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# Digeneans Parasitic in Freshwater Fishes (Osteichthyes) of Japan VI. Lissorchiidae

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**Abstract** Digeneans (Trematoda, Monorchioidea, Lissorchiidae) parasitic in freshwater fishes of Japan are reviewed: *Asymphylodora innominata* (Faust, 1924), *Asymphylodora japonica* Yamaguti, 1938, *Asymphylodora* sp. of Shimazu, Urabe, and Grygier, 2011, *Asymphylotrema monostyloides* (Ito, 1960) comb. nov., and *Palaeorchis diplorchis* (Yamaguti, 1936). Each species is described and figured with a summarized life cycle where known. Partial sequences (653 bp) of the cytochrome *c* oxidase I gene of the mitochondrial DNA (COI mtDNA) were identical between adults of *Asymphylotrema hamajimai* (Fujino and Kifune, 1991) (syn. *Anapalaeorchis hamajimai* Fujino and Kifune, 1991) and rediae of *Cercaria monostyloides* Ito, 1960. This result lends further support to the previous hypothesis that *C. monostyloides* is the cercaria of *As. hamajimai*. Since these two species are synonymous, the new combination *As. monostyloides* is made for *As. hamajimai*. A key to the three genera and five species of the family Lissorchiidae in Japan is presented.
**Key words:** Digeneans, Lissorchiidae, *Asymphylodora*, *Asymphylotrema*, *Palaeorchis*, *Asymphylotrema monostyloides* (Ito, 1960) comb. nov., partial COI sequences, freshwater fishes, Japan, review.

## Introduction

This is the sixth paper of a series that reviews adult digeneans (Trematoda) parasitic in freshwater fishes (Osteichthyes) of Japan (Shimazu, 2013). This contribution deals with the family Lissorchiidae Magath, 1917 *sensu* Bray (2008b) in the superfamily Monorchioidea Odhner, 1911 *sensu* Bray (2008a). Shimazu (2013) gave the Introduction and Materials and Methods for the review.

*Abbreviations used in the figures.* bp, birth pore; c, cercaria; cp, cirrus pouch; csd, common sperm duct; cvd, common vitelline duct; e, esophagus; ed, ejaculatory duct; egg, egg in uterus and metraterm; ep, excretory pore; ev, excretory vesicle; ga, genital atrium; gp, genital pore; i, intestine; Lc, Laurer's canal; m, metraterm; md, male duct; me, metacercaria; Mg, Mehlis' gland; o, ovary; od, oviduct; os, oral sucker; ot, ootype; p, pharynx; pc, prostatic cells; pp, pars prostatica; pr; prepharynx; s, stylet; sd, sperm duct; sr, seminal receptacle; sv, seminal vesicle; t, testis; tnc, transverse nerve commissure; u, uterus; usr, uterine seminal receptacle; v, vitellarium; vd, vitelline duct; vs, ventral sucker.

## Superfamily Monorchioidea Odhner, 1911
## Family Lissorchiidae Magath, 1917
## Genus ***Asymphylodora*** Looss, 1899
## ***Asymphylodora innominata*** (Faust, 1924)

(Figs. 1–9)

Cercaria H: Kobayashi, 1918: 70–73, 1 pl., fig. 16.
Cercaria VIII, or [Shin]: Ando, 1918: 616, 1 pl., fig. 8b.
Cercariaeum A: Kobayashi, 1922: 266–267.
*Cercariaeum innominatum* Faust, 1924: 295, table 1.
*Asymphylodora macrostoma* Ozaki, 1925: 104–106, fig. 4; Yamaguti, 1934: 393; Tang, 1962: 162–163, fig. 2; Shimazu, 1992: 8–10, figs. 6–11; Shimazu and Urabe, 2005: 11–12, figs. 18–20; Shimazu, 2008: 56–57, fig. 12.

Cercaria T: Ishii in Ueno, Ishii, and Abe, 1930: 974, fig. 27.
*Parasymphylodora macrostoma*: Szidat, 1943: 44–45, table 1, fig. 12.
*Cercaria innominatum* [*sic*, should be *innominata*]: Ito, Mochizuki, and Noguchi, 1959: 918; Ito, 1960: 59–72, fig. 13; Ito, 1964: 501, fig. 140.
*Asymphylodora* (*Asymphylodora*) *macrostoma*: Yamaguti, 1971: 97.
*Cercaria innominata*: Shimazu, 2007: 18.
*Asymphylodora innominata*: Shimazu, Urabe, and Grygier, 2011: 74, figs. 100–103.

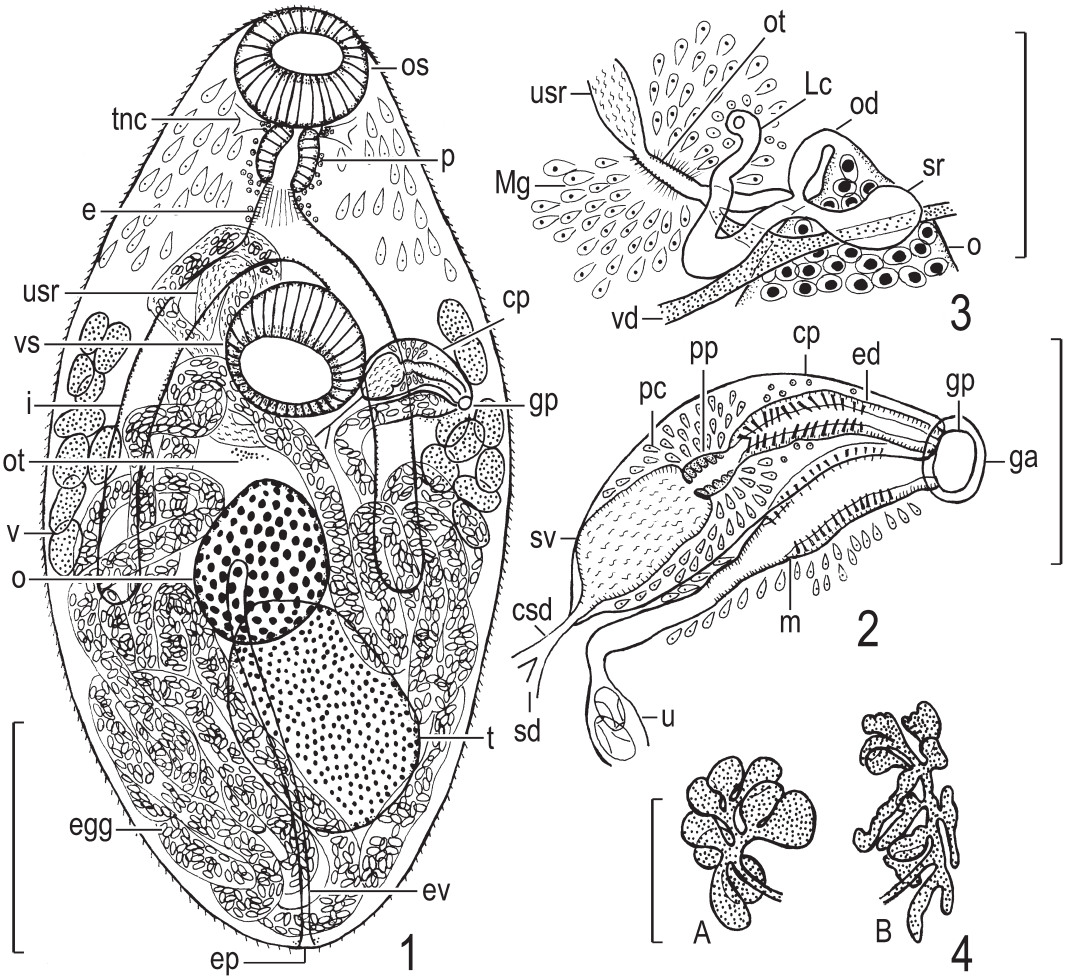
*Hosts in Japan. Odontobutis obscura* (Temminck and Schlegel, 1845) (Odontobutidae) (type host) including "[Gori]" (Ozaki, 1925; Yamaguti, 1934; Shimazu, 1992, 1995, 2015; this paper), *Opsariichthys uncirostris uncirostris* (Temminck and Schlegel, 1846) (Cyprinidae) (Yamaguti, 1934; Shimazu, 1992; Shimazu *et al.*, 2011), *Tribolodon hakonensis* (Günther, 1877) (Cyprinidae) (Yamaguti, 1934; Shimazu, 1992, 2007, 2008; Nakamura *et al.*, 2000; Shimazu and Urabe, 2005; Shimazu *et al.*, 2011; this paper), *Hemibarbus barbus* (Temminck and Schlegel, 1846) (Cyprinidae) (Yamaguti, 1934; Shimazu, 1992; Shimazu *et al.*, 2011), *Phoxinus steindachneri* Sauvage, 1883 (Cyprinidae) (Yamaguti, 1934; Shimazu, 1992), "[Ukikamatsuka?]" (*Hemibarbus longirostris* (Regan, 1908) [?]) (Shimazu 1992; Shimazu *et al.*, 2011), "[Bote]" (an acheilognathine) (Cyprinidae) (Shimazu, 1992), and *Gymnogobius isaza* (Tanaka, 1916) (Gobiidae) (Shimazu, 1992; Shimazu *et al.*, 2011).

*Sites of infection.* Intestine, rectum, and gut.

*Geographical distribution.* (1) Ibaraki Prefecture: Lake Kasumigaura at Tsuchiura City and Tsuchiura City (Yamaguti, 1934; Shimazu, 1992). (2) Saitama Prefecture: Oppe River at Ogose Town (Shimazu, 1992). (3) Toyama Prefecture: Namerikawa City (Yamaguti, 1934; Shimazu, 1992). (4) Nagano Prefecture: Hiroi River at Kotobuki, Iiyama City; Nogu River in Oomachi City; Torii River at Mure, Iizuna Town; and Lake Suwa at Suwa City (Shimazu, 1992, 2007; this paper). (5) Fukui Prefecture: Obama City (Shimazu, 1992). (6) Shiga Prefecture: Lake Biwa basin (Lake Biwa, Momose, Moriyama, Omastu, Onoe, and Tenjin River) (Yamaguti, 1934; Shimazu, 1992; Shimazu *et al.*, 2011; this paper). (7) Kyoto Prefecture: Yura River at Ayabe City and Lake Ogura (Yamaguti, 1934; Shimazu, 1992). (8) Nara Prefecture: Takami River at Kotsukawa, Higashiyoshino Village (Nakamura *et al.*, 2000; Shimazu and Urabe, 2005). (9) Wakayama Prefecture: Tonda River at Kurisugawa, Nakahechi-cho, Tanabe City (Shimazu, 2008). (10) Osaka Prefecture (?): Yodo River (Yamaguti, 1934; Shimazu, 1992). (11) Hiroshima Prefecture: a brook (type locality) in the vicinity of Saijo-cho, Matsuita River at Umaki, Saijo-cho, and Irasuke River at Kanesawa, Kurose-cho, Higashihiroshima City; Eno River at Yoshida, Yoshida-cho, Akitakata City; and Saijo River at Ooya, Saijo-cho, Shobara City (Ozaki, 1925; Shimazu, 1992; this paper). (12) Kochi Prefecture: Matsuda River at Idei, Hashikami-cho, Sukumo City (Shimazu, 2008). (13) Fukuoka Prefecture: Futatsu River at Takahatake, Mitsuhashi-machi, Yanagawa City (this paper). (14) Oita Prefecture: Chikugo River at Kobuchi Bridge, Miyoshikobuchi-machi, Hita City (this paper).

In Korea (cercaria) (Kobayashi, 1918) and China (*e.g.*, Tang, 1962).

*Material examined.* (1) 3 specimens (Ozaki's Collection, MPM Coll. No. 30028, species name not given, syntypes of *Asymphylodora macrostoma*) of *A. macrostoma*, adult, whole-mounted, ex "[Gori]" (*Odontobutis obscura* [syn. *Mogurnda obscura*]) (other data not given) (Shimazu, 1992, 1995, 2015; this paper). (2) 1 (Yamaguti's Collection, MPM Coll. No. 22280) of *A. macrostoma*, adult, whole-mounted, ex rectum of *O. obscura*, Lake Ogura, 4 May 1932 (Yamaguti, 1934; Shimazu, 1992). (3) 2 (NSMT-Pl 3683) of *A. macrostoma*, adult, whole-mounted, ex rectum of *O. obscura* (formalin-preserved), Matsuita River, 16 April 1991 (Shimazu, 1992). (4) 11 (NSMT-Pl 5785, MPM Coll. No. 21161), adult, whole-mounted, ex intestine of *O. obscura*, Matsuita River, 18 June 2009, 9 June 2011. (5) 2 (NSMT-Pl 5786), immature, adult, whole-mounted, ex intestine of *O. obscura*,




Figs. 1–4. *Asymphylodora innominata*, adult specimens. — 1, specimen (NSMT-Pl 3692) found in intestine of *Tribolodon hakonensis*, entire body, ventral view; 2, specimen (NSMT-Pl 5334) found in intestine of *T. hakonensis*, terminal genitalia, ventral view; 3, specimen (NSMT-Pl 5334), ovarian complex, dorsal view; 4, specimens (NSMT-Pl 3685) found in intestine of *Opsariichthys uncirostris uncirostris*, vitelline follicles in young adult specimen (A) and old adult specimen (B), ventral view. Fig. 4 redrawn from Shimazu *et al.* (2011). Scale bars: 0.3 mm in Figs. 1 and 4; 0.1 mm in Figs. 2–3.

Irasuke River, 18 June 2009. (6) 8 (Urabe's unpublished specimens) of *A. mocrostoma*, adult, whole-mounted, ex intestine of *O. obscura*, Futatsu River, 22–23 May 2003. (7) Specimens of *A. innominata*, whole-mounted, ex intestine of *Opsariichthys uncirostris uncirostris* (syn. *Opsariichthys uncirostris*), Lake Biwa basin (Yamaguti, 1934; Shimazu, 1992; Shimazu *et al.*, 2011): 3 (Yamaguti's Collection, MPM Coll. No. 22746), adult, Omatsu, 17 July 1927; 7 (NSMT-Pl 3700), adult, Omatsu, 30 April 1992; 119 (NSMT-Pl 3684–3686), immature, adult, Onoe, 5 May 1979, 3 June 1980, 11 November 1980; 9 (NSMT-Pl 3973), adult, Moriyama, 2 May 1992; and 58 (LBM 1-17 to -20), adult, Tenjin River, 21 May 1998; and 6 (LBM 5-8 to -10, hot formalin-fixed), adult, Momose, 1 May 2001. (8) 17 (Yamaguti's Collection, MPM Coll. Nos. 22284, published; 22745, unpublished) of *A. macrostoma*, adult, whole-mounted, ex intestine of *Op. uncirostris uncirostris*, Yodo River (locality not specified), 16 October 1929, 27 September 1928

(Yamaguti, 1934; Shimazu, 1992). (9) 2 (Yamaguti's Collection, MPM Coll. No. 22278) of *A. macrostoma*, adult, whole-mounted, ex intestine of *Tribolodon hakonensis* (syn. *Leuciscus hakonensis*), Namerikawa City, 28 October 1929 (Yamaguti, 1934; Shimazu, 1992). (10) 37 (NSMT-Pl 3691) of *A. macrostoma*, adult, whole-mounted, ex intestine of *T. hakonensis*, Nogu River, 1 July 1987 (Shimazu, 1992). (11) 247 (NSMT-Pl 3692, 5334, 5335) of *A. macrostoma*, immature, adult, whole-mounted, ex intestine of *T. hakonensis*, Torii River at Mure Village, now Mure, Iizuna Town, 16 August 1987, 7 May 1994, 11 November 1995 (Shimazu, 1992, 2007). (12) 146 (NSMT-Pl 3693, 3694, 3975, 3984, 5336) of *A. macrostoma*, immature, adult, whole-mounted, ex intestine of *T. hakonensis*, Lake Suwa, 5 October 1991, 14 November 1991, 19 May 1992, 2 June 1992, 14 September 1992 (Shimazu, 1992, 2007). (13) 50 (NSMT-Pl 5337–5341, slightly flattened and hot formalin-fixed) of *A. macrostoma*, immature, adult, whole-mounted, ex intestine of *T. hakonensis*, Hiroi River, 16 October 1999, 13 November 1999, 8 October 2004, 4–5 November 2004, 1 and 3 December 2004 (Shimazu, 2007). (14) 6 (NSMT-Pl 3689) of *A. macrostoma*, adult, whole-mounted, ex intestine of *T. hakonensis*, Oppe River, 13 October 1976 (Shimazu, 1992). (15) 2 (Yamaguti's Collection, MPM Coll. No. 22274, unpublished) of *A. macrostoma*, immature, adult, whole-mounted, ex intestine of *T. hakonensis*, Obama City, 27 March 1935 (Shimazu, 1992). (16) Specimens of *A. innominata*, adult, whole-mounted, ex intestine of *T. hakonensis*, Lake Biwa basin (Shimazu, 1992; Shimazu *et al.*, 2011): 8 (NSMT-Pl 3632, 3690), Onoe, 3 June 1980, 4 February 1980; 8 (NSMT-Pl 3974), Omatsu, 30 April 1992. (17) 46 (NSMT-Pl 5274, 5275) of *A. macrostoma*, immature, adult, whole-mounted, ex intestine of *T. hakonensis*, Takami River, 26 and 30 July 1999, 16 August 2000 (Shimazu and Urabe, 2005; see also Shimazu, 2013 for measurements). (18) 6 (NSMT-Pl 5556) of *A. macrostoma*, adult, whole-mounted, ex intestine of *T. hakonensis*, Tonda River, 4 August 1999 (Shimazu, 2008). (19) 8 (NSMT-Pl 3687) of *A. macrostoma*, adult, whole-mounted, ex intestine of *T. hakonensis*, Eno River, 30 October 1976 (Shimazu, 1992). (20) 19 (NSMT-Pl 3688) of *A. macrostoma*, adult, whole-mounted, ex intestine of *T. hakonensis*, Saijo River, 31 October 1976 (Shimazu, 1992). (21) 14 (NSMT-Pl 5557) of *A. macrostoma*, adult, whole-mounted, ex intestine of *T. hakonensis*, Matsuda River, 5 August 2000 (Shimazu, 2008). (22) 4 (Urabe's unpublished specimens) of *A. macrostoma*, adult, whole-mounted, ex intestine of *T. hakonensis*, Chikugo River, 25 August 2003. (23) 4 (Yamaguti's Collection, MPM Coll. Nos. 22277, 22743) of *A. macrostoma*, adult, whole-mounted, ex intestine of *Hemibarbus barbus*, Lake Kasumigaura at Tsuchiura, 16 April 1929 (Yamaguti, 1934; Shimazu, 1992). (24) 1 (NSMT-Pl 3976) of *A. macrostoma*, immature, whole-mounted, ex intestine of *H. barbus*, Onoe, 4 May 1992 (Shimazu, 1992; Shimazu *et al.*, 2011). (25) 1 (Yamaguti's Collection, MPM Coll. No. 22747, labeled "*Asymphylodora*," unpublished) of *A. innominata*, immature, whole-mounted, ex intestine of "[Ukikamatsuka?]" (*Hemibarbus longirostris* [?]), Lake Biwa, 4 December 1938 (Shimazu, 1992; Shimazu *et al.*, 2011). (26) 7 (NSMT-Pl 3977) of *A. innominata*, adult, whole-mounted, ex intestine of *Gymnogobius isaza* (syn. *Chaenogobius isaza*), Omatsu, 30 April 1992 (Shimazu, 1992; Shimazu *et al.*, 2011). (27) 1 (Yamaguti's Collection, MPM Coll. No. 22748, labeled "*Asymphylodora*," unpublished) of *A. macrostoma*, immature, whole-mounted, ex "[Bote]" (an acheilognathine), immature, whole-mounted, Tsuchiura, 4 April 1940 (Shimazu, 1992). (28) 3 (Yamaguti's Collection, MPM Coll. No. 22279) of *A. macrostoma*, adult, whole-mounted, ex intestine of *Phoxinus steindachneri* (syn. *Moroco steindachneri*), Yura River, 20 November 1929 (Yamaguti, 1934; Shimazu, 1992). (29) 10 (Yamaguti's Collection, MPM Coll. No. 22774, unpublished) of *A. macrostoma*, adult, whole-mounted, ex intestine of *Ph. steindachneri*, Obama City (date not given) (Shimazu, 1992).

*Description.* 1) Based on fully mature adult specimens (NSMT-Pl 3691–3694, 3975, 3984, 5334–5341); 10 of hot formalin-fixed ones of them (NSMT-Pl 5341) measured (Figs. 1–3). Body broad fusiform, pointed bluntly at both extremities, or ovate, tapering anteriorly, very small, 0.66–0.82 by 0.30–0.40; forebody 0.30–0.39 long, occupying 38–51% of body length. Tegument spinose; spines lanceolate, present all over body, sometimes not seen on median dorsal part of hindbody, becoming smaller and thinner posteriorly. Eyespot pigment absent. Many large cephalic gland cells seen in dorsal parenchyma of forebody, each opening on periphery of body wall around oral sucker; many globular gland cells including fine reddish granules present in parenchyma throughout. Transverse nerve commissure dorsal to prepharynx. Oral sucker subglobular, large, 0.10–0.13 by 0.13–0.15, anteroventral, its lumen not spinose; minute spines present in at least three circular rows around its aperture. Prepharynx very short, with gland cells on either side. Pharynx pyriform to oblong, 0.07–0.08 by 0.05–0.07. Esophagus short, 0.04–0.08 long, bifurcating anterior or dorsal to anterior part of ventral sucker. Intestines short, ending blindly at midlevel of hindbody or ovarian level. Ventral sucker subglobular, 0.11–0.14 by 0.13–0.15, slightly pre-equatorial; minute spines present in at least five circular rows around its aperture; sucker width ratio 1 : 0.9–1.0. Testis one, globular to elliptical, large, 0.12–0.17 by 0.10–0.16, usually in posterior half of hindbody, or rarely close to posterior end of body. Sperm ducts two, short; common sperm duct present, sometimes fairly long (Shimazu, 1992, fig. 10). Cirrus pouch club-shaped, slightly curved, fairly thick-walled, muscular, usually posterolateral or rarely anterolateral to ventral sucker, lying obliquely across left intestine, small, 0.15 by 0.04–0.05, including small seminal vesicle, extending slightly beyond left cecum (Fig. 1), or large, 0.17–0.20 by 0.06–0.08, including large seminal vesicle, extending slightly beyond median line of body (Shimazu, 1992, fig. 9), containing seminal vesicle, prostatic complex, and ejaculatory duct. Seminal vesicle spherical to elliptical, fairly thick-walled, sometimes weakly sinuate (Shimazu, 1992, fig. 10). Pars prostatica globular to elliptical, small, 0.01 in diameter; prostatic cells well developed. Ejaculatory duct club-shaped, 0.06–0.08 by 0.02, lined with spines (8–10 $\mu$m long) in posterior two-thirds, eversible. Genital atrium shallow. Genital pore sinistrally sublateral or lateral at level of ventral sucker. Ovary pyriform to ovate, 0.09–0.14 by 0.11–0.13, immediately pretesticular, usually overlapping testis slightly. Ovarian complex preovarian. Laurer's canal fairly long. Seminal receptacle canalicular, retort-shaped, small (0.03–0.05 by 0.02–0.04), empty or containing a small number of sperm. Ootype vesicular, large; Mehlis' gland well developed. Uterus ascending and descending on either side of ovary and testis, occupying all available space in hindbody, sometimes extending forward to esophagus in median field; uterine seminal receptacle well developed in proximal coils. Metraterm thick-walled, dilated in middle two-fourths to form globular to elliptical chamber 0.04–0.06 by 0.03, lined with spines (6–9 $\mu$m), surrounded by gland cells, eversible. Eggs numerous, narrow ovate, yellow to light brown, 22–25 by 13–14 $\mu$m, with small domed operculum at attenuated pole, sometimes with small hooklike protuberance at anoperculate pole, fully embryonated. Vitellaria follicular; follicles large, usually entire but rarely of irregular outline, probably nine forming bunch (0.17–0.22 long), lying laterally to ventral sucker along posterior two-thirds of intestine on either side of body. Excretory vesicle I-shaped, extending forward to middle of ovary; excretory pore posteroterminal.

*Remarks.* Ozaki (1925) described this species as a new species, *Asymphylodora macrostoma*, on the basis of adult specimens found in the cloaca [presumably the lowermost part of the rectum] of *Odontobutis obscura* (syn. *Mogurnda obscura*) (Japanese name: Donko, but Goppo in Ozaki) from a brook in the vicinity of Saijo, now Saijo-cho, Higashihiroshima City, Hiroshima Prefecture. He did not designate the holotype for

this species. Shimazu (2015) regarded the host fish "[Gori]" as *O. obscura.* I consider that the present three adult specimens of Ozaki (MPM Col. No. 30028) as syntypes of *A. macrostoma.* Shimazu (1992, fig. 7) briefly described and figured them. The name of the brook has not yet been determined.

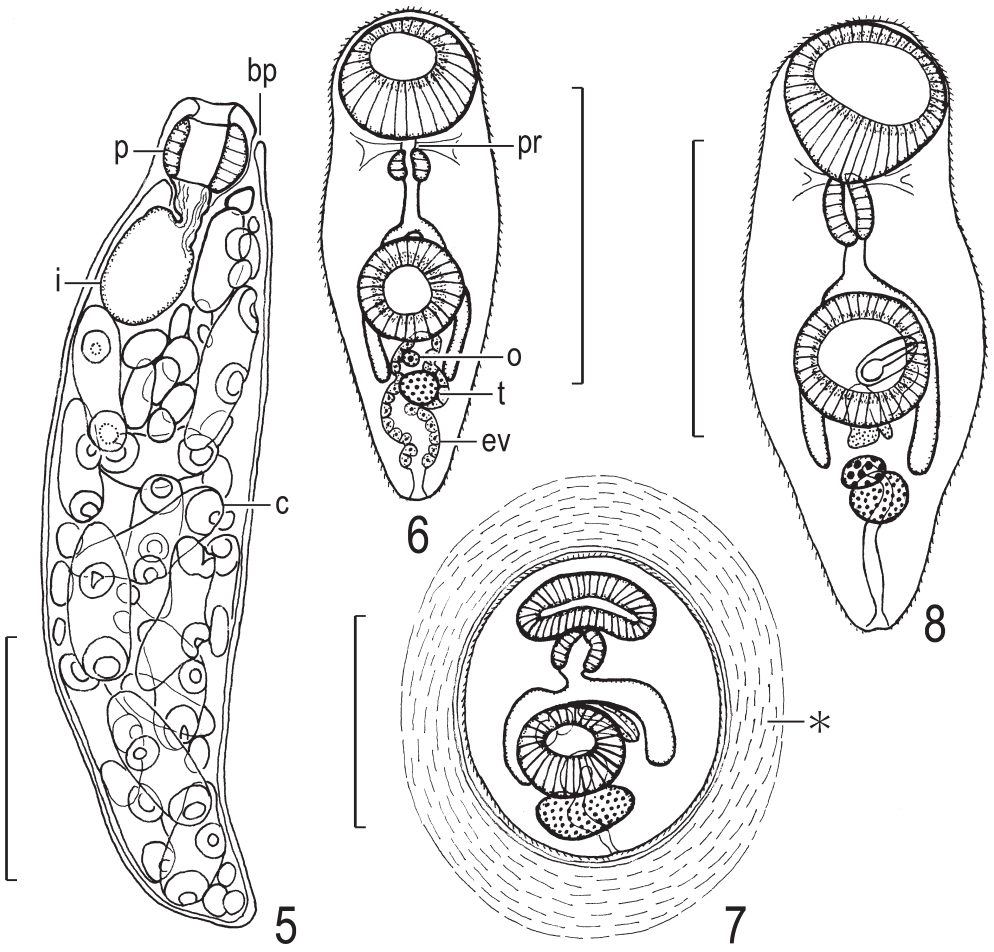
Tang (1962) erected a new genus, *Orientotrema*, for *Asymphylodora japonica* Yamaguti, 1938 (type species) and *A. macrostoma*; but he did not make a new combination for *A. macrostoma.* Shimazu (1992) and Bray (2008b) did not accept this new genus.

Kobayashi (1918) described a tailless cercaria (cercariaeum), Cercaria H, found in the gonads and around their ducts of *Melania* spp. [now *Semisulcospira* spp.] (Pleuroceridae) from Korea and added that this cercaria also occurred in Japan. Ando (1918) described Cercaria VIII, or [Shin], found in *Semisulcospira libertina* (Gould, 1859) (syn. *Melania libertina*) from Hiramaki Village, now in Kani City, and Kaminogo Village, now in Mitake Town, Gifu Prefecture, Japan. Cercaria H and Cercaria VIII are the same species (Ito, 1960, 1964). Faust (1924) named Cercaria H *Cercariaeum innominatum.* Since Shimazu (2007) had experimentally demonstrated that *Cercariaeum innominatum* or *Cercaria innominata* was the larva of *Asymphylodora macrostoma*, Shimazu *et al.* (2011) made a new combination, *A. innominata* (Faust, 1924), for *A. macrostoma.* Ito (1964) also assigned the cercaria of Okumura (1919) (locality not given) and Cercaria T of Ishii in Ueno *et al.* (1930) (locality not given) to *Cercaria innominata.*

Kobayashi (1922) grouped Cercaria H and Cercaria VIII into Cercariaeum A and stated that Cercariaeum A was parasitic in *Semisulcospira* spp. and the bithyniid snail *Parafossarulus manchouricus japonicus* (Pilsbry, 1901) (syn. *Bulimus striatulus japonicus*) in Okayama, Shiga, and Gifu Prefectures and Korea without citing any references concerning the cercaria in *Pa. manchouricus japonicus.* Shimazu *et al.* (2011) questioned whether *Cercariaeum innominatum* and Cercariaeum A were the same species. It seems evident that Kobayashi (1922) erroneously identified the cercaria of *Asymphylodora japonica* Yamaguti, 1938 in *Pa. manchouricus japonicus* with Cercariaeum A owing to a morphological similarity between these two cercariae (see the next species *A. japonica*).

Ozaki (1925) described that the oral sucker was larger than the ventral sucker, with a sucker width ratio of 1 : 0.8 (my calculation). The oral sucker was larger in cercariae (Fig. 6), metacercariae (Fig. 8) and barely matured adults, but sometimes slightly smaller in fully matured adults (Fig. 1) (Shimazu, 1992, 2008; Shimazu and Urabe, 2005; Shimazu *et al.*, 2011; this paper). Ozaki (1925) described that the cirrus pouch was slender and slightly larger than an elongated spined vagina, or the spined chamber of the metraterm (this paper). However, the cirrus pouch was about twice as long as the spined chamber (Shimazu, 1992, 2008; Shimazu and Urabe, 2005; Shimazu *et al.*, 2011; this paper) and in Ozaki's specimens (MPM Coll. No. 30028) (Shimazu, 1992, fig. 7) and the new specimens (NSMT-Pl 5785–5786) from Higashihiroshima City. Ozaki (1925, fig. 4) described that the vitellaria consist of a number of follicles. Probably, nine follicles form a bunch on either side of the body. They are large and entire in young adult specimens, but lobed (of irregular outline) in old adult specimens (Shimazu, 1992; Shimazu *et al.*, 2011; this paper, Fig. 4). The presence of minute spines around the apertures of both suckers has previously been overlooked in adult specimens. Ito (1960, 1964) described five and seven circular rows of pointed spines on the oral and ventral suckers, respectively, in the cercaria (*Cercaria innominata*). I counted at least three and five circular rows, respectively.

Yamaguti (1934) claimed to find *A. macrostoma* in *Pseudorasbora parva* (Temminck and Schlegel, 1846) (Cyprinidae) from Lake Kobata [Kohata Pond or Kowata Pond] in Uji City, Kyoto Prefecture; but Yamaguti's Collection contained no specimen from this fish species (Shimazu, 1992). Kuyama (1938) found adults, which were similar to a European species,




Figs. 5–8. *Asymphylodora innominata*, life cycle. — 5, daughter (?) redia (NSMT-Pl 5347) found in *Semisulcospira libertina*; 6, cercaria (NSMT-Pl 5345), ventral view; 7, encysted metacercaria (NSMT-Pl 5352) found in connective tissue of mucous membrane of gill raker of *Pseudorasbora parva*, 16 days after experimental infection, surrounded by tissue of host origin (*); 8, excysted metacercaria (NSMT-Pl 5349), 14 days after experimental infection. Scale bars: 0.5 mm in Fig. 5; 0.2 mm in Figs. 6–8.

*Asymphylodora tincae* (Modeer, 1790), in *Ps. parva* from an irrigation canal in Okita Village, now in Okayama City, Okayama Prefecture. It is possible that they may have belonged to *A. macrostoma*, though they have not been described. Tang (1962) found adults of *A. macrostoma* in a small cyprinid, *Punctius* sp., from Fujian Province, China, where *Melania peregrinorum* Heude was infected with cercariae.

*Asymphylodora innominata* and *A. japonica* (see below) have a single testis but two sperm ducts. Bray (2008b) regarded this as a reduction of two testes into one. However, it appears to me that this is an undivision of a single testicular primordium that fails to divide into two.

*Life cycle.* The first intermediate hosts in Japan are pleurocerid snails, *Semisulcospira libertina* (Japanese name: Kawanina), *Semisulcospira dolorosa* (Gould, 1859) (Japanese name: Kitano-kawanina), *Semisulcospira* (*Biwamelania*) *habei* Davis, 1969 (Japanese name: Habe-kawanina), *Semisulcospira* (*Biwamelania*) *niponica* (Smith, 1876) (Japanese name: Yamato-kawanina), and *Semisulcospira* (*Biwamelania*) *nakasekoae* Kuroda, 1929 (Japanese name Nakaseko-kawanina) (*e.g.*, Ando, 1918; Ito,

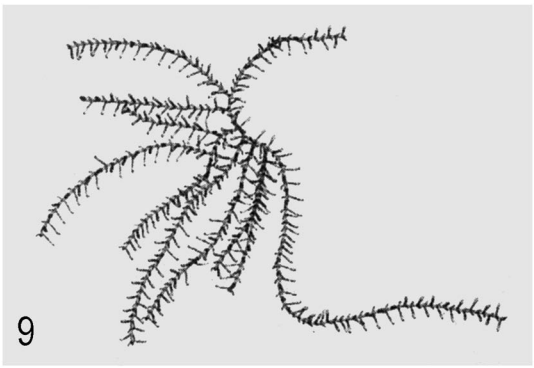



Fig. 9. *Asymphylodora innominata*, life cycle, photograph of an aggregation of cercariae, scale unknown. Reproduced from Shimazu (2007).

1960, 1964; Urabe, 2003; Shimazu, 2007). Tailless cercariae (*Cercaria innominata*) are produced in [daughter (?)] rediae in the gonads and around their duct (*e.g.*, Kobayashi, 1918; Ito, 1960; Shimazu, 2007, NSMT-Pl 5345–5347; this paper, Figs. 5–6). The testis, ovary, and terminal genitalia are already differentiated fairly well (Fig. 6). The flame-cell formula is 2[(3 + 3 + 3 + 3 + 3) + (3 + 3 + 3 + 3 + 3)] = 60 (Ito, 1960). When leaving snail hosts, many cercariae make aggregations of various sizes (Fig. 9) in the mantle cavity of the snails (Okumura, 1919; Shimazu, 2007).

The second intermediate hosts are small fish, such as *Tribolodon hakonensis*, *Pseudorasbora parva*, etc. (*e.g.*, Yamaguti, 1934, 1938; Komiya, 1965; Shimazu, 2007). Metacercariae are found encysted, surrounded by a tissue of host origin, in the connective tissue of the mucous membrane mainly of the gill arches and gill rakers, rarely of the buccal cavity, and more rarely of the esophagus [not pharynx] and intestine of the hosts (*e.g.*, Yamaguti, 1934, 1938; Shimazu, 2007, NSMT-Pl 5342–5344, 5348–5349; this paper, Figs. 7–8).

The final hosts are listed above. Adults live in the intestine of them. Tang (1962) studied the life cycle in China.

The aggregations of cercariae are flesh-colored and move slowly on the bottom of the water. Small fish (*T. hakonensis* and *Ps. parva*) ingested them quickly (Shimazu, 2007). Since cercariae are bottom-dwellers and incapable of invading second intermediate host fish cutaneously, they must be ingested by the fish. Obviously, ingestion of cercarial aggregations will be much more efficient than ingestion of individual cercariae for infection of cercariae to fish (Shimazu, 2007).

Adults have been reported from *T. hakonensis* (as small as 35 mm in standard body length), *Gymnogobius isaza*, "[Gote]" and *Ps. parva* (Shimazu, 1992; this paper). It is interesting how these fishes acquire infection with *A. innominata*, because they are unlikely to eat fish infected with metacercariae. Metacercariae are also found in the wall of the digestive tract (buccal cavity, esophagus, and intestine) as mentioned above. Possibly, metacercarial cysts may liberate from the wall of the digestive tract into the lumen by unknown mechanism after unknown days after encystment and are swallowed down into the intestinal lumen, excystment takes place there, and then now juveniles can attain sexual maturity there (see also Shimazu, 2007).

### ***Asymphylodora japonica*** Yamaguti, 1938

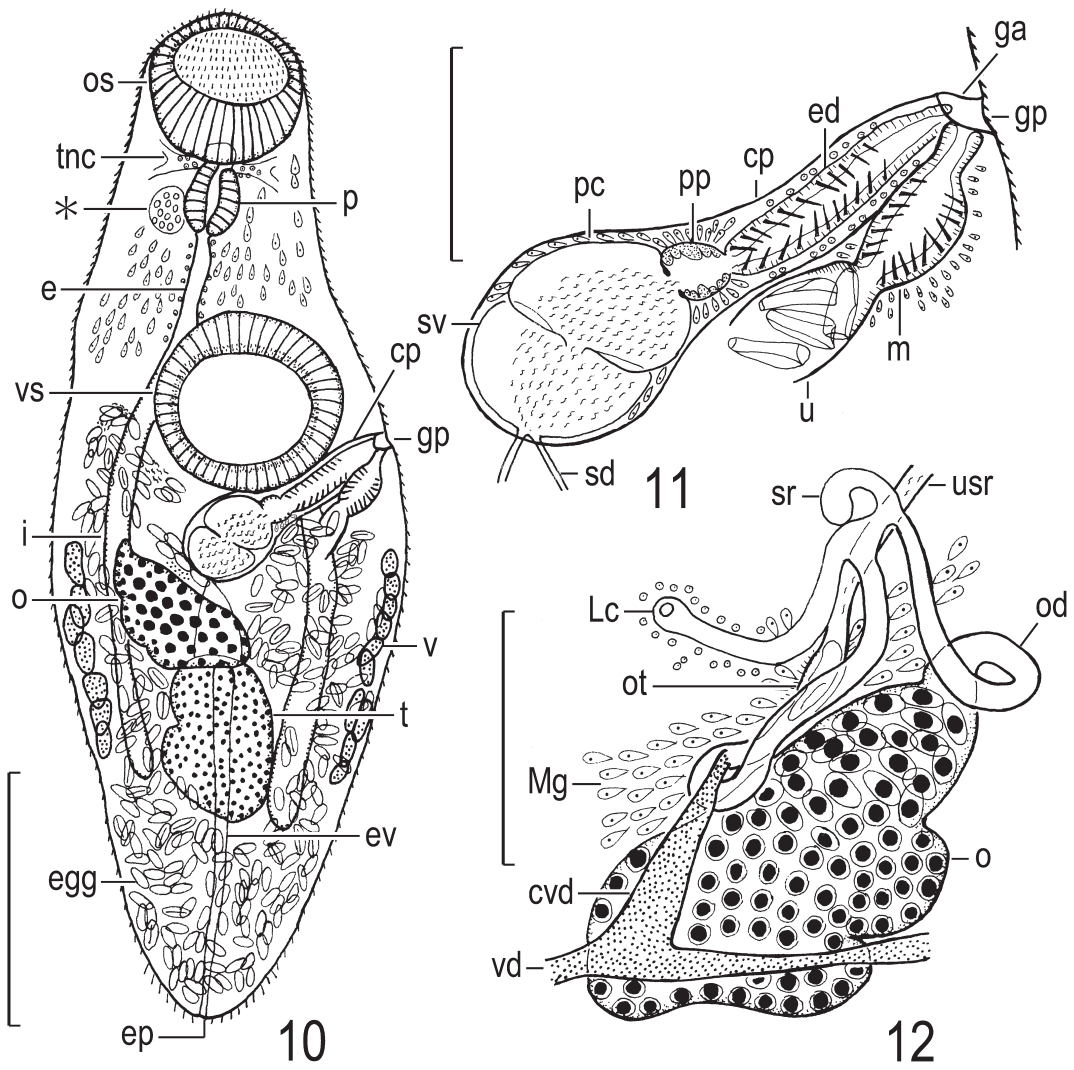
(Figs. 10–14)

Metacercaria IV: Kurokawa, 1935: 1797–1798, fig. 4.
Cercaria A: Kuyama, 1938: 351, figs. 36–37.
Cercaria B: Abe in Ueno, Ishii, and Abe, 1930: 967, pl. 1, fig. 3.
*Asymphylodora japonica* Yamaguti, 1938: 87–88, fig. 47; Yamaguti, 1942: 371; Shimazu, 1992: 3, 5, figs. 1–3; Shimazu *et al.*, 2011: 75–77, figs. 105–107.
*Orientotrema japonica* [*sic*, should be *japonicum*]: Tang, 1962: 169, 183.
*Parasymphilodora japonica* [*sic*, misspelling of *Parasymphylodora*]: Besprozvannykh, 2005: 138, 141, fig. 2A.

*Host in Japan. Cyprinus carpio* Linnaeus, 1758 (Cyprinidae) (type host) (Yamaguti, 1938, 1942; Shimazu, 1992; Shimazu *et al.*, 2011).

*Site of infection.* Intestine.

*Geographical distribution.* (1) Ibaraki Prefecture: Lake Kasumigaura at Tsuchiura City (Yamaguti, 1942; Shimazu, 1992). (2) Shiga Prefecture: Lake Biwa (Yamaguti, 1938; Shimazu, 1992; Shimazu *et al.*, 2011). (3) Okayama Pre-

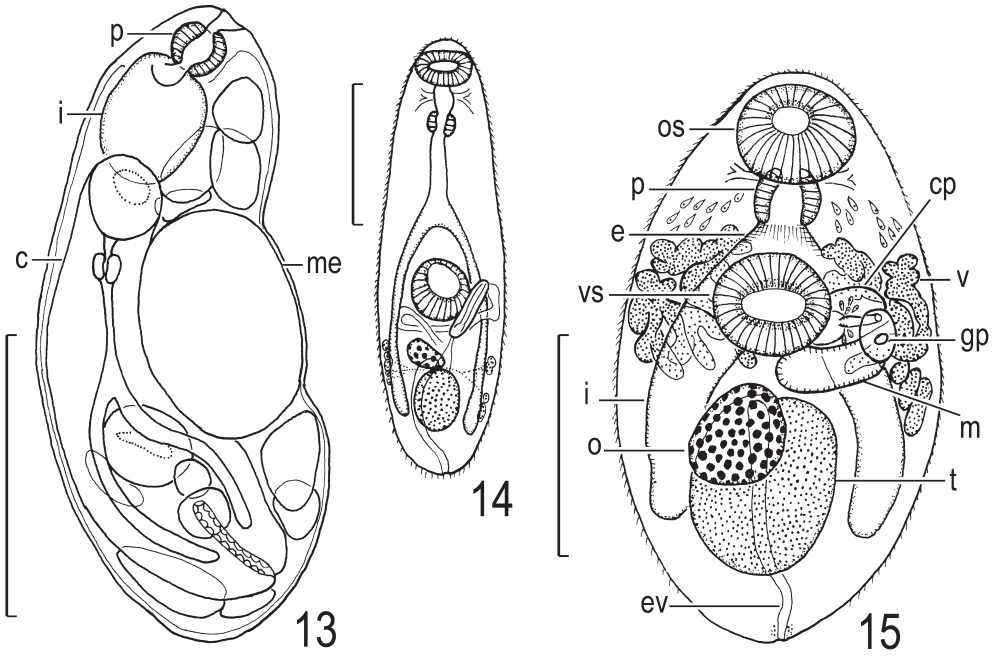



Figs. 10–12. *Asymphydora japonica*, adult, holotype (MPM Coll. No. 22271) found in intestine of *Cyprinus carpio* — 10, entire body, possibly a protozoan mass (*), ventral view; 11, terminal genitalia, ventral view; 12, ovarian complex, dorsal view. Scale bars: 0.3 mm in Fig. 10; 0.1 mm in Figs. 11–12.

fecture (type locality): Minami-ku, Okayama City (Yamaguti, 1938; Shimazu, 1992; this paper).

In Primorsky Region, Russia (Besprozvannykh, 2005); and China (?) (*e.g.*, Tang, 1962; Wang and Pan, 1984; Wang, 1991).

*Material examined.* (1) Many specimens (Yamaguti's Collection, MPM Coll. No. 22271, type series, holotype and paratypes) of *Asymphylodora japonica*, immature, adult, whole-mounted, ex intestine of *Cyprinus carpio*, Fukuda-son (Fukuda Village), now in Minami-ku, Okayama City, Okayama Prefecture, 26 November 1937 (Yamaguti, 1938; Shimazu, 1992). (2) Some 50 (Yamaguti's Collection, MPM Coll. No. 22272, paratypes) of *A. japonica*, adult, whole-mounted, ex intestine of *Cy. carpio*, Lake Biwa (locality not specified), 1 June 1936 (Yamaguti, 1938; Shimazu, 1992; Shimazu *et al.*, 2011). (3) 3 (Yamaguti's Collection, MPM Coll. No. 22270-a, number changed) of *A. japonica*, adult, whole-mounted, ex small intestine




Figs. 13–14. *Asymphylodora japonica*, life cycle. — 13, [daughter (?)] redia (MPM Coll. No. 22744) found in *Parafossarulus manchouricus japonicus*; 14, excysted metacercaria (MPM Coll. No. 22281) found in *Pa. manchouricus japonicus*, ventral view. Scale bars: 0.3 mm in Figs. 13–14.

Fig. 15. *Asymphylodora* sp., immature specimen (NSMT-Pl 3978) found in intestine of *Tridentiger brevispinis*, entire body, ventral view. Scale bar: 0.3 mm.

[*sic*] of *Cy. carpio*, Lake Kasumigaura, 4 April 1940 (Yamaguti, 1942; Shimazu, 1992).

*Description.* Based on adult ones of type specimens (MPM Coll. No. 22271–22272), after Shimazu (1992) and Shimazu *et al.* (2011), slightly modified from the present study (Figs. 10–12). Similar to the foregoing *Asymphylodora innominata* in morphology, except for spinose lumen of oral sucker and bipartite seminal vesicle. Body fusiform, small, 0.75–1.31 by 0.26–0.48 (holotype 1.16 by 0.41); forebody 0.30–0.48, occupying 35–42% of body length. Tegumental spines triangular, seen all over body but on median dorsal part of hindbody, becoming smaller and thinner posteriorly and larger and thicker again at posterior extremity of body. (A possibly protozoan mass seen on right side of pharynx in parenchyma of holotype, Fig. 10, *). Oral sucker 0.12–0.18 by 0.13–0.19; minute spines present on internal wall of lumen; small spines present in at least seven semicircular rows around anterior half of its aperture. Pharynx 0.06–0.09 by 0.05–0.07. Esophagus bifurcating usually anterodorsal but sometimes anterior to ventral sucker. Intestines extending usually to level of posterior border of testis or rarely to midlevel of post-testicular region. Ventral sucker 0.16–0.22 by 0.17–0.25; small spines present in at least five circular rows around its aperture; sucker width ratio 1 : 1.2–1.6. Testis 0.11–0.22 by 0.09–0.16, in middle third of hindbody. Sperm ducts two, short; common sperm duct absent. Cirrus pouch 0.19–0.27 by 0.07–0.09, extending a little farther than median line of body, usually reaching to ovary. Seminal vesicle bipartite, thin-walled, 0.09–0.12 by 0.06–0.10. Pars prostatica 0.02 in diameter. Ejaculatory duct 0.09–0.14 by 0.02–0.03, lined heavily with spines (14–24 $\mu$m long) in posterior four-fifths, eversible. Genital atrium fairly deep. Genital pore sinistrally lateral, at level slightly anterior to posterior border of ventral sucker. Ovary subglobular to pyriform,

sometimes of irregular outline, 0.09–0.14 by 0.06–0.16, dextrally submedian, immediately anterolateral to testis. Laurer's canal short. Seminal receptacle 0.02–0.03 by 0.01–0.02, empty. Uterus occupying all available space in hindbody, sometimes extending to bifurcal level; metraterm short, dilated in middle two-fourths to form globular to elliptical chamber (0.04–0.06 by 0.03–0.05) lined heavily with spines (17–29 $\mu$m long). Eggs narrow ovate, symmetrical, yellow, 32–40 by 16–22 $\mu$m, fully embryonated. Vitelline follicles about seven on either side of body, lateral to intestines in gonadal zone of hindbody. Excretory vesicle reaching to anterior border of testis or slightly farther than it; excretory pore posteroterminal.

*Remarks.* Yamaguti (1938) described this species as a new species, *Asymphylodora japonica*, based on the type series found in the intestine of *Cyprinus carpio* from Fukuda-son, Okayama Prefecture, and Lake Biwa, Shiga Prefecture (see also Shimazu, 1992). Later, Yamaguti (1942) found the species in *Cy. carpio* from Lake Kasumigaura, Ibaraki Prefecture. Kobayashi (1918) briefly mentioned that numerous specimens of a digenean were found in the intestine of *Cy. carpio* from Lake Biwa in November, 1911. He said that they resembled *Asymphylodora perlata* (Nordmann, 1832), or now *A. tincae*; but he must have encountered *A. japonica* then (Shimazu *et al.*, 2011). Shimazu (1992) redescribed the species on the basis of the holotype and paratypes and three of the seven specimens of Yamaguti (1942). Shimazu *et al.* (2011) redescribed the paratypes from Lake Biwa. Small spines around the apertures of both suckers and minute spines on the internal wall of the oral sucker have previously been overlooked.

Tang (1962) erected a new genus, *Orientotrema*, for *A. japonica* (type species) and *A. macrostoma.* Shimazu (1992) and Bray (2008b) did not accept this new genus.

Yamaguti (1938) merely stated that *A. japonica* very closely resembled *Asymphylodora tincae* but differed from it markedly in egg size. Shimazu (1992) distinguished *A. japonica* from *A. tincae* as described by Looss (1894) by not only the egg size (37–40 by 16–22 $\mu$m instead of 23–27 by 12–14 $\mu$m) but also the shape and size of the excretory vesicle (I-shaped and reaching to the ovary instead of sacciform and located posterior to the testis). *Asymphylodora japonica* morphologically differs from the foregoing *A. macrostoma* in that minute spines are present on the internal wall of the oral sucker; the intestines are longer, ending at the level of the posterior border of the testis instead of the level of the ovary; the sucker width ratio is higher, 1 : 1.2–1.6 instead of 1 : 0.9–1.3; the seminal vesicle is bipartite instead of unipartite; the vitelline follicles are posterior to the ventral sucker instead of lateral; and eggs are larger, 32–40 by 16–22 $\mu$m instead of 22–25 by 11–14 $\mu$m (see also Shimazu, 1992; Shimazu *et al.*, 2011).

Yamaguti (1938, plate fig. 7) found cercariae and metacercarial cysts of *A. japonica* in the snail *Parafossarulus manchouricus japonicus* (syn. *Bulimus striatulus japonicus*) (Bithyniidae) (Japanese name: Mametanishi) (their site of infection not given) from Okayama. Shimazu (1992, figs. 4–5) reexamined Yamaguti's specimens: [daughter (?)] rediae (MPM Coll. No. 22744, labeled "*Asymphylodora*") and excysted metacercariae (MPM Coll. No. 22281, labeled "*Asymphylodora*") from Kojo-son (Kojo Village), now in Minami-ku, Okayama City, Okayama Prefecture, on 15 September 1935 (see *Life cycle* below). The rediae each contained several developing cercariae, one of which usually was fully formed, and one encysted metacercaria. The encysted metacercariae were too deeply stained to discern internal organs.

Nagano (1930) found cercariae and encysted metacercariae in *Pa. manchouricus japonicus* (locality not given). Further, he recovered adults from the intestine of *Cy. carpio* and crucian carp [*Carassius* sp.], to which he had fed the metacercariae. Similar rediae and metacercariae were reported from *Pa. manchouricus japonicus*: Metacercaria IV from Okayama Prefecture (Kurokawa, 1935) and Cercaria A (with the excretory vesicle reaching to the ventral sucker)

from an irrigation canal in Okita Village, now in Naka-ku, Okayama City (Kuyama, 1938). Abe in Ueno *et al.* (1930) described Cercaria B found in "[Mame-mamedanishi]," possibly *Gabbia kiusiuensis* (Hirase, 1927) (Bithyniidae) (Japanese name: Hime-mametanishi), not *Stenothyra japonica* Kuroda as mentioned by Ito (1964), in Kumamoto Prefecture. Metacercariae were found encysted in some tissues and even in rediae (Nagano, 1930; Kurokawa, 1935; Kuyama, 1938) of both snails infected with the rediae and those free from them (Nagano, 1930). Nagano (1930), Kurokawa (1935), and Kuyama (1938) considered their parasites to be *A. tincae*. Although their rediae, cercariae, metacercariae, and adults lack detailed morphological descriptions, they are most likely referred to not *A. tincae* but *A. japonica* (Yamaguti, 1938; Shimazu, 1992, 1999, 2003; Shimazu *et al.*, 2011). Furthermore, *A. tincae* in the Czech Republic uses pulmonate snails as intermediate hosts and develops into adults, unusually without the metacercarial stage, in *Tinca tinca* (Linnaeus, 1758) (Cyprinidae) when this final host ingests snails harboring rediae with mature cercariae (Našincová and Scholz, 1994).

Tang (1962), Wang and Pan (1984), and Besprozvannykh (2005) studied the life cycle of the digeneans under the species name of *A. japonica* in China and Primorsky Region, Russia, respectively. Wang and Pan (1984) found rediae, cercariae, and metacercariae in snails of three species including *Parafossarulus* sp. The digeneans from China do resemble *A. japonica* from Japan in general morphology but differ from it in that the seminal vesicle in the adult is semicircular (Tang, 1962) or oblong (Wang and Pan, 1984; Wang, 1991); adults were found in the intestine of *Pseudorasbora parva* (Tang, 1962); and adults, which were smaller than cercariae, were found in the intestine of fry of *Ctenopharyngodon idellus* (Cyprinidae), etc., as small as 0.7–0.8 cm long (Wang and Pan, 1984). Since the above fishes are unlikely to eat snails harboring metacercariae (see *Life cycle* below), it is doubtful that their digeneans from China are assigned to *A. japonica* (see also Shimazu, 1992; Shimazu *et al.*, 2011).

*Life cycle.* The first and second intermediate hosts are *Parafossarulus manchouricus japonicus* and possibly *Gabbia kiusiuensis* (Nagano, 1930; Abe in Ueno *et al.*, 1930; Kurokawa, 1935; Kuyama, 1938; Yamaguti, 1938; this paper). Tailless cercariae (cercariaea) are produced in [daughter (?)] rediae (Fig. 13) in the midgut gland of snails. Metacercariae encyst in some organs (tissues) of snails and even in rediae (Figs. 13–14) in them. The final hosts are *Cyprinus carpio* (natural and experimental) and crucian carp (*Carassius* sp.) (experimental), in the intestine of which adults live (Kobayashi, 1918; Nagano, 1930; Yamaguti, 1938, 1942). Probably, *Cy. carpio* attains infection with *A. japonica* by eating snails harboring metacercariae (Shimazu, 1999, 2003).

Shimazu (1992) stated that cercariae encyst into metacercariae while still in rediae. However, it may be that, after emerging from snails infected with rediae, cercariae invade the same or other snails to become encysted metacercariae in some tissues of them and even in the rediae in them, because Nagano (1930) and Tang (1962) found metacercariae in not only snails infected with rediae but also those free from them.

### ***Asymphylodora*** sp. of Shimazu, Urabe, and Grygier, 2011

(Fig. 15)

*Asymphylodora macrostoma* (not of Ozaki, 1925): Shimazu, 1992: 8.

*Asymphyrodora* sp.: Shimazu, Urabe, and Grygier, 2011: 78, fig. 104.

*Host in Japan.* *Tridentiger brevispinis* Katsuyama, Arai, and Nakamura, 1972 (Gobiidae) (Shimazu, 1992; Shimazu *et al.*, 2011).

*Site of infection.* Intestine.

*Geographical distribution.* Shiga Prefecture: Lake Biwa basin (Omatsuzaki Point, Minamikomatsu, Otsu City) (Shimazu, 1992; Shimazu *et al.*, 2011).

*Material examined.* 1 specimen (NSMT-Pl 3978) of *Asymphylodora* sp., immature, whole-mounted, ex intestine of *Tridentiger brevispinis*, Omatsuzaki Point, 5 May 1992 (Shimazu, 1992; Shimazu *et al.*, 2011).

*Description.* Body broad elliptical, very small, 0.76 by 0.42; forebody 0.31 long, occupying 41% of body length. Oral sucker 0.14 by 0.16, with many minute spines on anterior half of its aperture. Pharynx 0.07 by 0.09. Esophagus bifurcating anterodorsally to ventral sucker. Intestines ending at junction between middle and posterior thirds of hindbody. Ventral sucker 0.14 by 0.16, with many minute spines around its aperture; sucker width ratio 1 : 1.0. Testis oblong, 0.26 by 0.19, located some distance from posterior extremity of body. Cirrus pouch 0.13 by 0.06, lateral to ventral sucker, lying across left cecum, not reaching median line of body. Seminal vesicle spherical, 0.06 by 0.05. Pars prostatica 0.02 in diameter; prostatic cells well developed. Ejaculatory duct 0.05 by 0.01, lined with spines (about 8$\mu$m long). Genital atrium large. Genital pore sinistrally submedian at level of ventral sucker. Ovary oval, 0.14 by 0.11, dextrally submedian, anteroventral to testis. Ovarian complex not clearly observed. Metraterm (or spined chamber) elongate, large, 0.16 by 0.06, lined heavily with small thornlike spines (about 6$\mu$m long), slightly everted into genital atrium. Vitelline follicles in irregular and complicated shape between lateral margins of body and ventral sucker, slightly overlapping ventral sucker. Excretory vesicle extending forward to anterior border of testis; excretory pore posteroterminal.

*Remarks.* This immature specimen of *Asymphylodora* morphologically differs from *A. macrostoma* and *A. japonica* in that the cirrus pouch is smaller than the metraterm; the metraterm is lined with much more numerous, much more smaller spines; and the vitelline follicles are in an irregular, much more complicated shape instead of globular. Furthermore, it is different from *A. japonica* in that the seminal vesicle is unipartite instead of bipartite and the vitelline follicles are lateral to the ventral sucker instead of posterior. It may represent an undescribed species of *Asymphylodora* (see also Shimazu *et al.*, 2011).

*Life cycle.* Not known.

## Genus ***Asymphylotrema*** Dvoryadkin and Besprozvannykh, 1985

## ***Asymphylotrema monostyloides*** (Ito, 1960) comb. nov.

(Figs. 16–20)

"*Asymphylodora japonica*?" (not of Yamaguti, 1938): Yamaguti, 1938: 88–89; Shimazu, 1992: 12.

*Cercaria monostyloides* Ito, 1960: 68–69, fig. 15.

*Anapalaeorchis hamajimai* Fujino and Kifune, 1991: 35–36, figs. 1–8; Shimazu, 1992: 12, 14, figs. 12–16.

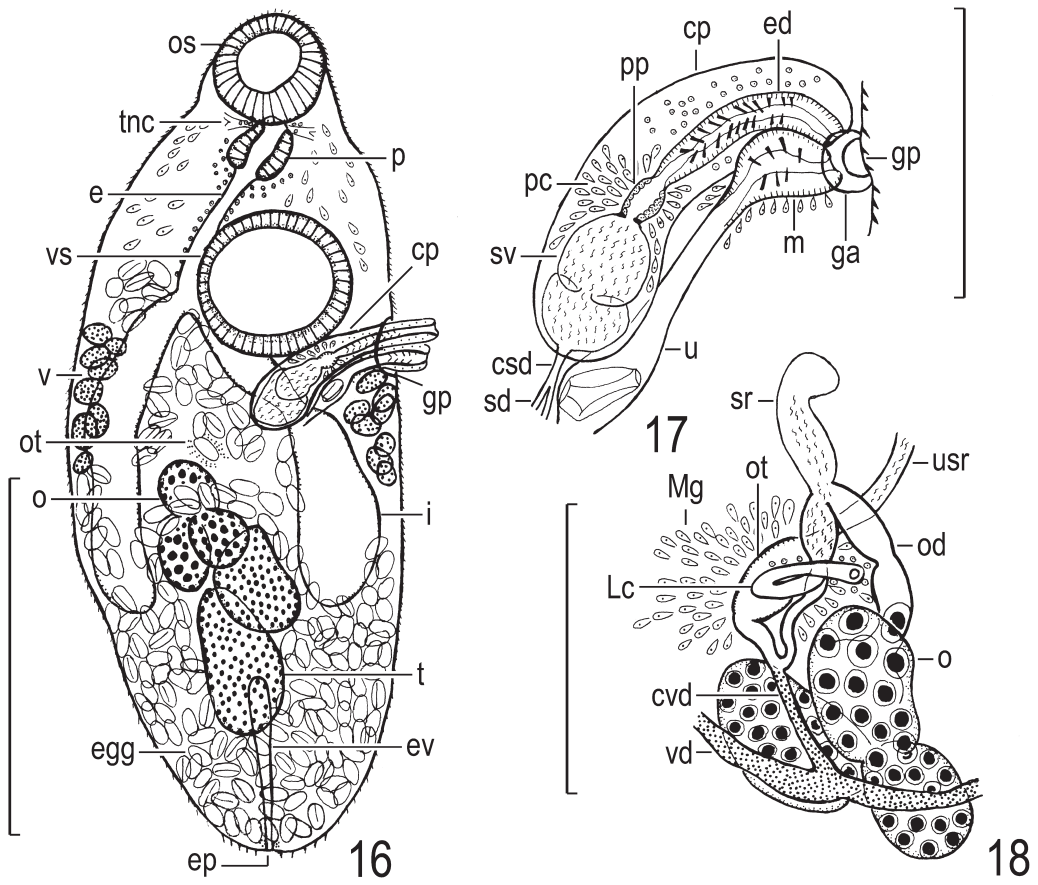
*Asymphylotrema hamajimai*: Bray, 2008b: 178; Shimazu *et al.*, 2011: 79–80, figs. 108–110.

*Host in Japan. Cobitis biwae* Jordan and Snyder, 1901 (Cobitidae) (type host) (Yamaguti, 1938; Fujino and Kifune, 1991; Shimazu, 1992, 2007; Shimazu *et al.*, 2011; this paper).

*Site of infection.* Intestine.

*Geographical distribution.* (1) Saitama Prefecture: Tokigawa River (type locality) at Tokigawa Village (Fujino and Kifune, 1991). (2) Nagano Prefecture: Metoba River at Hara and Asahi, Matsumoto City; and Aida River at Akashina-Nakagawate, Azumino City (Shimazu, 2007; this paper). (3) Shiga Prefecture: Lake Biwa basin (Hamabun) (Shimazu *et al.*, 2011). (4) Kyoto Prefecture: Katsura River (Yamaguti, 1938; Shimazu, 1992). (5) Hiroshima Prefecture: Seki River at Shiraki-cho, Asakita-ku, Hiroshima City (Shimazu, 1992).

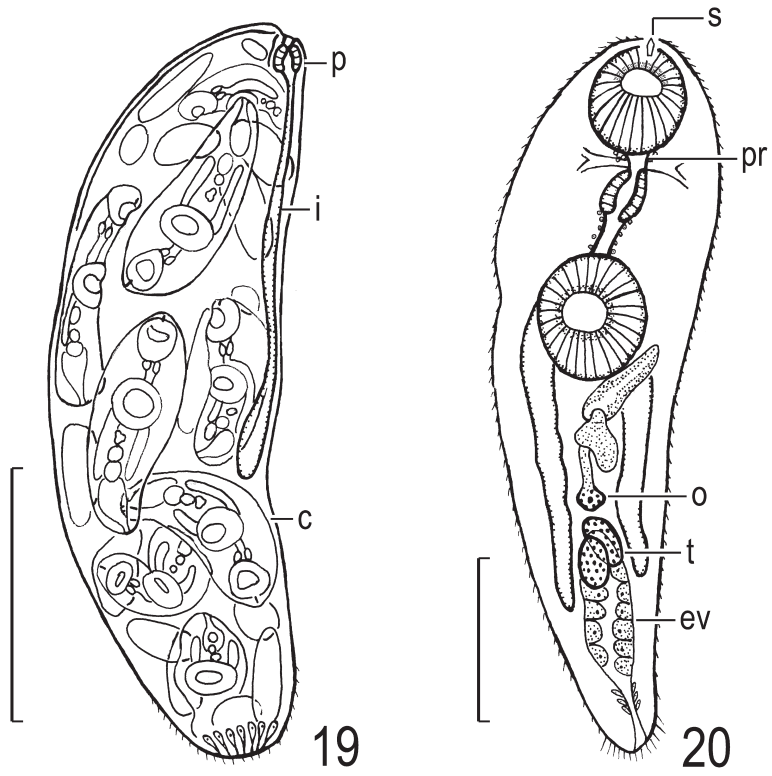
*Material examined.* (1) 7 specimens (NSMT-Pl 3776, type series, holotype and 6 paratypes, labeled "[Adult of *C. mono.*]") of *Anapalaeorchis hamajimai*, adult, whole-mounted, ex intestine of *Cobitis biwae*, Tokigawa River, 30 May 1985, 27 June 1985, 20 July 1988 (Fujino and Kifune, 1991; Shimazu, 1992). (2) 20 (NSMT-Pl 5493–5495) of *An. hamajimai*, immature, adult, ex intestine of *Co. biwae*, Metoba River at Hara, 23 November 1993, 19 August 1994, 21 November 1999 (Shimazu, 2007). (3) 1 (MPM Coll. No.




Figs. 16–18. *Asymphylotrema monostyloides*, adults found in intestine of *Cobitis biwae*. — 16, holotype (NSMT-Pl 3776), entire body, ventral view; 17, paratype (NSMT-Pl 3776), terminal genitalia, ventral view; 18, specimen (NSMT-Pl 5474), ovarian complex, dorsal view. Scale bars: 0.3 mm in Fig. 16; 0.1 mm in Figs. 17–18.

21163), adult, ex intestine of *Co. biwae*, Aida River, 8 November 2012. (4) 7 (LBM 1-55, 3-40) of *Asymphylotrema hamajimai*, adult, whole-mounted, ex intestine of *Co. biwae*, Hamabun, 24 October 1997, 1 November 2000 (Shimazu *et al.*, 2011). (5) 14 (Yamaguti's Collection, MPM Coll. No. 22034, labeled "*Asymphylodora japonica*?") of *An. hamajimai*, immature, adult, whole-mounted, ex intestine of *Co. biwae*, Katsura River, 25 May 1936 (Yamaguti, 1938; Shimazu, 1992). (6) 5 (NSMT-Pl 3682) of *An. hamajimai*, adult, whole-mounted, ex intestine of *Co. biwae*, Seki River, 25 July 1991 (Shimazu, 1992).

*Description.* Based on type series (NSMT-Pl 3776), after Shimazu (1992), slightly modified from the present study, supplemented by examination of other specimens (Figs. 16–18). Similar to the foregoing *Asymphylodora* in morphology, except for two tandem testes and 3-lobed ovary. Body elliptical, very small, 0.70–0.99 by 0.27–0.34 (holotype 0.70 by 0.28); forebody 0.22–0.37 long, occupying 32–37% of body length. Tegumental spines wedge-shaped, covering whole body but median ventral field of forebody, becoming smaller and thinner posteriorly and larger and thicker again around excretory pore. Oral sucker 0.09–0.13 in diameter, its lumen not spinose; minute spines present in at least three semicircular rows around anterior half of its aperture. Pharynx 0.05–0.07 in diameter. Esophagus bifurcating dorsal to ventral sucker. Intestines extending into testicular zoon. Ventral




Figs. 19–20. *Asymphylotrema monostyloides*, life cycle, [daughter (?)] redia (NSMT-Pl 5496) and cercaria (MPM Coll. No. 21164) found in *Semisulcospira libertina*. — 19, redia; 20, cercaria, penetration glands omitted. Scale bars: 0.3 mm in Fig. 19; 0.1 mm in Fig. 20.

sucker 0.13–0.19 in diameter; minute spines present in at least four circular rows around its aperture; sucker width ratio 1 : 1.2–1.5. Testes two, tandem or almost so, slightly overlapping each other, in third quarter of hindbody; anterior testis usually spherical, 0.08–0.14 by 0.08–0.11; posterior one usually elongate, 0.12–0.16 by 0.07–0.13. Common sperm duct short. Cirrus pouch 0.14–0.16 by 0.04–0.06, just posterior to ventral sucker, rarely extending slightly beyond median line of body. Seminal vesicle bipartite, thin-walled, 0.05–0.07 by 0.03–0.04. Pars prostatica 0.01–0.02 in diameter. Ejaculatory duct 0.06–0.09 by 0.01–0.02, lined heavily with spines (8–9 $\mu$m long) in posterior two-thirds, eversible. Genital atrium small. Genital pore sinistrally lateral, at level a little anterior to posterior border of ventral sucker. Ovary 3-lobed, 0.11–0.14 by 0.07–0.16, dextrally submedian, anterolateral to anterior testis. Laurer's canal fairly long, containing sperm. Seminal receptacle 0.05–0.06 by 0.02–0.03, empty or containing a small number of sperm. Uterus occupying all available space in hindbody, extending forward slightly beyond bifurcal level; metraterm about half as long as cirrus pouch, dilated in anterior half to form chamber lined thinly with spines (8–11 $\mu$m long). Eggs ovate, symmetrical, yellow, 27–30 by 16–19 $\mu$m, fully embryonated. Vitelline follicles six to seven on each side of body, distributed lateral and ventral to intestines between ventral sucker and midlevel of ovary [not anterior testis]. Excretory vesicle reaching to middle of posterior [not anterior] testis; excretory pore posteroterminal.

*Remarks.* Fujino and Kifune (1991) described a new genus and species, *Anapalaeorchis hamajimai*, based on the type series. Bray

(2008b) made a new combination, *Asymphylotrema hamajimai* (Fujino and Kifune, 1991), for *An. hamajimai*, synonymizing *Anapalaeorchis* with *Asymphylotrema* Dvoryadkin and Besprozvannykh, 1985, the type species of which is *Asymphylotrema macracetabulum* (Belous, 1953) in Primorsky Region, Russia (Dvoryadkin and Besprozvannykh, 1985). Shimazu *et al.* (2011) followed this. *Asymphylotrema* morphologically differs from *Asymphylodora* chiefly in that it has two tandem testes instead of one testis and the ovary is 3-lobed instead of globular.

Ito (1960) described a new cercaria, *Cercaria monostyloides*, from *Semisulcospira libertina* (see *Life cycle* below) from Shizuoka Prefecture. Shimazu (2007) concluded that *C. monostyloides* was the larva of *Anapalaeorchis hamajimai*, or now *Asymphylotrema hamajimai*, from positive circumstantial evidence, as follows (see also Shimazu, 1992, 1999, 2003). (1) Fujino and Kifune (1991) labeled the three slides of the type series of *An. hamajimai* "[Adult of *C. mono.*]" in Japanese, which, however, they did not mentioned at all in their paper. (2) Hamajima *et al.* (1982) found *C. monostyloides* in *S. libertina* collected at the type locality (Tokigawa River). (3) Fukuda *et al.* (1990) experimentally exposed eggs of *An. hamajimai* to *S. libertina* and subsequently recovered cercariae that were similar to *C. monostyloides* from the snail. (4) Shimazu (2007) found *An. hamajimai* and *C. monostyloides* in *Co. biwae* and *S. libertina*, respectively, from the Metoba River at Hara, where *An. hamajimai* was the only lissorchiid found. (5) *Cercaria monostyloides* and *An. hamajimai* were very similar to each other in morphology. Moreover, partial sequences (653 bp) of the cytochrome *c* oxidase I gene of the mitochondrial DNA (COI mtDNA) of adults of *An. hamajimai*, or now *As. hamajimai* (ex intestine of *Co. biwae*, Metoba River at Asahi-3chome, Matsumoto City, 15 October 2013) and rediae of *C. monostyloides* (ex midgut gland of *S. libertina*, the same locality, 1 October 2013) were identical between them (GenBank accession nos. LC114260 and LC114261, respectively, deposited by Urabe and Yano (2016)). This result supports Shimazu's (2007) conclusion.

Evidently, *C. monostyloides* and *As. hamajimai* are the same species. The specific name *monostyloides* Ito, 1960 has priority over the specific name *hamajimai* Fujino and Kifune, 1991. Therefore, I here make *Asymphylotrema monostyloides* (Ito, 1960) comb. nov. for the taxon according to Article 23.3.2.2 of the *International Code of Zoological Nomenclature* (International Commission on Zoological Nomenclature, 1999).

The stylet is present in the cercaria of *As. monostyloides* (Ito, 1960, 1964; this paper) but absent in that of *As. macracetabulum* (Dvoryadkin and Besprozvannykh, 1985). It is uncertain at present what taxonomic importance this difference has.

Ito (1960) included, in *C. monostyloides*, Cercaria XVII of Nakagawa (1915) found in *S. libertina* from Taiwan; Cercaria E of Yoshida (1917) found in *S. libertina* (2 subspecies) and *Semisulcospira reiniana* (Brot, 1876) (Japanese name: Chirimen-kawanina) from Osaka, Okayama, Tokushima, and Fukuoka Prefectures; and Cercaria XIII, or [Tora], of Ando (1918) found in *S. libertina* from Hiramaki Village, now in Kani City, Gifu Prefecture. I hesitate at present to follow Ito, because Cercaria XVII and Cercaria E and Cercaria XIII were recorded from Taiwan and Japan, respectively; and the three cercariae lack the stylet.

Shimazu (1992, fig. 12) erroneously described that the excretory vesicle reached forward to the middle of the anterior testis in the adult; but it is actually shorter, ending at the middle of the posterior testis (Shimazu *et al.*, 2011, fig. 108; this paper, Fig. 16).

*Life cycle.* The first intermediate hosts are *Semisulcospira libertina* and *S.* (*Biwamelania*) *nakasekoae*, in the midgut gland of which tailless cercariae (*Cercaria monostyloides*) are produced in [daughter (?)] rediae (Ito, 1960, 1964; Urabe, 2003; Shimazu, 2007; this paper). No second intermediate host is known. The final host is *Cobitis biwae*, in the intestine of which adults

live (Fujino and Kifune, 1991; Shimazu, 1992, 2007; Shimazu *et al.*, 2011; this paper).

I found [daughter (?)] rediae and cercariae (NSMT-Pl 5496–5500, MPM Coll. No. 21164; this paper, Figs. 19–20) in *S. libertina* from the Metoba River at Hara and Asahi 3-chome, Matsumoto City (see also Shimazu, 2007). Ito (1960) described that the genital primordium was still a cellular mass located posterior to the ventral sucker. However, two tandem testes, a pretesticular ovary, and preovarian terminal genitalia were already differentiated fairly well in my cercariae (Fig. 20). The flame cell formula is 2[(4 + 5 + 5) + (5 + 5 + 5)] = 58 (Ito, 1960, 1964).

## Genus ***Palaeorchis*** Szidat, 1942
## ***Palaeorchis diplorchis*** (Yamaguti, 1936)

(Figs. 21–23)

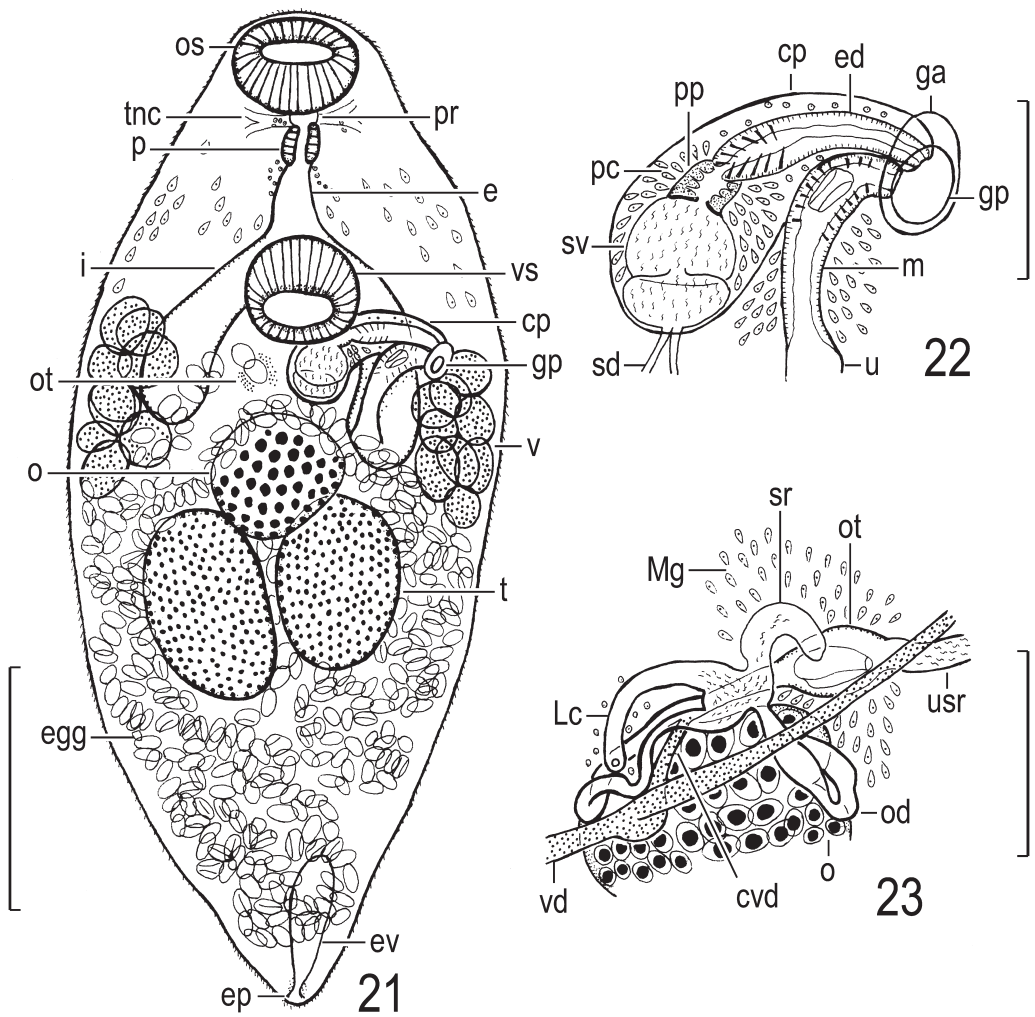
*Asymphylodora diplorchis* Yamaguti, 1936: 4–5, fig. 8.
*Steganoderma kamatukae* Takeuti, 1936: 582–583, 1 fig.; Yamaguti, 1954: 51.
*Palaeorchis diplorchis*: Szidat, 1943: 48, fig. 14; Shimazu, 1992: 15, 17, figs. 17–22; Shimazu and Urabe, 2005: 12–13, figs. 21–23; Shimazu *et al.*, 2011: 81, 83, figs. 111–115.

*Hosts in Japan. Pseudogobio esocinus esocinus* (Temminck and Schlegel, 1846) (Cyprinidae) (type host) (Yamaguti, 1936; Takeuti, 1936; Shimazu, 1992; this paper), *Biwia zezera* (Ishikawa, 1895) (Cyprinidae) (Shimazu *et al.*, 2011), *Hemibarbus barbus* (Cyprinidae) (Shimazu *et al.*, 2011), and *Anguilla japonica* Temminck and Schlegel, 1846 (Anguillidae) (Shimazu *et al.*, 2011).

*Sites of infection.* Intestine and stomach (accidental [?]).

*Geographical distribution.* (1) Nagano Prefecture: Lake Suwa (type locality) (Yamaguti, 1936; Shimazu, 1992). (2) Shiga Prefecture: Lake Biwa basin (Lake Biwa, Imazu, Momose, Moriyama, Omatsu, and Onoe) (Takeuti, 1936; Shimazu, 1992; Shimazu *et al.*, 2011). (3) Kyoto Prefecture: Uji River at Uji City (Shimazu and Urabe, 2005). (4) Hiroshima Prefecture: Furukawa River at Yoshikawa, Hachihonmatsu-cho, Higashihiroshima City (Shimazu, 1992). (5) Fukuoka Prefecture: Naka River at Terase Bridge, Narutake, Nakagawa Town (this paper).

*Material examined.* (1) 3 specimens (Yamaguti's Collection, MPM Coll. No. 22273, type series, holotype and 2 paratypes of *Asymphylodora diplorchis*) of *Palaeorchis diplorchis*, adult, whole-mounted, ex intestine of *Pseudogobio esocinus esocinus* (syn. *Pseudogobio esocinus*), Lake Suwa, 18 May 1935 (Yamaguti, 1936; Shimazu, 1992). (2) 37 (Yamaguti's Collection, MPM Coll. No. 23231, number changed, labeled "*Asymphylodora diplorchis*") of *P. diplorchis*, immature, adult, whole-mounted, ex intestine of *Ps. esocinus esocinus*, Lake Suwa, 30–31 March 1936 (Yamaguti, 1936, p. 6, a footnote; Shimazu, 1992). (3) Specimens of *P. diplorchis*, whole-mounted, ex intestine of *Ps. esocinus esocinus*, Lake Biwa basin: 26 (NSMT-Pl 3695, 3981), immature, adult, Onoe, 4 February 1980, 4 May 1992; 46 (NSMT-Pl 3980), adult, Moriyama, 2 May 1992; 21 (NSMT-Pl 3979, 3982, 3983), adult, Omatsu, 1, 5 and 6 May 1992; 3 (LBM 1-35, from intestine), adult, Imazu, 19 May 1998; 8 (LBM 1-35, 6-21 to -25, 8-50), immature, adult, Momose, 24 November 2007; and 1 (MPM Coll. No. 21162), adult, Momose, 14 May 2009 (Shimazu, 1992; Shimazu *et al.*, 2011; this paper). (4) 1 (NSMT-Pl 4013) of *P. diplorchis*, adult, whole-mounted, ex stomach of *Anguilla japonica*, Omatsu, 4 May 1992 (Shimazu *et al.*, 2011). (5) 2 (NSMT-Pl 5733) of *P. diplorchis*, adult, whole-mounted, ex intestine of *Hemibarbus barbus*, Moriyama, 2 May 1992 (Shimazu *et al.*, 2011). (6) 1 (NSMT-Pl 5736) of *P. diplorchis*, immature, whole-mounted, ex intestine of *Biwia zezera*, Onoe, 4 May 1992 (Shimazu *et al.*, 2011). (7) 38 (NSMT-Pl 5276, 5277) of *P. diplorchis*, adult, whole-mounted, ex intestine of *Ps. esocinus esocinus*, Uji River, 30 April 1998, 2 May 1998 (Shimazu and Urabe, 2005; see also Shimazu, 2013 for measurements). (8) 12 (NSMT-Pl 3696) of *P. diplorchis*, adult, whole-mounted, ex intestine of *Ps. esocinus esocinus*, Furukawa River, 25 July 1991 (Shimazu, 1992). (9) 13 (Urabe's unpublished specimens), imma-




Figs. 21–23. *Palaeorchis diplorchis*, adults found in intestine of *Pseudogobio esocinus esocinus.* — 21, holotype (MPM Coll. No. 22273), entire body, ventral view; 22, holotype, terminal genitalia, ventral view; 22, specimen (NSMT-Pl 3980), ovarian complex, dorsal view. Scale bars: 0.3 mm in Fig. 21; 0.1 mm in Figs. 22–23.

ture, adult, ex intestine of *Ps. esocinus esocinus*, Naka River, 25 November 2003.

*Description.* Based on specimens from Lake Biwa basin, after Shimazu (1992) and Shimazu *et al.* (2011), slightly modified from the present study (Figs. 21–23). Similar to the foregoing *Asymphylotrema monostyloides* in morphology, except for two symmetrical testes and globular ovary. Body elliptical to fusiform, small, 0.82–1.31 by 0.28–0.53 (holotype 1.22 by 0.53); forebody 0.28–0.46, occupying 28–39% of body length. Tegument spines scaly, present all over body, becoming lanceolate and thinner posteriorly and larger and thicker again at posterior extremity of body. Oral sucker 0.08–0.12 by 0.09–0.15; its lumen not spinose; minute spines present in about three semicircular rows around anterior half of its aperture. Pharynx 0.03–0.05 by 0.04–0.05. Esophagus bifurcating dorsally to ventral sucker. Intestines short, pretesticular. Ventral sucker 0.08–0.13 by 0.09–0.14, at about junction between anterior and middle thirds of body; minute spines arranged in about three circular rows around its aperture; sucker width ratio

1 : 0.9–1.0. Testes two, elliptical, symmetrical to slightly diagonal, contiguous or separated by uterus, submedian on either side of body, in about middle third of hindbody; right testis 0.19–0.30 by 0.09–0.14, left one 0.14–0.27 by 0.10–0.16. Common sperm duct absent. Cirrus pouch 0.13–0.26 by 0.06–0.09, reaching to median line of body or slightly beyond it. Seminal vesicle bipartite, thin-walled, 0.07–0.19 by 0.05–0.09. Pars prostatica 0.02–0.03 in diameter. Ejaculatory duct 0.07–0.14 by 0.02–0.03, lined heavily with spines (14–24 $\mu$m long) in proximal half, eversible. Genital atrium fairly deep. Genital pore sinistrally lateral or sublateral, slightly posterior to level of ventral sucker. Ovary irregular in shape (pyriform, subglobular, triangular, weakly 2-lobed, or weakly 3-lobed), 0.11–0.16 by 0.08–0.15, median or dextrally submedian, immediately pretesticular. Seminal receptacle kidney- to retort-shaped, 0.03–0.06 by 0.01, empty or containing a small number of sperm (and oocytes). Uterus much coiled in all available space in hindbody, proximally extending to midlevel of ventral sucker and acting as uterine seminal receptacle; metraterm fairly long, 0.06–0.13 long, dilated in middle three-fifths to form oblong chamber lined thinly with spines (9–17 $\mu$m long). Eggs oviform to pyriform, dark brown, 35–42 by 19–24 $\mu$m, fully embryonated. Vitelline follicles nine, large, distributed from intestinal shoulder to slightly beyond intestinal end on either side of body. Excretory vesicle short, posterior to testes in fully matured specimens, but reaching to testes in immature specimens; excretory pore posteroterminal.

*Remarks.* Yamaguti (10 September 1936) described a new species, *Asymphylodora diplorchis*, based on the type series found in the intestine of *Pseudogobio esocinus esocinus* from Lake Suwa. Takeuti (15 October 1936) described a new genus and species, *Steganoderma kamatukae*, based on adult specimens (type specimens not designated) found in the intestine of *Ps. esocinus esocinus* from Lake Biwa. Szidat (1943) established a new genus, *Palaeorchis*, with *A. diplorchis* as the type species; but he was unaware of *St. kamatukae* at that time. Yamaguti (1954) synonymized *St. kamatukae* with *P. diplorchis*. Shimazu (1992) agreed to this treatment. Takeuti's original material was not available, but it was probably lost (see the Materials and Methods in Shimazu, 2013).

The testes are slightly oblique in the holotype (Yamaguti, 1936; Shimazu, 1992; this paper, Fig. 21), but they are almost symmetrical to almost oblique in the other specimens (see Shimazu, 1992; Shimazu and Urabe, 2005; Shimazu *et al.*, 2011). The ovary varies widely in shape (pyriform, subglobular, triangular, or weakly 2- to 3-lobed) (see Shimazu, 1992; Shimazu and Urabe, 2005; Shimazu *et al.*, 2011). The excretory vesicle is small and posterior to the testes in fully matured adult specimens with well-developed uterine loops in the post-testicular region of the body, but it sometimes reaches to the testes in immature specimens without developed uterine loops in this region (Shimazu *et al.*, 2011). Apparently, *Palaeorchis* closely resembles *Asmphylotrema*, except for that the excretory vesicle is posterior to the testes instead of reaching to them. It is desirable that further critical studies be made of these two genera.

An adult specimen (NSMT-Pl 4013) of *P. diplorchis* was found in the stomach of *Anguilla japonica* from Omatsu (Shimazu *et al.*, 2011). Since this host fish had been raised on small fish from Lake Biwa in a fish preserve (Shimazu, 2015), it is considered that this infection was accidental from food fish, such as *Ps. esocinus esocinus*.

*Life cycle.* Not known. In Japan, another cercariaeum, *Cercariaeum incognitum* Faust, 1924 (syn. Cercariaeum C of Kobayashi, 1922), is known from *Semisulcospira libertina* (Kobayashi, 1922; Faust, 1924; Ito, 1964; Makita *et al.*, 1996; Urabe, 2003). It is possible that this cercaria is the larva of *P. diplorchis*, because it has shorter intestines ending at the level of the ventral sucker.

**Key to the genera and species of the family Lissorchiidae in this paper**

1.1. Testis one ..... 2
1.2. Testes two ..... 3
2.1. Seminal vesicle unipartite; cirrus pouch larger than metraterm; vitelline follicles lateral to ventral sucker ..... *Asymphylodora innominata*
2.2. Seminal vesicle unipartite; cirrus pouch smaller than metraterm; vitelline follicles lateral to ventral sucker ..... *Asymphylodora* sp.
2.3. Seminal vesicle bipartite; cirrus pouch larger than metraterm; vitelline follicles posterior to ventral sucker ..... *Asymphylodora japonica*
3.1. Testes symmetrical; ovary subglobular to weakly 3-lobed; excretory vesicle posterior to testes ..... *Palaeorchis diplorchis*
3.2. Testes tandem; ovary 3-lobed; excretory vesicle reaching to posterior testis ..... *Asymphylotrema monostyloides*

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