Digeneans Parasitic in Freshwater Fishes (Osteichthyes) of Japan. I.
Aporocotylidae, Bivesiculidae and Haploporidae

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Abstract Adult digenetic trematodes (digeneans) parasitic in freshwater fishes (Osteichthyes) of Japan are reviewed in a series of several papers. The current knowledge of each of the digenean species is provided from the existing specimens and literature, including a morphological description with figures, lists of its synonyms, hosts, localities and existing specimens, and a summary of its life cycle where known. The Aporocotylidae Odhner, 1912 (Sanguinicola ugui, Sanguinicola hasegawai sp. nov. and Sanguinicola sp.), Bivesiculidae Yamaguti, 1934 (Bivesicula sp.) and Haploporidae Nicoll, 1914 (Carassotrema koreanum) are reviewed. Sanguinicola hasegawai sp. nov. is described and figured on the basis of specimens found in the blood vessels and heart of Barbatula toni (Dybowski, 1869) (Nemacheilidae) from Hokkaido.

Key words: digeneans, freshwater fishes, Japan, review, Aporocotylidae, Bivesiculidae, Haploporidae, Sanguinicola hasegawai sp. nov.

Introduction
I wrote two reviews on adult digenetic trematodes (digeneans) parasitic in freshwater fishes (Osteichthyes) of Japan (Shimazu, 1999, 2003). Since then, several papers of taxonomic importance have been published in this field. The two previous reviews contain neither morphological descriptions nor figures for any of the digenean species.

This new review will be published in a series of several papers. The current knowledge of each of the digenean species from the existing specimens and literature will be provided, including a morphological description with figures, lists of its synonyms, hosts, localities and existing specimens and a summary of its life cycle where known. Keys to genera in families and species in genera will be given if necessary. In a final paper, a general discussion on the digenean fauna of Japanese freshwater fishes, host-parasite and parasite-host lists and a key to the families will be added.


In Japan, 150–170 species of freshwater fishes (mostly Osteichthyes) are distributed (Goto, 1987). Exorchis oviformis Kobayashi, 1915 was the first adult digenean that was reported from a freshwater fish in Japan (Shimazu, 1999, 2003). Since then, adult digeneans of Japanese freshwater fishes have been studied by many workers (e.g., H. Kobayashi, T. Fujita, Y. Ozaki, S. Yamaguti, S. Takahashi, S. Eguchi, T. Hasegawa, N. Ishii, T. Fujino, F. Moravec, T. Shimazu, M. Urabe and others). Their published papers will be cited in the respective species of digeneans (see also
Shimazu, 1999, 2003). Early studies, which were devoted chiefly on taxonomy and life cycle, prospered after 1915, reached to the heyday in early 1920 to late 1930, and have been gradually declining since (Shimazu, 1999, 2003). Y. Ozaki’s works were compiled by Shimazu (1995); and S. Yamaguti’s, by Kamegai and Ichihara (1972). Shimazu (1999, 2003) counted at least 41 species in 20 genera and 12 families of digeneans reported until then. Shimazu et al. (2011) recorded 24 previously known, 2 new and 4 unidentified species in 17 genera and 12 families of digeneans from fishes from the Lake Biwa basin, Shiga Prefecture. Studies of the taxonomy, phylogenetic relationship, life cycle, geographical distribution and others of some species of digeneans have recently been in progress from not only morphological but also molecular data. In addition, cercariae and metacercariae have been extensively studied in Japan from the viewpoint of medical importance (Ito, 1964; Komiya, 1965). Further studies of them would prove that the names of some of them have preoccupied the names of some adult digeneans, e.g., *Cercaria gotoi* Ariake, 1922 and *Azygia anguillae* Ozaki, 1924 in *Azygia gotoi* (Ariake, 1922) Shimazu, 1979. In *Life cycle* of each species, the life cycle will be summarized where known, and a possible cercaria or metacercaria will be referred to.

During the above-mentioned studies, many adult digeneans were described as new species. Holotypes were designated for some of them, but not for others. Many of the original specimens including syntypes and holotypes exist: Dr. T. Fujita’s Collection (Fujita’s Collection) deposited in the Hokkaido University Natural History Museum (HUNHM), Sapporo, Hokkaido; Dr. S. Yamaguti’s Collection (Yamaguti’s Collection) and Dr. Y. Ozaki’s Collection (Ozaki’s Collection) in the Meguro Parasitological Museum (MPM), Tokyo; and the collections of T. Shimazu and M. Urabe in the National Museum of Nature and Science (NMNS, collection code name NSMT-Pl), Tsukuba, Ibaraki Prefecture, formerly the National Science Museum, Tokyo.

Y. Ozaki deposited a total of 38 holotypes of his new species of platyhelminth parasites including digeneans in the collection of the Zoological Institute, Science Faculty, Tokyo Imperial University, Tokyo (Shimazu, 1995). This collection has been transferred to the Department of Zoology, The University Museum, The University of Tokyo, Tokyo. The latter collection today includes no holotypes of helminth parasite species including Ozaki’s ones (Shimazu and Araki, 2006). Obviously, all the holotypes of helminth parasite species deposited in the Zoological Institute were lost. Furthermore, none of the original specimens of some other workers could be traced for reexamination. Probably, they were also lost (see also Shimazu et al., 2011). Neotypes will be designated for some digeneans species if necessary and possible in this work.

### Materials and Methods

In the present work, the classification of the subclass Digenea Carus, 1863 in the class Trematoda Rudolphi, 1808 is fundamentally based on that in the series entitled "Keys to the Trematoda" (Volume 1 edited by Gibson et al., 2002; Volume 2 edited by Jones et al., 2005; and Volume 3 edited by Bray et al., 2008). Some of the technical terms used are different from those used in the series. Most of the family and species names of host fishes are given according to Nakabo (2013) and Eschmeyer (2013).

Synonym lists contain only the references for the original descriptions, subsequent descriptions of taxonomic importance from Japan in particular and establishments of new combinations. In *Geographical distribution*, only records of immature (juvenile) and mature (adult) worms from freshwater fishes are presented. The locality name “Japan” means that parasites were certainly obtained in Japan, but their exact localities have not yet been determined.

Specimens had been prepared by various methods, either whole-mounted in Canada balsam or serially sectioned. Many of them were borrowed from Fujita’s Collection (HUNHM),
Yamaguti's and Ozaki's Collections (MPM), the collection of the NMNS, Tokyo then, and the personal collection of M. Urabe. I also used some of the specimens deposited in the Lake Biwa Museum (LBM), Kusatsu, Shiga Prefecture, for this review while preparing the paper by Shimazu et al. (2011). Newly collected specimens of mine were also used, and they have been deposited in the NMNS.

Measurements (length by width) are given in millimeters unless otherwise stated. The body size is indicated as follows: very small, –1 mm long; small, 1–3; fairly small, 3–7; medium, 7–12; fairly large, 12–20; large, 20–35; and very large, 35– (Dawes, 1956). The sucker width ratio is the ratio of the width of the oral sucker to that of the ventral sucker. Most of the drawings were made with the aid of a camera lucida.

The name of the Japanese journal Dobutsugaku Zasshi is cited as merely Dobutsugaku Zasshi in this work, though it was cited as Zoological Magazine (Japan) (Dobutsugaku Zasshi) in some of my previous papers.

**Class** Trematoda Rudolphi, 1808  
**Subclass** Digenea Carus, 1863  
**Superfamily** Schistosomatoidea Stiles and Hassall, 1898  
**Family** Aporocotylidae Odhner, 1912  
**Genus** Sanguinicola Plehn, 1905  
**Sanguinicola ugui** Shimazu, 2007  
(Figs. 1–4)  

*Sanguinicola ugui* Shimazu, 2007: 2–4, figs. 1–6.

**Host in Japan.** *Tribolodon hakonensis* (Günther, 1877) (Cyprinidae) (type host) (Shimazu, 2007; this paper).

**Sites of infection.** Blood vessels and heart: afferent branchial artery of gills, thick branches of hepatic artery of liver, and undetermined blood vessel of kidneys; and arterial bulb of heart (Shimazu, 2007; this paper).

**Geographical distribution.** Nagano Prefecture: Hiroi River (type locality) at Kotobuki, iyama City; Lake Suwa at Suwa City; Tenryu River at Ina City; and Sai River at Akashina-Nakagawate, Azumino City (Shimazu, 2007; this paper).


**Description** (Figs. 1–3). After Shimazu (2007), altered slightly from the present study. Body flat dorsoventrally, elongate, widest at level of anterior part of testis, pointed at anterior extremity, gradually narrowing posteriorly but rounded at posterior extremity, small, 1.29–1.67 by 0.19–0.35 (holotype 1.32 by 0.24) (Fig. 1). Anterior proboscis absent. Testegument spino; fine setae may be present throughout body; ventrolateral spines present, short transverse rows of one to five spines forming one longitudinal row from near anterior extremity of body to midlevel of cirrus pouch on either side of body, one each in anterior first to sixth transverse rows, thick, 6–8 μm long, two to five (usually four) each in...
remainder, slender, 14–22 μm long (Fig. 2). Nerve chords conspicuous; transverse nerve commissure dorsal to esophagus, 10–12% of body length from anterior extremity. Oral and ventral sucker absent. Mouth small, slightly ventrally subterminal. Small globular sphincter- or sucker-like structure present around mouth, about 6–9 μm in diameter (Fig. 2). Esophagus narrow, slender, forming fusiform pharynxlike thickening measuring 0.06–0.08 by 0.02–0.04 slightly anteriorly to transverse nerve commissure, surrounded by gland cells anteriorly to ceca (Figs. 1–2). Intestine X-shaped, consisting of usually four but rarely five to six short ceca,
21–23% of body length from anterior extremity. Testis single, elongated longitudinally, median, between ceca and ovary, 0.44–0.60 by 0.09–0.19, with 21–25 lateral lobes on either side. Sperm flowing in dorsal bundle in each lobe, bundles joining together into dorsal median bundle running posteriorly within testis from anterior end of testis to posterior, these bundles apparently lacking any kind of duct. Sperm duct single, running posteriorly from median point of posterior margin of testis to cirrus pouch, ventral to ovary. Cirrus pouch spindle- or club-shaped, thin-walled, 0.14–0.16 by 0.05–0.07, sinistrally submedian, postovarian, directed posteriorly, surrounded by small gland cells, including seminal vesicle and large prostatic cells (Fig. 3). Seminal vesicle club-shaped, thin-walled. Small globular pars prostatica may be differentiated immediately before short eversible ejaculatory duct (cirrus interpreted in Shimazu, 2007). Male genital pore dorsal, sinistrally submedian, some distance from posterior extremity of body, lined with one-layered cuboidal cells, without sphincter around it.

Ovary single, bilobed laterally in shape of butterfly, 0.14–0.19 by 0.09–0.14; isthmus 65–71% of body length from anterior extremity. Oviduct originating from posterior margin of ovarian isthmus, passing posteriorly, slightly undulating, dorsal to sperm duct and vitelline duct, including sperm (acting as oviductal seminal receptacle). Seminal receptacle and Laurer’s canal absent. Ootype spherical, lined with one-layered columnar cells, 0.03–0.04 in diameter. Large gland cells (Mehlis’ gland (?)) seen posterior to ootype, possibly emptying into ootype. Uterus club-shaped, short, 0.06–0.10 by 0.03–0.05, directed posteriorly, bending dorsally at anterior end, lined with one-layered cuboidal cells, surrounded by small gland cells. Sphincter present between ootype and uterus; metraterm not seen. Female genital pore dorsal, dextrally submedian, antero-lateral to male genital pore, with well-developed sphincter around it, surrounded by small gland cells. Eggs triangular, one in ootype and 1–7 in uterus if present, 22–34 by 16 μm (collapsed in balsam), not operculate, not embryonated. Vitellaria follicular, follicles small, profuse mostly between nerve chords from pharynx-like thickening to cirrus pouch, some present laterally to nerve chords, almost confluent anteriorly, separately posteriorly. Common vitelline duct single, beginning at posterior end of pharynx-like thickening, median, in ventral parenchyma; short vitelline ducts joining this duct in places; vitelline reservoir postovarian, in ventral parenchyma, uniting with oviduct to form short ovovitelline duct entering ootype dorsally. Excretory vesicle V-shaped, small, far posterior to ootype; excretory pore posteroterminal.

Excretory system. In living adult specimens, excretory system found arranged asymmetrically (Fig. 1). Right arm of excretory vesicle longer than left. Right main collecting canal long, divided into two short collecting canals between transverse nerve commissure and ceca; left short, divided into two short collecting canals laterally to cirrus pouch. Each collecting canal with one flame cell. Flame cell formula $2 \cdot [(1 + 1)] = 4$.

Eggs (Fig. 4). Adults laying unembryonated triangular eggs. Fully embryonated eggs triangular, 40–48 by 34–40 μm in life. In serial sections of liver and heart, eggs found in capillaries (or sinusoids) of lobules of liver, capillaries of cardiac muscle of atrium and ventricle of heart, and capillaries of smooth muscle of arterial bulb of heart, surrounded by granulomatous tissue of host origin of various sizes, usually one egg but rarely two to three eggs per granuloma. No eggs found in gill filaments. Miracidia 30–32 by 18 μm in life, no hatched miracidia seen in either blood vessels or heart.

Remarks. The present study shows that the tegument may be covered by fine cetae throughout the body; the esophagus is surrounded by small gland cells anteriorly to the ceca; and the ovary is slightly larger than described and figured by Shimazu (2007), 0.14–0.19 long (Figs. 1–2) instead of 0.12–0.17 long (Figs. 1–2). In addition, Shimazu (2007) misinterpreted the arterial bulb as the ventricle and the afferent branchial artery as the efferent branchial artery. In the figure legend (Shimazu, 2007: 3), “0.2 mm” in Figs. 3 and...
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Prevalence and intensity. Not recorded.

Type specimens. (1) Holotype (NSMT-Pl 5834), adult, hot formalin-fixed, whole-mounted, ex atrium of Barbatula toni, Mamachi River, 20 May 2011. (2) 13 paratypes: 4 (NSMT-Pl 5833), adult, flattened, whole-mounted, ex liver of B. toni, Mamachi River, 18 April 2011; 2 (NSMT-Pl 5835) and 5 (NSMT-Pl 5836), adult, hot formalin-fixed, whole-mounted, ex liver of B. toni, Mamachi River, 20 May 2011 and 9 August 2011, respectively; 2 (NSMT-Pl 5837), adult, hot formalin-fixed, whole-mounted, ex atrium of B. toni, Izari River, 1 June 2011.

Vouchers. 1 specimen (NSMT-Pl 5838), adult, hot formalin-fixed, whole-mounted, ex atrium of B. toni, Mamachi River, 20 May 2011; 5 (NSMT-Pl 5839), adult, hot formalin-fixed, whole-mounted, ex atrium of B. toni, Izari River, 1 June 2011; serial sections of livers (NSMT-Pl 5840–5841) and hearts (NSMT-Pl 5842, 5844) of B. toni, Mamachi River, 9 August 2011; and egg masses (NSMT-Pl 5843), ex atrium of B. toni, Mamachi River, 9 August 2011.

Etymology. The specific name hasegawai is dedicated to Dr. Koh Hasegawa, who generously collected the host fish several times for the present study.

Description (Figs. 5–7). Based on the type series; 10 specimens including holotype measured. Similar to the foregoing Sanguinicola ugui in general morphology. Body flat dorsoventrally, elongate, widest at level of anterior part of testis, rounded at anterior extremity, gradually narrowing posteriorly but rounded at posterior extremity, small, 0.76–1.13 by 0.19–0.30 (holotype 1.13 by 0.30) (Fig. 5). Fine setae may be present throughout body; ventrolateral spines somewhat curved, lying transversely, large, 8–24 by 2–3μm, becoming smaller especially in part of body anterior to transverse nerve commissure, forming single longitudinal row from near anterior extremity to midlevel of cirrus pouch (Figs. 5–6). Transverse nerve commissure 0.12–0.15% of body length from anterior extremity. Sucker-like structure present around mouth, about 8μm
in diameter. Pharynx-like thickening of esophagus 0.05–0.08 by 0.02–0.03, immediately anterior to transverse nerve commissure. Small gland cells surrounding esophagus anteriorly to ceca. Ceca four, short, 0.05–0.11 by 0.08–0.17, 29–33% of body length from anterior extremity. Testis 0.20–0.29 by 0.10–0.19, with 9–11 lateral lobes on either side. Cirrus pouch club-shaped, curved in S-shape, thin-walled, 0.11–0.17 by 0.03–0.05. Seminal vesicle club-shaped, thin-walled. Pars prostatica not clearly seen. Ejaculatory duct may be differentiated, short, eversible (Fig. 7). Male genital pore dorsal, sinistrally submedian, midway in postovarian region of body, encircled by
sphincter, surrounded by small gland cells. Ovary bilobed laterally, 0.05–0.08 by 0.09–0.17, lobes globular or slightly irregular in outline; isthmus 62–68% of body length from anterior extremity. Ootype spherical, 0.03–0.05 in diameter, almost median, posterior to male genital pore. Large cells (Mehlis’ gland (?)) seen posterior to ootype, possibly emptying into ootype. Uterus clavate, short, 0.05–0.11 by 0.04–0.06. Sphincter present between ootype and uterus. Female genital pore dorsal, dextrally submedian or median, anterior to male genital pore, encircled by well-developed sphincter. Eggs elliptical, thin-shelled, pale-white, not operculate, one in ootype and 1–13 in uterus if present, 27–32 by 13–17 μm, not embryonated. Vitelline follicles profuse mostly between nerve chords from midlevel of pharynxlike thickening to midlevel of cirrus pouch, some present laterally to nerve chords, almost confluent anteriorly, separate posteriorly. Common vitelline duct beginning at midlevel of pharynxlike thickening. Excretory vesicle V-shaped, small, far posterior to ootype; excretory pore posterterminal.

Excretory system. In living adult specimens, excretory system found arranged asymmetrically (Fig. 5). Right arm of excretory vesicle longer than left. Right main collecting canal long, divided into two short collecting canals between transverse nerve commissure and ceca; left short, divided into two short collecting canals laterally to cirrus pouch. Each collecting canal with one flame cell. Flame cell formula 2 [(1 + 1)] = 4 or 2 [(2)] = 4

Eggs (Figs. 8–9). Adults laying unembryonated elliptical eggs. Fully embryonated eggs elliptical, 49–60 by 27–36 μm; miracidia ciliated, 40–48 by 19–25 μm (Fig. 8). Central stylet near anterior extremity of body, 14 by 1 μm; four small rodlets on either side of it, 5 by 2 μm. Eye-spot 8–9 by 5–6 μm, median, slightly pre-equatorial, tightened in middle. Germ cell ball globular, 16–21 μm in diameter, near posterior extremity of body. A pair of flame cells anterior to germ cell ball. No hatched miracidia seen in blood vessels and heart.

After being laid, two or more eggs agglutinated by host cells into masses, both solitary eggs and egg masses encapsulated by host cells in a few layers of various shapes and sizes (Fig. 9A, B) at least in heart and liver, but not in gills: in heart, in lumen of atrium, ventricle and arterial bulb and in lumen of branches of coronary artery; in liver, in lumen of thick branches and arterioles of hepatic artery. No new granulomatous tissue observed around encapsulated eggs.
even in arterioles.

Remarks. *Sanguinicola hasegawai* sp. nov. most closely resembles *Sanguinicola megalobrane* Li, 1980 found in *Megalobrama amblycephala* Yih, 1955 (Cyprinidae) from Hubei Province, China (Li, 1980), in having a single longitudinal row of the ventrolateral tegumental spines on either side of the body, an asymmetrically arranged excretory system and elliptical eggs. However, it differs from the latter in having a smaller body, 0.76–1.13 by 0.19–0.30 instead of 1.23–1.74 by 0.23–0.42; no long setae at the posterior part of the body; four short ceca instead of one cecum; fewer lateral lobes of the testis, 9–11 instead of 18–22 on either side; and larger fully embryonated eggs, 49–60 by 27–36 μm instead of 29.5–35.7 by 18.7–20.4 μm.

The asymmetrically arranged excretory system is also known in *Sanguinicola idahoensis* Schell, 1974 found in *Oncorhynchus mykiss* (Walbaum, 1792) (syn. *Salmo gairdneri* Richardson, 1836) (Salmonidae) from Idaho, USA (Schell, 1974); and *S. ugui* found in *Tribolodon hakonensis* (Shimazu, 2007; this paper). *Sanguinicola idahoensis* has elliptical eggs but a larger body, 1.2–2.3 by 0.3–0.5; and more lateral lobes of the testis, 14–18 on either side. *Sanguinicola ugui* has a single longitudinal row of short transverse rows of one to five ventrolateral spines on either side of the body; more lateral lobes of the testis, 21–25 on either side; and triangular eggs.

Adults were found in the lumen of thick branches of the hepatic artery of the liver and the lumen of the atrium, ventricle and arterial bulb of the heart of the host fish. Solitary eggs and egg masses were found encapsulated by host cells in a few layers: in the liver, in the lumen of thick branches and arterioles of the hepatic artery, but not in the lobules; in the heart, in the lumen of the atrium, ventricle and arterial bulb and in the lumen of the branches of the coronary artery. Eggs became fully embryonated in the capsules. No new granulomatous lesions were observed around the encapsulated eggs even in the arterioles. These findings suggest that, after being deposited at the sites of infection of adults, eggs stand solitarily or two or more eggs become agglutinated into masses by host cells; the solitary eggs and egg masses become encapsulated by host cells; these encapsulated solitary eggs and egg masses are carried by blood flow into the arterioles of the liver and the branches of the coronary artery of the heart to clog them as emboli. It appears that no new granulomas of host origin are added around the emboli. In this histopathology, the present new species differs from some other species of *Sanguinicola*, in which solitary naked eggs are carried by blood flow into the capillaries to lodge there as emboli and then granulomatous tissue of host origin develops around them to occlude the capillaries (see *S. ugui* in this paper). It is not known how fully embryonated eggs or hatched miracidia exit the host fish into the water.

Life cycle. Not known.

*Sanguinicola* sp. of Shimazu, 1999

*Sanguinicolidae* gen. sp.: Shimazu, 1999: 67.

Host in Japan. *Acheilognathus tabira tabira* Jordan and Thompson, 1914 (Cyprinidae) (Shimazu, 1999).


Remarks. An immature specimen of *Sanguinicola* sp. was found in the gill of *A. tabira tabira* on 3 June 1980 (Shimazu, 1999, 2003; Shimazu et al., 2011). This specimen was lost before morphological observations for species identification and description could be undertaken.

Life cycle. Not known.

Discussion on *Sanguinicola*

In *Sanguinicola*, sanguinicolid [or lophocercous-brevifurcate-apharyngeate] cercariae are produced in sporocysts and rediae [?] in molluscan intermediate hosts, and cercariae directly penetrate the skin of fish final hosts (Smith,
Several species of sanguinicolid cercariae have been reported in Japan: *Cercaria andoi* Faust, 1924 and some similar cercariae in *Semisulcospira* spp. (Pleuroceridae); *Cercaria senoi* Faust, 1924 in *Viviparus malleatus* Reeve, 1863 (Viviparidae) and a similar cercaria in *Bulimus manchouricus japonicus* (now *Parafossarulus manchouricus japonicus* (Pilsbry, 1901)) (Stenothyridae); *Cercaria cristhophora* Ito, 1978 in *Austropeplea ollula* (Gould, 1859) and *Lymnaea japonica* (Jay, 1857) (Lymnaeidae) and a similar cercaria in *L. japonica*; and a cercaria in *Sinotaia quadrata historica* (Gould, 1859) (Viviparidae) (Ito, 1964, 1988; Shimazu, 2007). This suggests that, besides *S. ugui* and *S. hasegawai*, some unpublished species of *Sanguinicola* also occur in Japan, though the cercariae themselves need further critical studies.

Judging from my experience, it is not easy to foresee, while examining fish for parasites, which fish harbors small worms of *Sanguinicola* in organs or tissues, especially in case no granulomas caused by laid eggs are seen in the gill filaments as in *S. ugui* and *S. hasegawai*. Fish should be examined as soon as possible after killing, or worms would disappear (perhaps collapse) from the heart and blood vessels. The heart and liver of the fish should be examined first. Worms should be fixed as soon as possible after collecting, or they would disappear (perhaps collapse) in saline in a short time.

The name and authorship of the family, to which *Sanguinicola* belongs, is Aporocotylidae Odhner, 1912 (Bullard *et al.*, 2009), not Sanguinicicolidae von Graff, 1907 (Smith, 2002a, b).

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### Key to two species in *Sanguinicola* in this paper

1.1. Ventrolateral tegumental spines arranged in a single longitudinal row on either side of body; testis having 9–11 lateral lobes on either side; eggs elliptical ............................ *S. hasegawai* sp. nov.

1.2. Ventrolateral tegumental spines arranged in a single longitudinal row of short transverse rows of 1–5 spines on either side of body; testis having 21–25 lateral lobes on either side; eggs triangular ................................................................................................................................................... *S. ugui*

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**Superfamily Bivesiculoidea** Yamaguti, 1934  
**Family Bivesiculidae** Yamaguti, 1934  
**Bivesicula** sp. of Shimazu, 1994  
(Fig. 10)

*Bivesicula* sp.: Shimazu, 1994: 17, 19, fig. 1.

*Host in Japan.* Monopterus albus (Zuiew, 1793) (Synbranchidae) (Shimazu, 1994).  
*Site of infection.* Intestine (Shimazu, 1994).  
*Geographical distribution.* Okinawa Prefecture: Naha (Shimazu, 1994).  

*Description* (Fig. 10). After Shimazu (1994), slightly altered from the present study. Body flattened dorsoventrally, broad-ovate, rounded anteriorly, attenuated posteriorly, small, 1.95–2.00 by 1.25–1.30. Tegmental spines not seen (most likely lost by maceration of body). Eyespot pigment fine, dispersed in forebody. Transverse nerve commissure dorsal to middle of esophagus. Oral and ventral suckers absent. Mouth anter terminal. Prepharynx not seen. Pharynx globular, 0.12 by 0.14–0.16. Esophagus 0.28–0.31 long, thicker-walled in anterior third (0.08 by 0.02–0.03), surrounded by small gland cells, bifurcating at about junction of first and second fourths of body. Intestinal ceca terminating blindly at about midlevel of testis. Testis single, entire, 0.35–0.43 by 0.43–0.51, median, in third fourth of body. Sperm ducts two, diverging from right
and left anterolateral margin of testis, running dorsally to cirrus pouch. External seminal vesicle oblong, 0.16 by 0.06–0.09, anterodorsal to cirrus pouch. Cirrus pouch elliptical, thin-walled, 0.43–0.50 by 0.35–0.39, median, slightly pre-equatorial, pretesticular. Internal seminal vesicle spherical, constricted posteriorly, 0.16–0.19 by 0.09–0.14. Male terminal duct complex, extending backward. Pars prostatica thick, 0.10–0.12 by 0.09–0.10, with two diverticula, surrounded by numerous prostatic cells. Ejaculatory duct thick, 0.10 long, surrounded by numerous small gland cells slightly smaller than prostatic cells. Genital atrium may be fairly long. Genital pore on posterior margin of cirrus pouch. Ovary almost globular, 0.16–0.18 by 0.15–0.16, pretesticular, dextrally submedian, posterolateral to cirrus pouch, about level with genital pore. Ovarian complex between ovary and testis, not observed in more detail. Uterus coiled a few times between two
arms of excretory vesicle from near posterior extremity of body to midlevel of cirrus pouch, embracing testis dextrally, acting as uterine seminal receptacle in proximal portion; metraterm not observed clearly. Eggs fairly numerous, thick-shelled, operculate, light brown, 90–100 by 40–60 μm (collapsed), not embryonated. Vitellaria follicular, follicles distributed from midlevel of esophagus to midlevel of testis along ceca, or slightly beyond testis, confluent anteriorly to cirrus pouch, separate posteriorly; most follicles in dorsal and lateral fields of body; some extending ventral to excretory vesicle and ceca. Excretory vesicle V-shaped, large, in ventral parenchyma; arms reaching to midlevel of esophagus; excretory pore posteroterminal.

Remarks. These specimens are somewhat similar to *Bivesicula synodi* Yamaguti, 1938, a marine species, described from *Synodus japonicus* (Houttuyn) [sic, now *Synodus ulae* Schultz, 1953 (Synodontidae) (?)] at Numazu, Shizuoka Prefecture (Yamaguti, 1938); but they are different from the latter in several morphological respects (Shimazu, 1994). They have remained unidentified.

Life cycle. Not known.

Discussion on *Bivesicula*

Species of *Bivesicula* have previously been recorded from marine fishes (Cribb, 2002). *Monopterus albus* is a freshwater fish. It is quite unknown at present whether the present specimens represent a freshwater species of *Bivesicula*. Shimazu (1994) discussed the infection route of the parasite to the host. The freshwater fish *M. albus* devours worms, worms, crustaceans and other small aquatic animals. If the present specimens belong to a marine species, the occurrence in *M. albus* suggests that *M. albus* acquired infection with this parasite by eating some marine fish or animals that had transferred juvenile or adult worms within them from sea to fresh water. It seems unlikely that planktonic free-living cercariae are directly ingested by *M. albus* after entering fresh water. Otherwise, the host may have been artificially fed on marine fish harboring worms.
Figs. 11–17. *Carassotrema koreanum*, adults found in intestine of *Carassius* sp. and life cycle. — 11, Entire body, ventral view; 12, terminal genitalia, ventral view; 13, ovarian complex, ventral view; 14, fully embryonated egg; 15, [most likely daughter] redia; 16, cercaria; 17, encysted metacercaria. Figs. 14–17 redrawn from Tang and Lin (1979). Scale bars: 0.5 mm in Figs. 11 and 15; 0.3 mm in Fig. 12; 0.1 mm in Figs. 13 and 16; 0.05 mm in Fig. 17; 0.03 mm in Fig. 14.

**Description** (Figs. 11–13). After Shimazu (2005), slightly altered from the present study. Body elongate-oval, small, 1.52–2.26 by 0.60–0.85; forebody 0.51–0.72 long, occupying 32–38% of body length. Tegument spinose, spines dense in anterior part of body, becoming sparser posteriorly. Eyespot pigment scattered on both sides of prepharynx. Numerous large glands cells of two types (with fine and coarse granular cytoplasm, respectively) seen in ventral and dorsal parenchyma, especially in forebody. Transverse nerve commissure dorsal to prepharynx. Oral sucker subglobular, 0.17–0.23 by 0.19–0.24, ventrally subterminal; its rim and inner wall bearing small spines. Prepharynx present, 0.05–0.12 long [small gland cells not seen between oral sucker and prepharynx]. Pharynx globular, large, 0.14–0.19 by 0.14–0.17. Esophagus longer than pharynx, 0.16–0.29 long, surrounded by small gland cells, bifurcating dorsally to ventral sucker. Small gland cells massed together between pharynx and esophagus. Intestinal ceca ending blindly at level of posterior margin of testis or slightly farther, but not reaching to posterior extremity of body. Ventral sucker subglobular, 0.21–0.26 by 0.25–0.30, at about junction of anterior and middle thirds of body; its rim and inner wall bearing small spines; sucker width ratio 1:1.2–1.3. Testis single, cordate, irregularly indented, large, 0.39–0.62 by 0.26–0.55, almost intercecal, located some distance anterior to posterior extremity of body. Sperm ducts two, arising from respective anterolateral corners of testis; common sperm duct absent. External seminal vesicle large, retort- to club-shaped, between ventral sucker and ovary. Hermaphroditic sac elongate, fairly thick-walled, muscular, longer than ventral sucker, 0.23–0.39 by 0.15–0.22, usually dextral or sinistral but rarely dorsal to ventral sucker, usually bent backward in anterior part, containing internal seminal vesicle, prostatic complex, ejaculatory duct and hermaphroditic duct. Internal seminal vesicle elongate, connecting to pars prostatica with short inverted U-shaped male duct. Pars prostatica oblong, small, surrounded by weakly developed prostatic cells. Ejaculatory duct short, entering lateral wall of metraterm at junction of metraterm and hermaphroditic duct, projecting (forming cirrus of Park, 1938) into hermaphroditic duct. Hermaphroditic duct long, everted, surrounded by small gland cells, apparently being continuation of metraterm in structure. Genital atrium small. Genital pore usually slightly dextral to esophagus between pharynx and ventral sucker. Ovary single, reniform to pyriform, 0.15–0.32 by 0.08–0.16, median or submedian, immediately pretesticular. Ovarian complex anteromedial [not anterolateral] to ovary. Oviduct dilated to contain sperm. Laurer’s canal proceeding backward obliquely to open dorsally at ovarian level, dilated in proximal portion to contain sperm (as rudimentary seminal receptacle). Seminal receptacle absent. Ootype small, may not be vesicular; Mehlis’ gland large, massive. Uterus coiled a few times on both sides of external seminal vesicle between testis and ventral sucker, overlapping ceca; uterine seminal receptacle well developed; metraterm well developed in hermaphroditic sac. Uterine eggs not numerous, thin-shelled, operculate, light brown, 56–62 by 34–42 μm (collapsed), with small hooklike projection on anteroopercular pole, not embryonated. Vitellaria follicular, follicles elliptical to tubular, rarely branched in parts, spreading in lateral fields of hindbody between level of intestinal bifurcation and posterior extremity of body, ventral and dorsal to intestines, separate anteriorly, confluent post-testicularly; vitelline ducts transverse, ventral to ceca, dorsal to testis; common vitelline duct running forward. Excretory vesicle Y-shaped; stem dorsal to testis, bifurcating dorsally to anterior part of testis; arms in ventral parenchyma, extending anteriorly to level of ventral sucker; excretory pore posterodorsal.

**Remarks.** Park (1938) described *Carassotrema koreanum* as a new genus and species on the basis of adults found in the alimentary canal of *Carassius auratus* (Linnaeus, 1758) caught in the vicinity of Seoul, Korea. Yamaguti (1942) supplemented Park’s original description by
describing two and three adult specimens found
in the intestine of *Cyprinus carpio* from Lake
Kasumigaura and *Ca. carassius* from Kawai-
[Kawachi] Province (the ancient name of the
southeastern part of Osaka Prefecture), respec-
tively, in Japan. Yamaguti had used five speci-
mens then, but Yamaguti’s Collection included
only one adult specimen (MPM Coll. No. 22270-
b) from *Cy. carpio*. Since *Carassius carassius*
do not inhabit Japan (Nakabo, 2013), Yamaguti
may have examined another species or subspe-
cies of *Carassius* Nilsson, 1832 at that time.

Shimazu (2005) described adult specimens found
in the intestine of *Carassius* sp. and
*Tribolodon hakonensis* from Lake Ogawara.
The present reexamination has shown that the
rims and inner walls of the two suckers are
armed with small spines (Fig. 11). Park (1938),
Yamaguti (1942) and Shimazu (2005) over-
looked the spines. Kulakova and Ha Ky (1976)
first described that the suckers were spinose in
their materials of *C. koreanum* and *C. ginezinskajae*
Kulakova and Ha Ky, 1976, not Kha Ki
[Ha Ky], 1969, from northern Vietnam. Tang
and Lin (1979) presented a figure (fig. 3) to suggest
that the two suckers were spinose in their mate-
rial of *C. koreanum* from China, though they did
not mention this. Wang and Pan (1984) illus-
trated the two smooth suckers in their materials
of *C. koreanum* and six other species of *Carassot-
rema* from China.

**Life cycle** (Figs. 14–17). Not known in
Japan. Tang and Lin (1979) elucidated the life
cycle in China. Eggs develop after being laid.
Miracidia in fully embryonated eggs have a pig-
mented eyespot (Fig. 14). The first intermediate
host is *Stenothyra toucheana* Heude, 1880 (Gas-
tropoda, Stenothyridae), in which gymnocepha-
lous cercariae (Fig. 16) are produced in [most
likely daughter] rediae (Fig. 15). Mother sporo-
cysts were not described. After emerging from
the snail host, cercariae encyst upon algae or
other tender plants on the bottom of the water.
Encysted metacercariae (Fig. 17) were fed to
goldfish, from the intestine of which subse-
quently immature and adult worms of *C. korea-
um* were recovered. Final host fishes are likely
to become infected with the parasite by eating
algae and tender plants on which metacercariae
have encysted. In Japan, Abe in Ueno et al.
(1930) reported a similar gymnocephalous cer-
caria (Cercaria D, pl. 1, figs. 7–8) from
“Bithynella” sp. (Gastropoda, Hydrobiidae) in the
neighborhood of Kumamoto [Kumamoto City,
Kumamato Prefecture (?)]. Since Abe stated that
this *Bithynella* sp. was very similar to *Parafossa-
rulus manchouricus japonicus* (Pilsbry, 1901)
(Stenothyridae) and common in fresh water in
Japan, it may have been a stenothylid.

**Discussion on Carassotrema**

Moravec and Sey (1989) also recorded *Caras-
sotrema koreanum* from northern Vietnam and
considered *C. ginezinskajae* to be a junior syn-
onym of *C. koreanum*. However, Moravec and
Sey’s (1989) material is slightly different from
Park’s (1938) and the present materials in the egg
size, 63–84 by 39–51 μm instead 57–64 by
24–35 μm and 57–72 by 31–38 μm (collapsed),
respectively. *Carassotrema ginezinskajae* has
eggs measuring 58–68 by 27–43 μm and the
vitelline follicles not extending into the extrace-
ccal fields of the body (Kulakova and Ha Ky,
1976). These Vietnamese materials need reex-
amination for definitive species identification.

The oral and ventral suckers are spinose in *C.
koreanum* and *C. ginezinskajae* (Kulakova and
Ha Ky, 1976; this paper). Tang and Lin (1979)
said nothing about this in *C. koreanum* and other
five species of *Carassotrema* from China, but
their figures (figs. 3 and 17 for *C. koreanum*
and fig. 16 for *Carassotrema wui* Tang and Lin,
1963) suggest that the two suckers were spinose.
Wang and Pan (1984) illustrated the two smooth
suckers in their materials of *C. koreanum* and six
other species of *Carassotrema* from China.

A single testis gives off two sperm ducts in *C.
koreanum* (Fig. 12, sd). Yamaguti (1942), Kula-
kova and Ha Ky (1976), Moravec and Sey (1989)
and Shimazu (2005) said nothing about
the sperm ducts. Park (1938), Long and Lee
(1958) and Tang and Lin (1979) described a single sperm duct. Tang and Lin (1979) also described a single sperm duct in *Carassotrema estuarinum* Tang and Lin, 1979, *C. wui* and *Carassotrema kui* Tang and Lin, 1963 from China. The testis protrudes two anterolateral lobes to various extents. The sperm duct arises out of the anterior corner of each lobe. These suggest that the testicular primordium remains undivided even in the adult stage in *Carassotrema*. The Haploporidae includes a few genera with two testes (Overstreet and Curran, 2005).

*Carassotrema wui* Tang and Lin, 1963 [sic, should be 1979 (?)], and *Carassotrema kui* Tang and Lin, 1963 [sic, should be 1979 (?)] appear in Tang and Li (1979) and Wang and Pan (1984). Since I have not yet read the abstracts on these two species, I cannot tell at present whether their names are available in accordance with Article 13.1.1 of the International Code of Zoological Nomenclature (ICZN) (International Commission on Zoological Nomenclature, 1999). The known species should be fully redescribed from the type materials, and *Carassotrema* requires a revision (see also Overstreet and Curran, 2005).

Overstreet and Curran (2005) reviewed the Haploporidae. They were wrong in saying eggs as anoperculate and miracidia as lacking eyespots in the diagnosis of *Carassotrema* (see *Life cycle*). They did not referred to the presence of spines on the two suckers. With regards to the author and date of the Haploporidae, they have been cited as "Nicoll, 1914" (e.g., Overstreet and Curran, 2005). Nicoll (1914) used the family name Haploporidae abruptly without discussing it at all. Nicoll’s family is identical with the group that Looss (1902) regarded for the time being as a subfamily Haploporinae (Poche, 1926). The author and date of the family and superfamily may be correctly Looss, 1902.

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