteriorly, slightly attenuated posteriorly, small, 1.30–2.54 by 0.73–1.30, including many large (gland (?)) cells in ventral parenchyma at cecal level (not illustrated). Tegumental spines thick in anterior part of body, large, often splitting into 2–4 points, becoming smaller and thinner posteriorly, sometimes not seen in posterior part of body. Rhynchus simple, sucker-like, round, 0.22–0.37 by 0.26–0.38, anteroventral. Mouth ventral, opening on tip of pharynx, encircled by large gland cells. Pharynx directed anteriorly, spherical, 0.10–0.14 in diameter, median, about equatorial (slightly postequatorial in small specimens 1.30–1.74 by 0.73–0.87 but slightly pre-equatorial in large ones 1.90–2.54 by 0.95–1.30); sphincter may be present between mouth aperture and pharynx; distance from anterior end of body to pharynx 0.74–1.14 long, occupying 42–57% of body length. Esophagus directed anteriorly, 0.11–0.17 long. Intestinal cecum elongate, median, directed first anteriorly and then recurving posteriorly, reaching beyond pharynx to ootype complex, posteriorly directed part 0.26–0.61 by 0.16–0.25. Testes two (abnormally single in one of 11 specimens), elliptical, slightly diagonal, nearly contiguous, postovarian, dextrally submedian; anterior or right testis 0.23–0.36 by 0.16–0.32, posterior or left testis 0.23–0.38 by 0.14–0.28. Sperm ducts short; common sperm duct long. Cirrus pouch tubular, almost straight, thick-walled, muscular, 0.42–0.51 by 0.09–0.14, sinistrally submedian to lateral, proximal end in testicular zoon of body. Seminal vesicle almost spherical, 0.11–0.16 by 0.06–0.08. Pars prostatica straight or somewhat undulating, about two-thirds of cirrus pouch length; prostatic cells well developed. Ejaculatory duct short. Cirrus (or genital lobe) projecting in form of crooked lobe into genital atrium, 0.08–0.14 long. Genital atrium deep, with sphincter at aperture. Genital pore almost median, posteroterminal or slightly posteroventral. Ovary nearly round, 0.22–0.44 by 0.19–0.41, pretesticular, dextrally submedian, lateral to intestinal cecum. Ovarian complex posteromedial to ovary. Oviduct forming dilatation before giving off Laurer's canal; no sperm seen in dilatation. Ootype complex median. Laurer's canal fairly long, running posteriorly, opening dorsally. Uterus much winding, extending anteriorly between rhynchus and intestinal cecum, more folded in sinistrally submedian, pretesticular field, extending backward to near posterior extremity of body, then turning forward, expanding there; metraterm short, opening into genital atrium at its base; uterine seminal receptacle well developed. Eggs numerous, elongate-elliptical, sometimes curved, thin-shelled, operculate, light brown, 32–41 by 12–16μm, embryonated. Vitelline follicles globular, small, 14–15 each forming two compact clusters, each posterolateral to rhynchus on either side of body. Excretory vesicle I-shaped, thick, extending anteriorly to near rhynchus; excretory pore posterodorsal.

Remarks. Ozaki (1928) described *Bucephalopsis ovatus* Ozaki, 1928 [*sic*, should be *ovata*] on the basis of adult specimens found in the small intestine [*sic*] of *Silurus asotus* (syn. *Parasilurus asotus*) from Korea (locality not specified). Because this species became a junior secondary homonym of *Bucephalopsis ovatus* (Linton, 1900) [*sic*, should be *ovata*], Nagaty (1937) proposed a new replacement, *Bucephalopsis ozakii*, for the taxon. Srivastava and Chauhan (1973) stated that all the species then included under *Bucephalopsis* Diesing, 1855 should be transferred to *Prosorhynchoides* Dollfus, 1929, but they did not propose a new combination for *B. ozakii.* Margolis and Arthur (1979) first made a new combination, *Prosorhynchoides ozakii* (Nagaty, 1937).

Prosorhynchoides differs from *Parabucephalopsis* in that the ovary is pretesticular instead of post-testicular, the vitelline follicles are immediately posterior to the rhynchus instead of in the middle of the body, and the excretory vesicle extends to near the rhynchus instead of slightly beyond the cirrus pouch.

It is believed that the holotype (No. p. 291) of *B. ovata* was lost (Shimazu, 2013). Ozaki's Collection included two whole-mounted adult specimens (MPM Coll. No. 30082, labeled "*Bucephalus* Namazu Korea"). Shimazu (1995b) identified them as *Bucephalopsis ovatus* Ozaki, 1928 found in *S. asotus* from Korea (see also Urabe, Ogawa, Nakatsugawa, Nakai *et al.*, 2007). Although neither of them is labeled a paratype, they must be part of Ozaki's original specimens of *B. ovata.*

Urabe (2006) and Urabe, Ogawa, Nakatsugawa and Wang (2007) first reported this species as *Bucephalopsis basargini* Layman, 1930. Later, Urabe, Ogawa, Nakatsugawa, Nakai *et al.* (2007) described the present specimens under the species name *Prosorhynchoides ozakii.* Ozaki (1928) did not mention the anterior extent of the excretory vesicle. The excretory vesicle extends forward to the level of the vitelline follicles, or to near the rhynchus, in young adult specimens (Urabe, Ogawa, Nakatsugawa, Nakai *et al.*, 2007; this paper, Figs. 23) as in Ozaki's two specimens (my unpublished data).

The present parasite has been recorded as *Bucephalopsis ovata* from *Silurus asotus* in Korea (Ozaki, 1928); as *Bucephalopsis ozakii* from *S. asotus* and *Pelteobagrus vachellii* (Richardson, 1846) (Bagridae) in China (Jiang, 1991; Wang and Wang, 1998); as *B. ozakii* from *Pe. vachellii* and *Saurogobius dobryi* (Gobiidae) [*sic*, correctly *Saurogobio dabryi* Bleeker, 1871 (Cyprinidae) (?)] in northern Vietnam (Moravec and Sey, 1989); and as *Prosorhynchoides ozakii* from *Silurus biwaensis* and *S. asotus* of the Uji River (Urabe, Ogawa, Nakatsugawa, Nakai *et al.*, 2007; Baba and Urabe, 2011a; Baba, 1913; this paper). The egg size differs from material to material: 37–39 by 14–17 μm (Ozaki, 1928) and 31–40 by 11–14 μm (my measurements on Ozaki's two specimens) from Korea; 20–23 by 6–8 μm (Jiang, 1991) and 37–39 by 14–17 μm (Wang and Wang, 1998) in China; 27–33 by 15–18 μm (Moravec and Sey, 1989) in northern Vietnam; and 29–43 by 8–13 μm (Urabe, Ogawa, Nakatsugawa, Nakai *et al.*, 2007) and 32–41 by 12–16 μm (this paper) in Japan. These materials need comparison with a new material from Korea using morphological and molecular data. Margolis and Arthur (1979) listed *Pr. ozakii* as a parasite of *Salvelinus malma* (Walbaum, 1792) (Salmonidae) from British Columbia, Canada. However, this species identification of the parasite is doubtful (Gibson, 1996).

Life cycle. A natural first intermediate host is *Limnoperna fortunei* in the Uji and Seta rivers (Baba and Urabe, 2011a; Baba *et al.*, 2012; Baba, 2013). Neither sporocysts nor cercariae have yet been described.

Natural second intermediate hosts, chiefly in the body muscles of which metacercariae encyst, are freshwater fishes of many species (chiefly cyprinids) in the Yodo and Uji rivers (Ogawa *et al.*, 2004; Baba and Urabe, 2011a, b; Baba, 2013). Metacercaria B of Ogawa *et al.* (2004, fig. [3b], fig. 4d) is assigned to *Pr. ozakii* because of a long excretory vesicle (see also Urabe, Ogawa, Nakatsugawa, Nakai *et al.*, 2007). Metacercariae have not yet been fully described.

Natural final hosts, usually in the intestine of which adults develop, are *S. biwaensis* in the Seta, Uji and Yodo rivers (Urabe, Ogawa, Nakatsugawa, Nakai *et al.*, 2007; Baba and Urabe, 2011a; Baba, 2013; this paper), *S. asotus* in the Uji River (Baba and Urabe, 2011a; Baba, 2013) and *S. lithophilus* in the Seta River (Baba, 2013). The entire life cycle is as yet unknown in Korea, China and northern Vietnam.

Key to the genera and species of the Bucephalidae in this paper

1.1. Ovary post-testicular; vitelline follicles in middle of body; excretory vesicle extending slightly beyond cirrus pouch. . . . . *Parabucephalopsi parasiluri*

1.2. Ovary pretesticular; vitelline follicles immediately posterior to rhynchus; excretory vesicle extending to near rhynchus. . . . . *Prosorhynchoides ozakii*

Discussion on the Bucephalidae in this paper

When I wrote a review on adult digeneans of freshwater fishes of Japan (Shimazu, 1999, 2003), no bucephalid species had been recorded from Japan. However, the two bucephalid species *Parabucephalopsis parasiluri* and *Prosorhynchoides ozakii* have since established themselves in the Seta, Uji and Yodo rivers. *Parabucephalopsis parasiluri* has already invaded Lake Biwa possibly through the Seta River, because sporocysts were molecularly identified from *Limnoperna fortunei* collected at Otsu City, Shiga Prefecture (Baba and Urabe, 2011a; Baba *et al.*, 2012; Baba, 2013). Neither adults nor metacercariae have as yet been recorded from Lake Biwa (Shimazu *et al.*, 2011; Baba, 2013).

Urabe *et al.* (2001) first briefly mentioned adults of an unidentified bucephalid in *Silurus biwaensis*, metacercariae in cyprinid fishes and cercariae in *Limnoperna fortunei* from the Uji River (see also Urabe, 2002). Ogawa *et al.* (2004) briefly described two types of bucephalid metacercariae found in cyprinids from the Uji River. Later, Urabe, Ogawa, Nakatsugawa, Nakai *et al.* (2007) described adults found in *S. biwaensis* from the Uji River under the species names *Parabucephalopsis parasiluri* and *Prosorhynchoides ozakii.*

In the Uji River, metacercariae were first detected in cyprinids in 1999–2000, and adults were first found in *S. biwaensis* in 2000 (Urabe *et al.*, 2001; Urabe, Ogawa, Nakatsugawa, Nakai *et al.*, 2007). It is thought that the two bucephalid species were introduced artificially and accidentally, together with their common first intermediate host *Limnoperna fortunei*, from China into the Uji River as a contaminant of edible corbiculid bivalves (Baba *et al.*, 2012; Baba, 2013), possibly not once but repeatedly (Shimazu *et al.*, 2011). *Limnoperna fortunei* appeared in Lake Biwa in about 1992 and then in the Yodo River system (including the Seta and Uji rivers) in about 1994 (Urabe *et al.*, 2001). Probably, some individuals of *L. fortunei* that had acquired infection with sporocysts of the two bucephalid species in China were mixed in some time ago, so that eventually the parasites also have settled themselves in the Uji River and then in the Seta River (Urabe *et al.*, 2008; Urabe *et al.*, 2009; Baba and Urabe, 2011a; Baba, 2013).

Wang (1985) described *Parabucephalopsis parasiluri* Wang, 1985 in the genus *Parabucephalopsis* Tang and Tang, 1963. The type species of the genus is *Parabucephalopsis prosthorchis* Tang and Tang, 1963 (Tang and Tang, 1964b; Tang and Tang, 1976; Overstreet and Curran, 2002).

Tang and Tang proposed a new genus and four new species of gasterostomes (bucephalids) at the National Congress of Parasitology held in Peking in 1963 (Tang and Tang, 1976). With regard to the new genus and its type species, only "*Parabucephalopsis prosthorchis* gen. et sp. nov." and the two names of its final hosts appear in the published abstract of their presentation at the Congress (Tang and Tang, 1964b). Tang and Tang (1976) referred to only the published abstract concerning the new genus and its type species. This means that none of the new generic and species names had been published anywhere until then. Even though they appeared in Tang and Tang's material for the Congress, such a material does not constitute a published work (Article 9.10 of the Code) (ICZN, 2012). Therefore, neither the generic name *Parabucephalopsis* Tang and Tang, 1963 nor the species name *Parabucephalopsis prosthorchis* Tang and Tang, 1963 was published, and so they deem to be unavailable. In addition, the generic name *Parabucephalopsis* Tang and Tang, 1964 and the species name *Parabucephalopsis prosthorchis* Tang and Tang, 1964 are *nomina nuda*, because neither of them was accompanied by a description or definition (Article 13.1.1 of the Code) (ICZN, 1999).

Although the generic name *Parabucephalopsis* Tang and Tang, 1963 is unavailable, the species name *Parabucephalopsis parasiluri* Wang, 1985 is available (Article 11.9.3.1 of the Code) (ICZN, 1999).

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