Bull. Natl. Mus. Nat. Sci., Ser. A, 41(4), pp. 201–216, November 20, 2015

# Digeneans Parasitic in Freshwater Fishes (Osteichthyes) of Japan V. Didymozoidae and Isoparorchiidae

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(Received 4 September 2015; accepted 24 September 2015)

**Abstract** Digeneans (Trematoda) parasitic in freshwater fishes of Japan are reviewed: *Paraphilopinna* sp. of Shimazu, 2006, *Philopinna higai* Yamaguti, 1936 and *Philopinna kawamutsu* Shimazu, Urabe and Grygier, 2011 (Didymozoidae); and *Isoparorchis eurytremus* (Kobayashi, 1915) (Isoparorchiidae). Each species is described and figured with a summarized life cycle where known. The life cycle and site of infection of the Didymozoidae in the present paper are discussed. It is considered that the site of infection of *Ph. higai* in the fin membrane of the host is the lymph capillary of the connective tissue. A key to the genera and species of the Didymozoidae in the present paper is given.

**Key words:** Digeneans, *Paraphilopinna*, *Philopinna*, *Isoparorchis*, freshwater fishes, Japan, review.

## Introduction

This is the fifth paper of a series that reviews adult digeneans (Trematoda) parasitic in freshwater fishes (Osteichthyes) of Japan (Shimazu, 2013). This contribution deals with the families Didymozoidae Monticelli, 1888 *sensu* Pozdnyakov and Gibson (2008) and Isoparorchiidae Travassos, 1922 *sensu* Gibson (2002b) in the superfamily Hemiuroidea Looss, 1899 *sensu* Gibson (2002a). Gibson (2002b) stated that the Drüsenmagen is absent in the diagnosis of the Isoparorchiidae, but this organ is well developed on either side of the body in *Isoparorchis* Southwell, 1913 (*e.g.*, Shimazu *et al.*, 2011; Shimazu *et al.*, 2014; this paper). Shimazu (2013) gave the Introduction and Materials and Methods for the review.

Some of the present specimens have also been deposited in the Swedish Museum of Natural History (SMNH), Stockholm, Sweden; The Natural History Museum (NHMUK), London, UK; the Institute of Parasitology (IPCAS), Biology Centre, Czech Academy of Sciences, Branišovská, Czech Republic; and the Queensland Museum (QM), Brisbane, Australia (Shimazu *et al.*, 2014).

*Abbreviations used in the figures.* bp, birth pore; c, cercaria; cbp, cercarial body proper; cc, cyclocoel; csd, common sperm duct; ct, cercarial (cystophorous) tail; cvd, common vitelline duct; Dm, Drüsenmagen; e, esophagus; ed, ejaculatory duct; egg, egg in uterus and metraterm; ep, excretory pore; ev, excretory vesicle; ga, genital atrium; gp, genital pore; hd, hermaphroditic duct; i, intestine; Lc, Laurer's canal; m, metraterm; md, male duct; Mg, Mehlis' gland; o, ovary; os, oral sucker; ot, ootype; p, pharynx; pac, parturient canal; pc, prostatic cells; pic, posterior intestinal cecum; pp, pars prostatica; rsr, rudimentary seminal receptacle; sd, sperm duct; so, sinus organ; sr, seminal receptacle; ss, sinus sac; sv, seminal vesicle; t, testis; tnc, transverse nerve commissure; u, uterus; usr, uterine seminal receptacle; v, vitellarium; vd, vitelline duct; vs, ventral sucker.

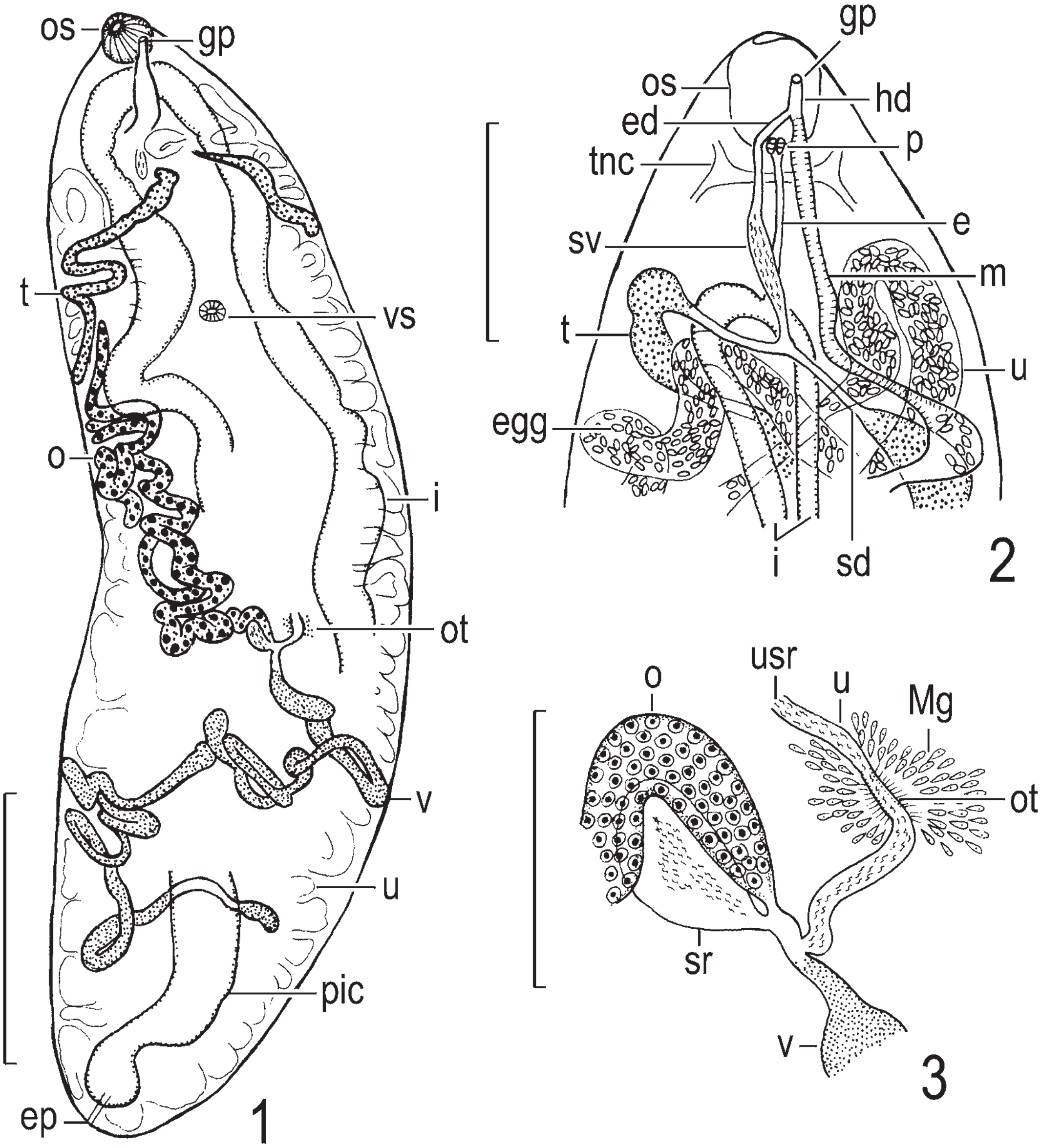
Superfamily Hemiuroidea Looss, 1899
Family Didymozoidae Monticelli, 1888
Genus ***Paraphilopinna*** Zhukov, 1971
***Paraphilopinna* sp.** of Shimazu, 2006
(Figs. 1–3)

*Paraphilopinna* sp.: Shimazu, 2006: 65–67, figs. 1–3.

*Host in Japan*. *Misgurnus anguillicaudatus* (Cantor, 1842) (Cobitidae) (Shimazu, 2006).

*Site of infection*. Unknown (see the Discussion below).

*Geographical distribution*. Eastern Hokkaido: Sarurunto (or Saruruto-numa, a shallow pond in




Figs. 1–3. *Paraphilopinna* sp., adult specimens (NSMT-Pl 5513) found in *Misgurnus anguillicaudatus* (site of infection unknown). — 1, entire body, ventral view; 2, anterior part of body, ventral view; 3, ovarian complex, ventral view. Redrawn from Shimazu (2006), modified, most part of uterus omitted. Scale bars: 1 mm in Fig. 1; 0.3 mm in Fig. 2; 0.2 mm in Fig. 3.

a marsh) in Toro, Shibecha Town (Shimazu, 2006).

*Material examined*. 3 specimens (NSMT-Pl 5513) of *Paraphilopinna* sp., adult, whole-mounted, ex *M. anguillicudatus*, Sarurunto, 4 July 1984 (Shimazu, 2006).

*Description*. After Shimazu (2006), 2 intact ones of 3 specimens measured, modified from the present study (Figs. 1–3). Body hermaphroditic, elongate, slightly flattened dorsoventrally, anoculate, fairly small, 2.96–4.32 by 0.64–1.22; forebody 1.12 long (occupying 26% of body length) in 1 specimen. Ecsoma absent. Tegument smooth. Transverse nerve commissure dorsal to esophagus. Oral sucker subglobular, 0.11–0.17 by 0.14–0.18, almost anteroterminal. Prepharynx absent. Pharynx very small, 0.03 by 0.04. Esophagus slender, short, 0.01–0.18 long. Intestines thick, undulating, extending posteriorly to near middle of body, but obscured in more posterior part by many folds of uterus; posterior intestinal cecum present, 1.04 long or 24% of body length, reaching to posterior extremity of body. Ventral sucker feeble, flat, 0.08 by 0.07 in 1 specimen, postbifurcal, located at about junction of first and second fourths of body; sucker width ratio 1 : 0.4. Testes two, tubular, long, postbifurcal, symmetrical, lateral and ventral to intestines, winding irregularly; right one longer than left one, beginning slightly posteriorly to ventral sucker; left one in forebody; testicular region 0.80 long or 18–27% of body length. Sperm ducts short, arising from anterior ends of their respective testes, following straight diagonal course; common sperm duct not seen. Seminal vesicle spindle-shaped, almost median, prebifurcal, ventral to esophagus, 0.11–0.12 by 0.08. Cirrus pouch absent. Prostatic complex absent. Ejaculatory duct thin-walled, fairly long, running anteriorly, uniting with metraterm ventrally to oral sucker to form hermaphroditic duct [not tubular genital atrium]; hermaphroditic duct tubular, short. Genital pore sinistrally submedian, midventral to oral sucker. Ovary single, tubular, long, dextrally submedian, dorsal to intestine, much convoluted irregularly, descending from level of proximal part of testes to ovarian complex; ovarian region 1.20–1.28 long or 29–40% of body length; oviduct short. Ovarian complex postovarian, median, slightly postequatorial, lying 1.68–2.40 or 55–57% of body length from anterior extremity of body. Seminal receptacle elliptical, large, 0.06–0.14 by 0.02–0.08. Juel's organ absent. Laurer's canal absent. Ovovitelline duct fairly long. Ootype vesicular, small, median or submedian, opposite seminal receptacle; Mehlis' gland well developed. Uterus highly folded possibly in dorsal and ventral parenchyma of all available space of body except in prebifurcal region; metraterm long, median, pretesticular, running forward in ventral parenchyma. Eggs numerous, scattered together with malformed ones in uterus, thin-shelled, operculate, elliptical, yellow, 24–27 by 14–16 $\mu$m, fully embryonated. Vitellarium single, tubular, long, dorsal to posterior intestinal cecum, much convoluted irregularly, commencing from near posterior extremity of body or more anteriorly; vitelline region 1.28 long or 29–43% of body length. Excretory vesicle not observed clearly; excretory pore posteroterminal.

*Remarks*. These specimens were poorly prepared, and so it could not be revealed where the intestines fused into the single posterior intestinal cecum. The testes, ovary and vitellarium were very convoluted irregularly that their actual lengths could not be measured (see also Shimazu, 2006).

*Paraphilopinna* Zhukov, 1971 has previously contained the type and only species *Paraphilopinna fluvialis* Zhukov, 1971, which Zhukov (1971) found in the ureter of *Misgurnus anguillicaudatus* from the Liao River in the basin of the Yellow Sea. The present specimens are different from *P. fluvialis* as described by Zhukov (1971) as follows. The oral sucker is larger, 0.11–0.17 by 0.13–0.18 instead of 0.054–0.083 by 0.050–0.062. The two intestines possibly fuse more posteriorly, near the middle of the body, instead of near the junction between the anterior and middle thirds of the body. The ventral sucker is larger, 0.08 by 0.07 instead of about 0.050–0.058 by 0.054–0.058. The testes, ovary and vitellarium are longer and more convoluted irregularly. The ovarian complex is located more posterior, slightly postequatorial instead of at the junction between the middle and posterior thirds of the body. Eggs are smaller, 24–27 by 14–16 $\mu$m instead of 29–31 by 14–20 $\mu$m (Shimazu, 2006). Furthermore, the seminal vesicle is spindle-shaped and prebifurcal instead of elliptical and postbifurcal. They may represent an undescribed species of *Paraphilopinna*, but they remain to be definitively identified to species until additional better-prepared specimens are made available.

*Life cycle*. Not known (see the Discussion below).

## Genus ***Philopinna*** Yamaguti, 1936
## ***Philopinna higai*** Yamaguti, 1936

(Figs. 4–6)

*Philopinna higai* Yamaguti, 1936: 1–2, figs. 1–7; Shimazu *et al.*, 2011: 8–10, figs. 6–8.

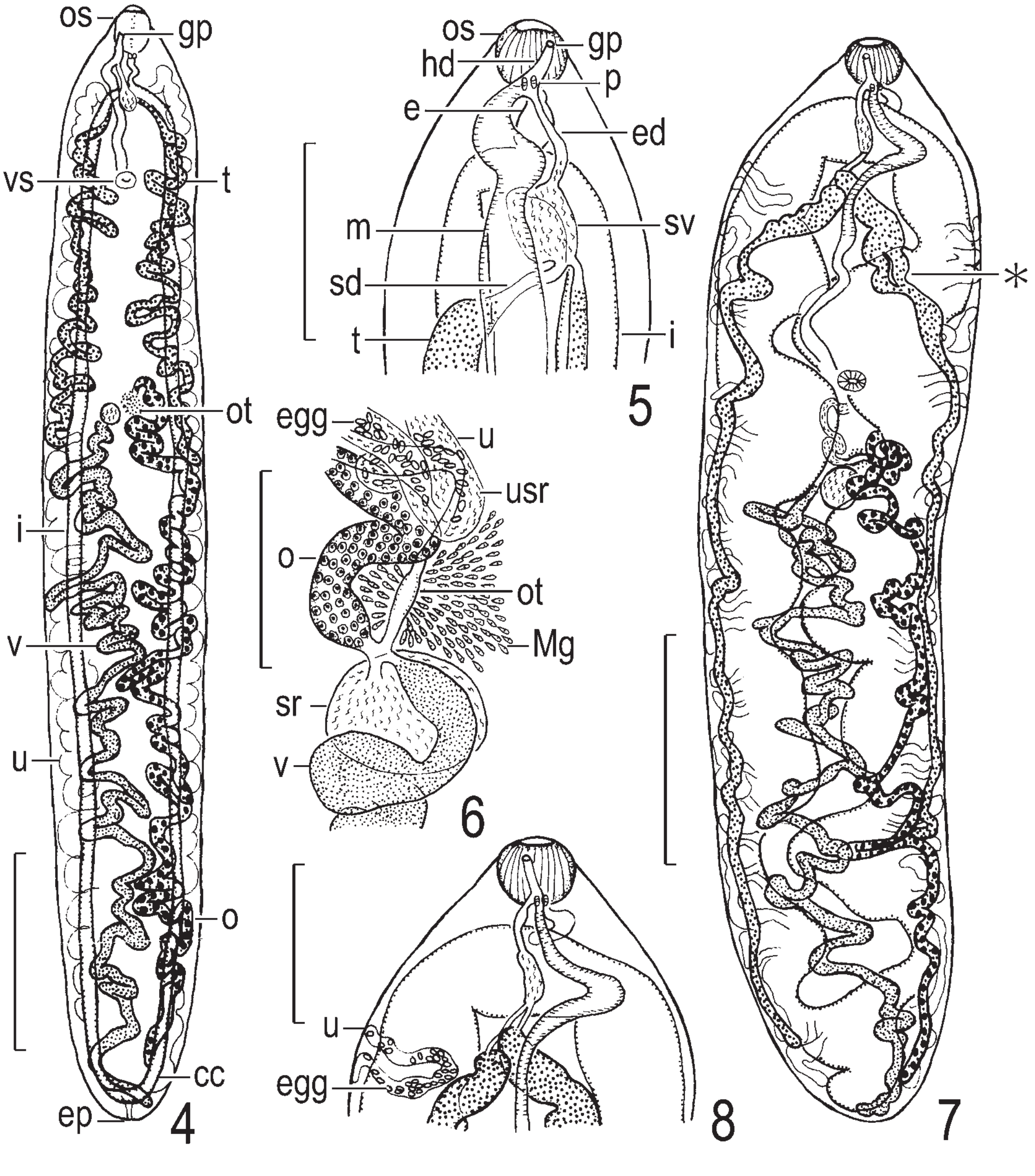
*Hosts in Japan*. "*Sarcocheilichthys variegatus* (Temm. et Schleg.)" (type host), *Sarcocheilichthys variegatus microoculus* Mori, 1927, *Sarcocheilichthys biwaensis* Hosoya, 1982 and *Sarcocheilichthys variegatus variegatus* (Temminck and Schlegel, 1846) (Yamaguti, 1936; Shimazu, 2007; Shimazu *et al.*, 2011; this paper).

*Sites of infection*. Fins and orbits (see the Discussion below).

*Geographical distribution*. (1) Ibaraki Prefecture: Lake Kitaura at Namegata City (Shimazu, 2007). (2) Nagano Prefecture: Lake Suwa (type locality) at Suwa City; Lake Kizaki in Oomachi City; and Hiroi River at Kotobuki, Iiyama City (Yamaguti, 1936; Shimazu, 2007). (3) Shiga Prefecture: Lake Biwa basin at Imazu-cho, Momose (Momose-gyoko Fishing Port), Chinai, Makino-cho, Harie River at Harie, Shin'asahi-cho, all Takashima City; Onoe, Kohoku-cho, and Minamihama-cho, both Nagahama City (Shimazu, 2007; Shimazu *et al.*, 2011; this paper). (4) Kyoto Prefecture: Uji River at Uji City (Shimazu, 2007). (5) Okayama Prefecture: Yoshii River at Joto, Okayama City (Shimazu, 2007). (6) Hiroshima Prefecture: Ashida River at Honjo, Fukuyama City (Shimazu, 2007). (7) Fukuoka Prefecture: Yanagawa City (Shimazu, 2007); and Futatsu River at Takahatake, Mitsuhashi-machi, Yanagawa City (this paper). (8) Kumamoto Prefecture: Midori River at Kosa Town (Shimazu, 2007).

In Korea (*e.g.*, Shimazu, 2007).

*Material examined*. (1) Type specimens (Yamaguti's Collection, MPM Coll. No. 22297) of *Philopinna higai*, ex fins and orbits of "*Sarcocheilichthys variegatus* (Temm. et Schleg.)," Lake Suwa: holotype, adult, whole-mounted, ex fin, 31 March 1936 [not 18 May 1935]; and 36 paratypes, adult, whole-mounted, serially sectioned, ex fins and orbits, 12 June 1932, 31 March 1935, 8 and 18 May 1935, 30 and 31 March 1936, 12 June 1936 (Yamaguti, 1936; Shimazu, 2007). (2) Specimens of *Ph. higai*, ex fins and orbits of *S. variegatus microoculus*, Nagano Prefecture: 20 (NSMT-Pl 5374–5376), adult, whole-mounted, Lake Kizaki, 5 November 1980, 16 May 1981, 5 May 1983; more than 36 (NSMT-Pl 5377–5380), immature, adult, whole-mounted, serially sectioned *in situ* in fins, Lake Suwa, 5 October 1991, 14 November 1991, 19 May 1992, 5, 30 October 1993; and 29 (NSMT-Pl 5381–5387), immature, adult, whole-mounted, Hiroi River, 19 October 1995, 26 September 1999, 16 October 1999, 13 November 1999, 20 May 2000, 10 June 2000, 5 November 2000 (Shimazu, 2007). (3) 9 (NSMT-Pl 5388) of *Ph. higai*, immature, adult, whole-mounted, ex fins of *S. variegatus microoculus*, Lake Kitaura at Kitaura Village, now in Namegata City, Ibaraki Prefecture, 6 August 1994 (Shimazu, 2007). (4) 10 (NSMT-Pl 5389–5390) of *Ph. higai*, adult, whole-mounted, ex fins of *S. variegatus microoculus* (formalin-preserved), Lake Biwa at Onoe, Kohoku Town, now Kohoku-cho, Nagahama City, Shiga Prefecture, 28 October 1976; and Minamihama-cho, Nagahama City, 15 June 1982 (Shimazu, 2007; Shimazu *et al.*, 2011). (5) 3 (LBM 3-25 to -26, 3-47) of *Ph. higai*, adult, whole-mounted, ex fins of *S. variegatus microoculus*, Lake Biwa, Imazu-cho, 5 May 2000; and




Figs. 4–6. *Philopinna higai*, adult specimens. — 4, holotype (MPM Coll. No. 22297) found in fin of "*Sarcocheilichthys variegatus* (Temm. et Schleg.)," entire body, ventral view; 5, LBM 3-26 found in fin of *S. variegatus microoculus*, anterior part of body, ventral view; 6, LBM 3-26, ovarian complex, dorsal view. Fig. 4 redrawn from Yamaguti (1936), modified, most part of uterus omitted; Figs. 5–6 redrawn from Shimazu *et al.* (2011), modified. Scale bars: 1 mm in Fig. 4; 0.5 mm in Fig. 5; 0.3 mm in Fig. 6.

Figs. 7–8. *Philopinna kawamutsu*, adult specimen, holotype (LBM 1340000066) found in connective tissue between vertebrae and air bladder near esophagus of *Nipponocypris temminckii*. — 7, entire body, ovary and vitellarium dorsal to intestines, but figured ventral for convenience's sake, partial abnormality in testis (*), ventral view; 8, anterior part of body, ventral view. Figs. 7–8 redrawn from Shimazu *et al.* (2011), modified, most part of uterus omitted in Fig. 7. Scale bars: 1 mm in Fig. 7; 0.5 mm in Fig. 8.

Harie River, 19 October 2000 (Shimazu *et al.*, 2011). (6) 2 (NSMT-Pl 5392) of *Ph. higai*, adult, whole-mounted, ex fins of *S. biwaensis* (formalin-preserved), Lake Biwa, Minamihama-cho, 15 June 1982 (Shimazu, 2007; Shimazu *et al.*, 2011). (7) Several (NSMT-Pl 5913), adult, whole-mounted, serially sectioned *in situ* in fins of *S. variegatus microoculus*, Lake Biwa, Momose, 14 December 2011, 26 April 2012. (8) 12 (NSMT-Pl 5391) of *Ph. higai*, adult, whole-mounted, ex fins of *S. variegatus microoculus* (formalin-preserved), Uji River, 2 August 1979 (Shimazu, 2007). (9) Specimens of *Ph. higai*, whole-mounted, ex fins of *S. variegatus variegatus* (formalin-preserved): 14 (NSMT-Pl 5393), immature, adult, Yoshii River, 10 February 1996; 6 (NSMT-Pl 5394), adult, Ashida River, 6 January 1980; 2 (NSMT-Pl 5395), adult, Yanagawa City, 29 June 1976; and 5 (NSMT-Pl 5396), adult, Midori River, 29 October 1977 (Shimazu, 2007). (10) 9 (Urabe's personal collection) of *Ph. higai*, immature, adult, whole-mounted, ex fins of *S. variegatus variegatus*, Futatsu River, 20 August 2002, 10 November 2002, 2, 4 and 16 September 2003, 10 October 2003.

*Description*. 1) After Yamaguti (1936) and Shimazu *et al.* (2011), measurements given by Shimazu *et al.* (2011), modified from the present study (Figs. 4–6). Similar to the foregoing *Paraphilopinna* sp. in general morphology except in shape of intestines and position of ovary. Body elongate, fairly small, 2.24–4.80 by 0.45–0.86 (holotype 5.63 by 0.84); forebody 0.56–0.96 long, occupying 18–25% of body length. Transverse nerve commissure dorsal to esophagus (not illustrated). Oral sucker napiform, 0.10–0.18 by 0.10–0.16, almost anteroterminal. Pharynx 0.02–0.04 by 0.03–0.05. Esophagus straight or curved, 0.09–0.17 long, with small sphincterlike structure surrounded with gland cells at posterior end (not illustrated). Intestines thin, sinuate, posteriorly fusing together to form cyclocoel near posterior extremity of body. Ventral sucker feeble, flat, small, 0.07–0.09 by 0.05–0.11, located at about junction of first and second fifths of body [not at about junction of anterior and middle thirds of body]; sucker width ratio 1 : 0.6–0.9. Testes tubular, about the same length, much convoluted, ventral to intestines, submedian, ascending from a short distance posterior to ootype to near intestinal bifurcation. Seminal vesicle pyriform, voluminous, 0.08–0.20 by 0.06–0.17, almost median, immediately posterior to and slightly overlapping intestinal bifurcation ventrally. Ejaculatory duct long. Hermaphroditic duct fairly long. Genital pore ventral to oral sucker, submedian, slightly anterior to midlevel of oral sucker. Ovary tubular, long, much convoluted, dorsal to intestine, dextrally or sinistrally submedian, ascending from near cyclocoel to a little anterior to ootype, then turning back to ootype. Ovarian complex median, greatly posterior to ventral sucker, about equatorial. Seminal receptacle almost spherical, large, 0.06–0.17 by 0.06–0.23, median. Ootype anterior or anterolateral to seminal receptacle; Mehlis' gland well developed. Uterus highly folded transversely in ventral and dorsal parenchyma of all available space on either side of body except in prebifurcal region; uterine seminal receptacle present in proximal part of uterus; metraterm beginning at level posterior to seminal vesicle. Eggs numerous, rounded rectangular, light brown, 21–25 by 14–17 $\mu$m, fully embryonated. Vitellarium tubular, long, much convoluted, dorsal to intestine, submedian and median, opposite ovary, ascending from posterior extremity of body to ovarian complex. Excretory vesicle I-shaped, straight, extending in ventral parenchyma to seminal vesicle (not illustrated); excretory pore posteroterminal.

*Remarks*. Yamaguti (1936) erected a new genus and species, *Philopinna higai*, on the basis of the type specimens found in the type host "*Sarcocheilichthys variegatus* (Temm. et Schleg.)" [Japanese name: Higai] from Lake Suwa (type locality). This fish will have been *S. variegatus microoculus* (Shimazu *et al.*, 2011), because the so-called Higai was artificially introduced from Lake Biwa into Lake Suwa in 1912 (Takei, 2007), and only this subspecies lives in

Lake Suwa these days (Shimazu, 1999, 2003, 2007). Similarly, it is considered that *Ph. higai* was artificially introduced, together with *S. variegatus microoculus*, from Lake Biwa into Lake Kitaura, Ibaraki Prefecture, and the Hiroi River (via the Chikuma River) and Lake Kizaki (via the Nogu River), Nagano Prefecture.

*Philopinna* differs from the foregoing *Paraphiloppina* in that the intestines fuse to form a cyclocoel near the posterior extremity of the body instead a single posterior intestinal cecum in the middle body and the ovary is posterior to the ootype instead of anterior.

No morphological difference significant enough to separate species was observed between the specimens found in the fins and those found in the orbits. *Philopinna higai* has also been recorded from *S. variegatus variegatus* in western Japan (Shimazu, 2007; this paper) and *Sarcocheilichthys variegatus wakiyae* Mori, 1927 (formalin-preserved) from the Puk'an-gang River at Anbo-ri, So-myon, Chunsong-gun, Kangwon-do, Korea, on [16 August 1984] (Shimazu, 2007, NSMT-Pl 5397). It is desirable that specimens found in *S. variegatus variegatus* from western Japan and *S. variegatus wakiyae* from Korea be compared with those found in *S. variegatus microoculus* from Lake Biwa with molecular data in order to see whether they really belong to the same species.

*Life cycle*. Not known (see the Discussion below).

### ***Philopinna kawamutsu*** Shimazu, Urabe and Grygier, 2011

(Figs. 7–8)

*Philopinna kawamutsu* Shimazu, Urabe and Grygier, 2011: 10, 12, figs. 9–13.

*Host in Japan*. *Nipponocypris temminckii* (Temminck and Schlegel, 1846) (Cyprinidae) (type host) (Shimazu *et al.*, 2011).

*Site of infection*. Connective tissue between vertebrae and air bladder near esophagus (see the Discussion below).

*Geographical distribution*. Shiga Prefecture: Yasu River (type locality) at Maeno, Tsuchiyama-cho, Koka City (Shimazu *et al.*, 2011).

*Material examined*. Type and voucher specimens of *Philopinna kawamutsu*: holotype (LBM 1340000066), adult, whole-mounted; 8 paratypes (LBM 1340000067, 1340000069, 1340000070, 1340000072), adult, whole-mounted; 2 paratypes and 4 vouchers (NSMT-Pl 5740), adult, whole-mounted; 18 vouchers (LBM 1340000068, 1340000071, 1340000073–1340000075), immature, whole-mounted; and 5 vouchers (LBM 1340000076), adult, serially sectioned, all ex connective tissue between vertebrae and air bladder near esophagus of *Nipponocypris temminckii*, Yasu River, 18 October 1997 (Shimazu *et al.*, 2011).

*Description*. After Shimazu *et al.* (2011), modified from the present study (Figs. 7–8). Similar to the foregoing *Ph. higai* in general morphology. Body elongate, fairly small, 2.96–5.16 by 0.77–1.15 (holotype 4.92 by 1.16); forebody 1.04–1.52 long, occupying 22–36% of body length. Oral sucker 0.16–0.21 by 0.16–0.23. Pharynx 0.02–0.03 by 0.02–0.04. Esophagus 0.14–0.16 long. Intestines thick, undulating. Ventral sucker located at about junction between anterior and second fourths of body, 0.05–0.07 by 0.05–0.11; sucker width ratio 1 : 0.3–0.5. Testes long; right testis longer than left one, beginning in posterior fourth of hindbody; left one beginning in third fourth of hindbody. Seminal vesicle spindle-shaped, 0.09–0.14 by 0.02–0.05, bifurcal. Genital pore submedian, slightly anterior to midlevel of oral sucker. Ovary sinistrally submedian and lateral, dorsal to intestine (but illustrated as ventral in Fig. 7). Ovarian complex slightly posterior to ventral sucker or at about junction between anterior and middle thirds of body. Seminal receptacle spherical, 0.12–0.16 in diameter. Ootype small, anterolateral to seminal receptacle; Mehlis' gland well developed. Uterus highly folded transversely except in prebifurcal region; metraterm almost median, beginning posterior to seminal vesicle. Eggs scattered in uterus, elliptical, yellow, 13–22 by 9–14 $\mu$m;

miracidia in them not clearly observed. Vitellarium almost median, dorsal to intestines (but illustrated as ventral in Fig. 7), beginning near posterior extremity of body. Excretory vesicle not clearly observed.

*Remarks*. Shimazu *et al*. (2011) described this form as a new species on the basis of specimens found in *Nipponocypris temminckii*. The species differs from *Ph. higai* (this paper) as follows. The ventral sucker is located more posteriorly, at about junction between anterior and second fourths of body instead of at about the junction of first and second fifths of body. The testes are much longer, beginning in the third and posterior fourths of the hindbody instead of in the posterior part of the anterior third of the hindbody. The seminal vesicle is spindle-shaped and bifurcal instead of pyriform and postbifurcal. The ovarian complex is located slightly, instead of greatly, posterior to the ventral sucker. Uterine eggs are smaller, 13–22 by 9–14 $\mu$m instead of 21–25 by 14–17 $\mu$m (Shimazu *et al*., 2011).

The following morphological abnormalities were observed in the present specimens. The intestines rarely fused halfway and rarely separate posteriorly in immature vouchers (Shimazu *et al*., 2011, figs. 12–13). The testis was partially split into two in the holotype (Shimazu *et al*., 2011, fig. 9; this paper, * in Fig. 7).

*Life cycle*. Not known (see the Discussion below).

## Discussion on the life cycle and site of infection of the Didymozoidae in this paper

Didymozoids are parasitic as adults in various tissues or organs of marine, exceptionally freshwater, fishes (Yamaguti, 1971). There is still no species whose entire life cycle is known (Kearn, 1998; Pozdnyakov and Gibson, 2008). Considering it easier to study the life cycles of freshwater species than those of marine species, I have long attempted to elucidate the life cycle of *Philoppina higai* in the field without success.

The site of infection of *Paraphilopinna* sp. has remained undetermined, because the present adult specimens were found in the saline, in which the opened bodies of the three fish hosts had been washed (Shimazu, 2006). Potentially, they lived free either in the body cavity or in the connective tissue of the peritoneum forming the body cavity.

The site of infection of *Ph. higai* is the connective tissue of the fin membranes and adipose tissue of the eyeballs and orbits. Examination of serial sections of infected fins showed that (1) worms were found free in elongate, wide spaces lined with a simple squamous endothelium in the connective tissue of the fin membranes; (2) a small number of cells like lymphocytes and neutrophils were seen in the intestines of the worms and around the worms in the spaces; (3) the intestines of the worms were filled with liquid and the above-mentioned cells; and (4) no hemorrhagic lesion was seen around or near the worms in the connective tissue. In addition, no blood current was seen in the spaces in living host fish. It is considered from these findings that the infected space, or the exact site of infection, is the lymph capillary. Shinpei Wada (personal communication, 2013) agreed to this, kindly examining the serial sections.

When some host fish were being examined for parasites, worms of *Ph. kawamutsu* were found free on or among their visceral organs. The site of infection was thought to be the body cavity at that time. Fortunately, it was by chance that a fish (about 4 cm in standard length) sectioned serially was found infected. Several immature worms (LBM 1340000077, vouchers) were found free in elongate, wide spaces lined with a simple squamous endothelium in the connective tissue, as in *Ph. higai*, of the peritoneum around small skeletal muscles between the vertebrae and air bladder near the esophagus of the fish. Some blood cells were seen in the intestines of the worms and around the worms in the spaces. No hemorrhagic lesion was seen around or near the worms (Shimazu *et al*., 2011). Shimazu *et al*. (2011) concluded, owing to the presence of the blood cells, that the infected space was the capillary. However, their conclusion is questionable. The site of infection might be not the capillary but the lymph capillary as in *Ph. higai*.

Little is known where and how didymozoid adults lay eggs (Kearn, 1998). No eggs were detected within the infected organs even though adult worms contained fully embryonated uterine eggs in *Ph. higai* and *Ph. kawamutsu* (Shimazu *et al.*, 2011; this paper). Accordingly, it does not appear that even fully gravid adults lay eggs there. On one occasion, a living adult worm of *Ph. higai* was found sticking the anterior half of the body out from the fin membrane of a living host fish (my unpublished data, 2011). The genital pore opens near the anterior extremity of the body. Adult worms might migrate out of the lymph capillary into the subcutaneous tissue, make a hole in the skin, stick the anterior part of the body out of the skin into the water and finally emit eggs into the water. Otherwise, eggs might be released from adult worms when infected fish are being digested in the digestive tract of predators.

**Key to the genera and species of the Didymozoidae in this paper**

1.1. Intestines fusing to form median, posterior cecum ........................................... *Paraphilopinna* sp.
1.2. Intestines fusing to form cyclocoel ........................................................................ *Philopinna*.... 2
2.1. Testes commencing at near middle of body ....................................................... *Ph. higai*
2.2. Testes commencing at near posterior extremity of body ......................................... *Ph. kawamutsu*

Family Isoparorchiidae Travassos, 1922
Genus ***Isoparorchis*** Southwell, 1913
***Isoparorchis eurytremus*** (Kobayashi, 1915)

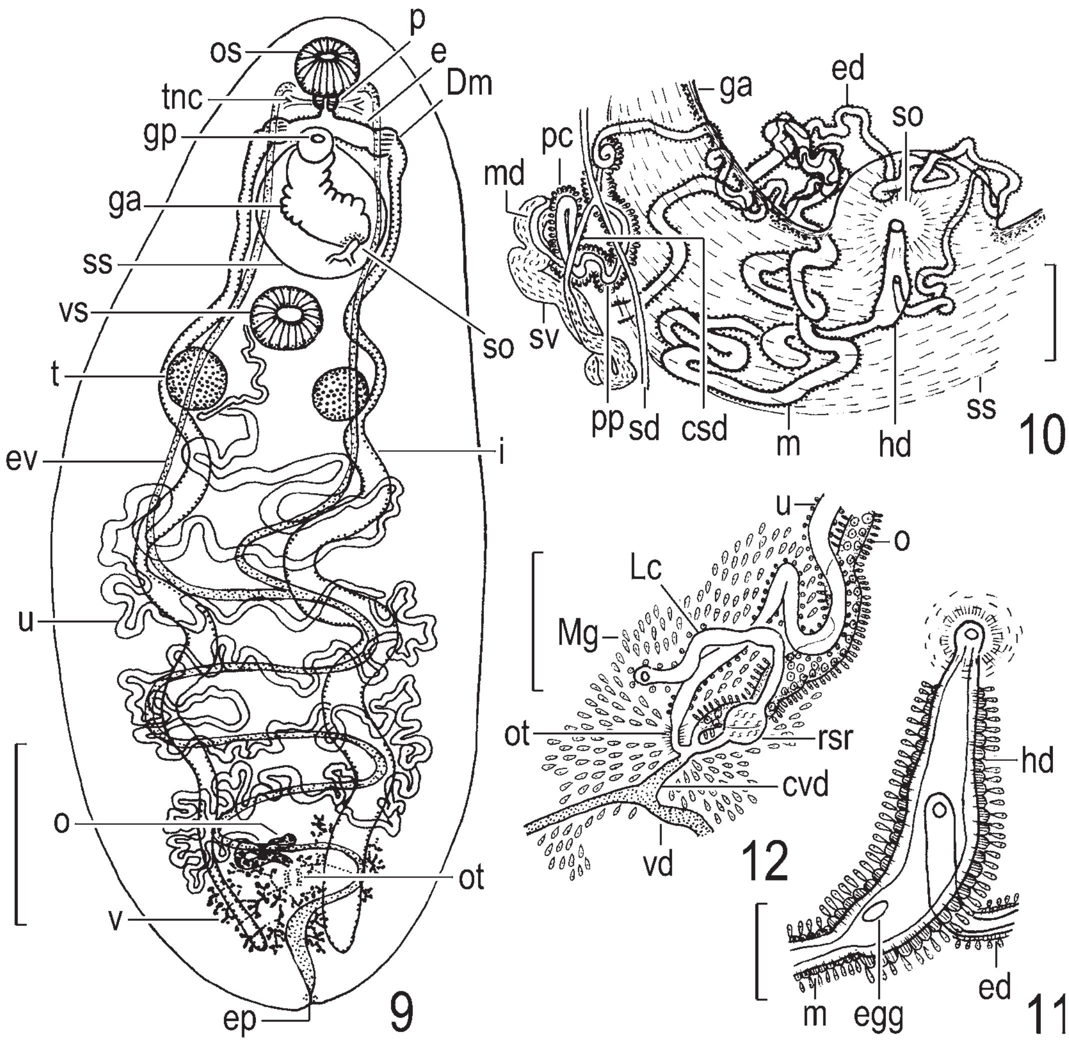
(Figs. 9–15)

*Leptolecithum eurytremum* Kobayashi, 1915: 50–52, pl. 2, figs. 1–3; Kobayashi, 1921: 397–399, pl. 26, fig. 1.
Cercaria XIV, or [U]: Ando, 1918: 619, fig. 14b.
*Isoparorchis euritremen* [*sic*, should be *eurytremus*]: Travassos, 1922a: 20.
*Isoparorchis eurytrema* [*sic*, should be *eurytremus*]: Travassos, 1922b: 230.
*Cercaria cystophora* B: Kobayashi, 1922: 268.
*Cercaria introverta* Faust, 1924: 294, table 1; Ito, 1953: 487, fig. 1; Ito, 1964: 478, fig. 97; Makita, Urabe and Nagoshi, 1996: 313, fig. 8.
*Isoparorchis eurytremum* [*sic*, should be *eurytremus*]: Odhner, 1927: 2, fig. 1; Shimazu, Urabe and Grygier, 2011: 27; Shimazu, Cribb, Miller, Urabe, Nguyen Van Ha, Tran Thi Binh and Shedʹko, 2014: 31, 33, figs. 12–19.
*Isoparorchis trisimilitubis* (not of Southwell, 1913): Bhalerao, 1926: 248; Yamaguti, 1934: 502, fig. 129.
*Isoparorchis eurytremus*: Ejsmont, 1932: 456.
*Isoparorchis hypselobagri* (not of (Billet, 1898) Ejsmont, 1932): Ejsmont, 1932: 456; Sawada and Osako, 1969: figs. 5, 7; Shimazu, Urabe and Grygier, 2011: 24–26, figs. 30–33.

*Hosts in Japan*. *Silurus asotus* Linnaeus, 1758 (Siluridae) (type host), *Silurus biwaensis* (Tomoda, 1961) and (?) “*Pseudobagrus aurantiacus*” (Bagridae) (type host) (Kobayashi, 1915, 1921; Yamaguti, 1934; Sawada and Osako, 1969; Shimazu, 2007; Shimazu *et al.*, 2011; Nagasawa *et al.*, 2013; Shimazu *et al.*, 2014).

*Site of infection*. Air bladder.

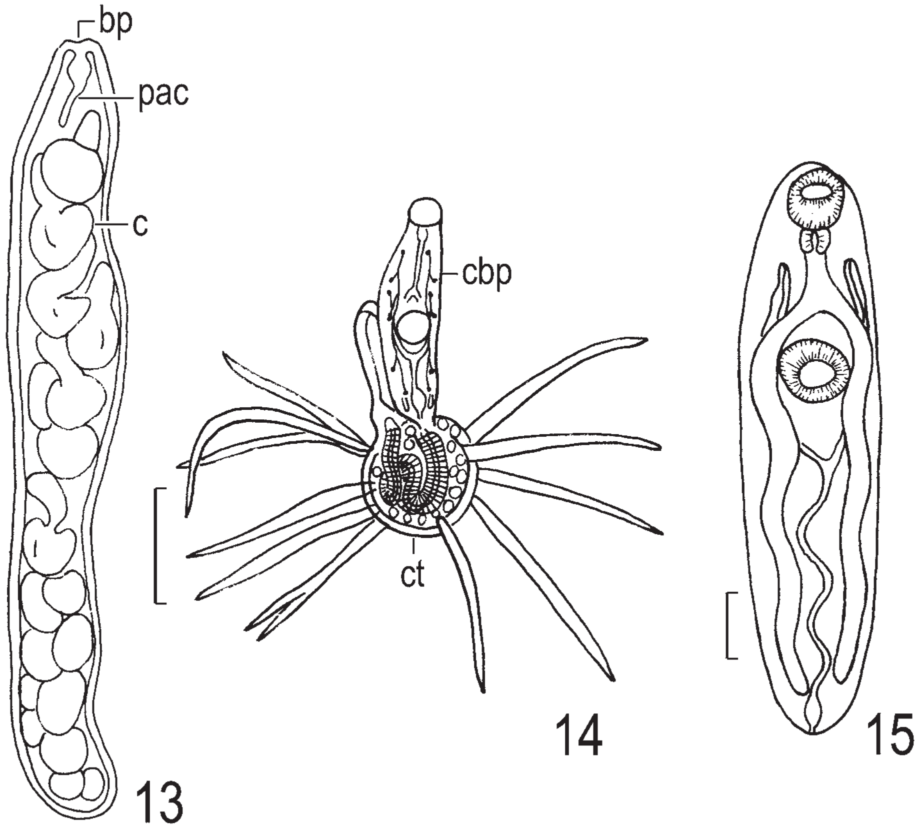
*Geographical distribution*. (1) Ibaraki Prefecture: Lake Kasumigaura (type locality) (Kobayashi, 1915, 1921). (2) Chiba Prefecture: Sawara (type locality), now in Katori City (Kobayashi, 1915, 1921). (3) Nagano Prefecture: Lake Kizaki in Oomachi City and Lake Suwa at Suwa City (Shimazu, 2007; Shimazu *et al.*, 2014). (4) Shiga Prefecture: Lake Biwa basin; Lake Biwa (type locality) (not specified); Lake Biwa off Imazuhama, Takashima City; off Isakifudo, Shirao-cho, Omihachiman City; off Katayama, Takatsuki Town; off Minamifunaki, Adogawa-cho, Takashima City; off Momose (Momose-gyoko Fishing Port), Chinai, Makinocho, Takashima City; off mouth of Echi River, Higashiomi City; off Onoe, Kohoku-cho, Nagahama City; and off Notogawa-cho, Higashiomi




Figs. 9–12. *Isoparorchis eurytremus*, adult specimen (NSMT-Pl 5866) found in air bladder of *Silurus asotus*. — 9, entire body, ventral view; 10, posterior part of terminal genitalia, ventral view; 11, terminal thickening of metraterm (hermaphroditic duct), ventral view; 12, ovarian complex, dorsal view. Redrawn from Shimazu *et al.* (2014), modified. Scale bars: 1 mm in Fig. 9; 0.5 mm in Figs. 10 and 12; 0.2 mm in Fig. 11.

City; Daido River at Ishizue, Otsu City; Kita River at Oyabu-cho, Hikone City; and Iba-naiko (a lake connected to Lake Biwa by Daido River), Higashiomi City; (Kobayashi, 1915, 1921; Shimazu *et al.*, 2011; Shimazu *et al.*, 2014). (5) Kyoto Prefecture: Kizu River at Kasagi Town and Uji River at Uji City (Sawada and Osako, 1969; Shimazu *et al.*, 2014). (6) Okayama Prefecture: various places (not specified) (type localities); Lake Kojima at Urayasuminami-cho, Minami-ku, Okayama City; and Mino, Kita-ku, Okayama City (Kobayashi, 1915, 1921; this paper). (7) Hiroshima Prefecture: Furuto River at Higashihiroshima City (Nagasawa *et al.*, 2013). (8) Yamaguchi Prefecture: Awano River at Shimonoseki City (Nagasawa *et al.*, 2013). (9) Oita Prefecture: Chikugo River at Kobuchi Bridge, Miyoshikobuchi-machi, Hita City (Shimazu *et al.*, 2014).

In Russia: Primorskiy Kray (Primorsky




Figs. 13–15. *Isoparorchis eurytremus*, life cycle. — 13, sporocyst, scale not given; 14, cercaria (*Cercaria introverta*); 15, 21-day-old metacercaria found in an experimental second intermediate host (species not given). Figs. 13 and 15 redrawn from Besprozvannykh (2000), fig. 14 redrawn from Ito (1953), modified. Scale bars: 0.1 mm in Figs. 14–15.

Region); and (?) China (*e.g.*, Shimazu *et al.*, 2014).

*Material examined*. (1) 1 specimen (SMNH Register Nos. 121146–121147, syntype of *Leptolecithum eurytremum*, neither host fish nor locality nor date indicated) of *I. eurytremum*, adult, serially sectioned, Japan (Odhner, 1927; Shimazu *et al.*, 2014). (2) Specimens of *I. eurytremum*, ex air bladder of *Silurus asotus*, Nagano Prefecture: 3 (NSMT-Pl 5407), adult, whole-mounted, Lake Kizaki, 14 October 1980; and 10 (NSMT-Pl 5408–5409), immature, adult, whole-mounted, Lake Suwa, 19 May 1992, 29 May 1993 (Shimazu, 2007; Shimazu *et al.*, 2014). (3) Specimens of *I. hypselobagri*, Lake Biwa basin: 5 (Yamaguti's collection, MPM Coll. No. 22009), immature, adult, whole-mounted, ex air bladder of *S. asotus*, Lake Biwa, 9 July 1927; 2 (Urabe's personal collection), immature, adult, ex air bladder of *S. biwaensis*, Lake Biwa, Katayama, 20 July 2006; 4 (LBM 8-74 to -77), adult, whole-mounted, Lake Biwa, Minamifunaki, 24 April 2007; 15 (LBM 8-59 to -73), immature, adult, Lake Biwa, Momose, 24 April 2007, 24 November 2007; and 1 (NSMT-Pl 5561), adult, whole-mounted, ex air bladder of *S. asotus*, Lake Biwa, Onoe, 4 May 1992; 1 (LBM 3-36), adult, whole-mounted, ex air bladder of *S. asotus*, Daido River, 1 May 2000; and 1 (Urabe's personal collection), adult, whole-mounted, Kita River, 12 November 2005 (Shimazu *et al.*, 2011). (4) Specimens of *I. eurytremum*, ex air bladder of

*S. asotus*, Lake Biwa basin: 10 (NSMT-Pl 5862–5864), adult, whole-mounted, Lake Biwa, Imazuhama, 2 and 5 December 2010; 2 (NSMT-Pl 5861), adult, whole-mounted, Lake Biwa, Isakifudo, 30 November 2010; 7 (6, NSMT-Pl 5865; 1, NHMUK 2013.7.16.3), adult, whole-mounted, Lake Biwa, mouth of Echi River, 17 December 2010; 11 (6, NSMT-Pl 5866; 2, IPCAS D-695; 2, QM G 234254; 1, Nguyen Van Ha's personal collection), adult, whole-mounted, Lake Biwa, Notogawa-cho, 18 December 2010; and 2 (1, NSMT-Pl 5867; 1, NHMUK 2013.7.16.4), adult, whole-mounted, Iba-naiko, 20 December 2010 (Shimazu *et al.*, 2014). (5) 20 (19, NSMT-Pl 5868; 1, QM G 234255) of *I. eurytremum*, adult, whole-mounted, serially sectioned, ex air bladder of *S. asotus*, Uji River, 20 May 2012 (Shimazu *et al.*, 2014). (6) 6 (NSMT-Pl 5910), adult, whole-mounted, ex air bladder of *S. asotus*, Mino, 22 April 2012. (7) 2 (NSMT-Pl 5911), adult, whole-mounted, ex air bladder of *S. asotus*, Lake Kojima, 12 May 2012. (8) 4 (Urabe's personal collection) of *I. eurytremum*, adult, whole-mounted, ex air bladder of *S. asotus*, Chikugo River, 25 August 2003 (Shimazu *et al.*, 2014).

*Description*. After Shimazu *et al.* (2014), modified from the present study (Figs. 9–12). Body ovate, very large, 20–47 by 11–18, anoculate; forebody without squaring shoulders, 7–15 long, occupying 27–37% of body length; hindbody elliptical. Ecsoma absent. Tegument smooth. Preoral lobe present. Oral sucker subglobular, 1.26–2.46 by 1.39–2.22, ventral, near anterior extremity of body. Prepharynx absent. Pharynx almost globular, 0.63–1.27 in diameter, posterodorsal to oral sucker. Esophagus inverted T- or Y-shaped, short, 0.08–0.24 by 1.05–3.65, surrounded by small gland cells. Drüsenmagen globular, small, 0.32–1.19 by 0.51–1.03, with several internal longitudinal grooves, between esophageal arm and intestine on either side of body. Intestines undulating about seven times in hindbody, terminating blindly near posterior extremity of body. Ventral sucker subglobular, larger than oral sucker, 1.11–3.49 by 1.66–3.65, located usually anterior to junction of anterior and middle thirds of body; sucker width ratio 1 : 1.2–1.4. Testes two, globular to elliptical, 1.26–4.44 by 1.19–3.96, symmetrical, directly medial to or overlapping intestines, posterolateral to ventral sucker. Sperm ducts long; common sperm duct short, anterior to ventral sucker. Seminal vesicle tubular, thick, long, thin-walled, convoluted, anterior to or overlapping ventral sucker, posterior to sinus sac. Male duct present between seminal vesicle and pars prostatica, not surrounded by gland cells, thin, short, lined with small epithelial cells. Pars prostatica tubular, thin, long, convoluted, surrounded by prostatic cells, free in parenchyma, between ventral sucker and sinus sac. Ejaculatory duct thin, very long, convoluted in dorsal wall of sinus sac, surrounded by small gland cells, leading to middle of hermaphroditic duct dorsally. Sinus sac elliptical, large, 3.09–11.11 by 2.06–7.93, occupying 43–74% of forebody length, weakly differentiated, with diffuse musculature, anterior to or overlapping ventral sucker; genital atrium elliptical, elongated, 2.30–6.66 by 1.34–2.85, occupying 72–95% of sinus sac length, divided into small anterior and large posterior portions, their border protruded like valve running obliquely posteriorly from dorsal to ventral. Sinus organ domelike, small, 0.40–0.79 by 1.59, situated on bottom of genital atrium. Genital pore large, median, immediately posterior to esophagus, encircled by sphincter. Ovary single, tubular, long, lying transversely, straight or folded once or twice, 3.28–10.92 by 0.14–0.21, dextrally or sinistrally submedian, greatly post-testicular, at about junction of third and posterior quarters of body, surrounded by tall gland cells. Oviduct and ovovitelline duct very short. Laurer's canal fairly long, storing sperm or not, opening dorsally, making elliptical dilatation to store sperm as rudimentary seminal receptacle 0.06–0.14 by 0.09–0.14 at near its proximal end. Juel's organ and canalicular seminal receptacle absent. Ootype vesicular, small, median; Mehlis' gland well developed. Uterus in dorsal parenchyma, surrounded by small gland cells, folded transversely some seven times between intestines in hindbody; metraterm tubular, thin, long, surrounded by small gland cells, convoluted anterior to ventral sucker and then in wall of sinus sac; uterine seminal receptacle present. Hermaphroditic duct (terminal thickening of metraterm for convenience's sake, Figs. 10–11) clavate, thick-walled, 0.51–0.92 by 0.10–0.22, in sinus organ, surrounded by tall gland cells, opening on tip of small protuberance in center of small shallow depression on apex of sinus organ. Eggs numerous, ovate, operculate, light brown, fully embryonated, 41–54 by 24–32 $\mu$m; small spherical malformed eggs also included. Vitellaria two, dendritic, postovarian, diagonal, dorsal, median; vitelline area overlapping intestines, between ovary and intestinal ends, 2.06–6.82 by 2.78–12.22. Vitelline ducts short; common vitelline duct or vitelline reservoir very short. Excretory vesicle Y-shaped, in ventral parenchyma; stem lined with epithelial cells in posterior part of longitudinal portion, folded transversely five times between intestines, bifurcating a little anterior to middle of hindbody; arms running forward on either side of body, turning backward laterally to pharynx, separate there; well developed elongate sphincter present at posterior end of excretory vesicle; excretory pore postero-terminal or -ventral.

*Remarks*. Kobayashi (1915) established a new genus and species, *Leptolecithum eurytremum*, in a Japanese paper. Later, Kobayashi (1921) gave a generic diagnosis of this new genus and a description of the type species in an English paper. He found his specimens in *Silurus asotus* and "*Pseudobagrus aurantiacus*" (Japanese name: "Gigi" in Kobayashi) from Lake Kasumigaura, Sawara, Lake Biwa and various places in Okayama Prefecture. It is quite uncertain where "*Pseudobagrus aurantiacus*" was really found infected (Shimazu *et al.*, 2011; Nagawasa *et al.*, 2013; Shimazu *et al.*, 2014). He seems to have presented measurements of only a single adult of his specimens. He did not designate a holotype nor a type host nor a type locality. Shimazu *et al.* (2014) reexamined Odhner's specimen (SMNH Register Nos. 121146–121147) and regarded it as the only existent syntype of *L. eurytremum*.

Synonymizing *Leptolecithum* with *Isoparorchis* Southwell, 1913, Travassos (1922a, b) transferred *L. eurytremum* to *Isoparorchis* as "*I. euritremen*" and "*I. eurytrema*," respectively. Bhalerao (1926) and Ejsmont (1932) regarded *L. eurytremum* as a synonym of *Isoparorchis trisimilitubis* Southwell, 1913; and the latter author used the species name *I. eurytremus* for *L. eurytremum*. Odhner (1927), Shimazu *et al.* (2011) and Shimazu *et al.* (2014) used the species name *I. eurytremum*. Shimazu *et al.* (2014) made a morphological and molecular study of species of *Isoparorchis* and demonstrated that *I. eurytremum* is valid, not a synonym of either *I. trisimilitubis* or *Isoparorchis hypselobagri* (Billet, 1898).

I here discuss the nomenclatural problem of the specific name *eurytremum* of *Leptolecithum eurytremum*. The question is whether the specific name is a noun or an adjective. Shimazu *et al.* (2011) treated it as a noun (a Greek word) in apposition to *Leptolecithum* in accordance with Article 31.2.2 of the *International Code of Zoological Nomenclature*, hereafter the Code (International Commission on Zoological Nomenclature, 1999), using the species name *Isoparorchis eurytremum*. However, I have recently received a contrary opinion from M. A. Alonso-Zarazaga (personal communication to M. J. Grygier, 17 May 2015) about the matter. He is of the opinion that no Greek word can finish in *-um*; and that the original author Kobayashi did mark his specific epithet "*eurytremum*" as an adjective, since he modified the Greek noun "*trema*" to adopt the Latin ending *-um*, which is only understandable as an adjective in gender agreement with *Leptolecithum* (gender neuter). Following his opinion, I use the specific name *eurytremus* (gender masculine) in gender agreement with *Isoparorchis* (gender masculine) (Article 31.2 of the Code). Ejsmont (1932) was correct in using the species name *I. eurytremus*.

The posterior part of the longitudinal portion of the excretory vesicle is lined with the epithelial cells. This liner of the epithelial cells is also seen in the metacercaria of *Isoparorchis tandani*

Johnston, 1927 (Shimazu *et al.*, 2014, fig. 29). The cercaria, metacercaria and adult of *I. eurytremus* have a dilatation in the longitudinal portion (*e.g.*, Sawada and Osako, 1969, figs. 5 and 7; this paper, Figs. 14 and 15). The liner is present in this dilatation.

*Life cycle*. Besprozvannykh and Ermolenko (1989) and Besprozvannykh (2000) studied the life cycle of *I. eurytremus* as *I. hypselobagri* in Primorsky Region, Russia. Natural first intermediate hosts were *Parajuga tegulata* and *Parajuga extersa* (Gastropoda, Pachychilidae). Sporocysts (Fig. 13) produced cystophorous cercariae (Cercaria XIV, or [U] of Ando, 1918, or *Cercaria introverta* Faust, 1924) (Fig. 14). Metacercariae (Fig. 15) developed unencysted in the body cavity of cyclops, a mayfly larva and an amphipod (experimental second intermediate hosts). They were infective to larvae of fish (experimental third intermediate hosts) [or paratenic hosts], in which they developed into juveniles. Natural final hosts there are *S. asotus* and *Silurus soldatovi* Nikolskii and Soin, 1948 (Shimazu *et al.*, 2014). A molecular study made by Shimazu *et al.* (2014) showed that *Cercaria introverta* was the cercaria of *I. eurytremus*. In Japan, *Cercaria introverta* has been recorded from the midgut gland of *Semisulcospira libertina* (syn. *Melania libertina*) and other species of *Semisulcospira* in various places (*e.g.*, Ando, 1918; Kobayashi, 1922; Ito, 1953, 1964; Makita *et al.*, 1996; Urabe, 2003). No natural second intermediate host is known.

The sporocyst has a long parturient canal (Fig. 13), which is complicated in anatomical structure, lacking a pharynx and leading from the body cavity to the birth pore at the anterior tip of the body (Shimazu *et al.*, 2014). Ando (1918) and Kobayashi (1922) interpreted this germinal sac as a redia; but Ito (1953, 1964), Besprozvannykh and Ermolenko (1989), Besprozvannykh (2000) and Shimazu *et al.* (2014) interpreted it as a sporocyst. It might be best referred to as a redia as in the life cycle of *Azygia* Looss, 1899 (Azygioidea) (Shimazu, 2014).

Juveniles (neither metacercariae nor larvae) and adult worms have been recorded from the body cavity, internal organs and musculature of fishes (perhaps paratenic hosts) of various species, and even from the air bladder of *Odontobutis obscura* (Temminck and Schlegel, 1845) (Yamaguti, 1934; Komiya, 1965; Shimazu, 1999, 2003; Shimazu *et al.*, 2011; Nagawasa *et al.*, 2013; Shimazu *et al.*, 2014). I also found specimens in the body cavity of *Pseudogobio esocinus esocinus* (Temminck and Schlegel, 1846) and *Zacco platypus* (Temminck and Schlegel, 1846) (NSMT-Pl 5777) and *Pungutungia herzi* Herzenstein, 1892 (NSMT-Pl 5778) from the Eno River at Yoshida, Yoshida-cho, Akitakata City, Hiroshima Prefecture, on 30 October 1976. Some of the worms were found gravid in organs other than the air bladder of *Nipponocypris temminckii* (Temminck and Schlegel, 1846), *Z. platypus*, *Pu. herzi* (Cyprinidae), *S. asotus, S. biwaensis* (Siluridae), *Lepomis macrochirus* Rafinesque, 1819, *Micropterus salmoides* (Lacepède, 1802) (Centrarchidae) and *Channa argus* (Cantor, 1842) (Channidae). It remains to be explained why worms can attain sexual maturity in the other organs of these fishes (Shimazu *et al.*, 2011; Shimazu *et al.*, 2014).

Adults live in the air bladder of final hosts, *Silurus asotus* and *S. biwaensis*, in Japan (Shimazu *et al.*, 2014). Kobayashi (1915, 1921) claimed to have found adults in "*Pseudobagrus aurantiacus*," but no adults have been recorded from the air bladder of bagrids in Japan since then. These final hosts are predatory. It will be essential that some paratenic hosts (fishes) harboring juveniles should be involved to transport metacercariae in second intermediate hosts to the final hosts (Shimazu *et al.*, 2014). Yamaguti (1934) experimentally showed that, in *S. asotus*, juveniles in the intestine penetrated the intestinal wall into the body cavity and, eventually, entered the air bladder to mature sexually there, without taking the route through the pneumatic duct. The infection route in the final host by way of the pneumatic duct as postulated by Kobayashi (1915) is untenable (Yamaguti, 1934). In the final hosts, eggs laid in the air bladder are probably carried out into the digestive tract via the pneumatic duct and eventually into the water. Nagasawa *et al.* (2013) reviewed this species under the species name *I. hypselobagri* in western Japan.

## Acknowledgments

I am grateful to Mr. Makoto Mizumoto (Okayama) for collecting some fishes for the present study, Prof. Shinpei Wada (Graduate School of Veterinary Medicine and Life Science, Nippon Veterinay and Life Science University, Musashino) for determining the site of infection of *Philopinna higai* in the host fish, Dr. Miguel A. Alonso-Zarazaga (Departamento de Biodiversidad y Biología Evolutiva, Museo Nacional de Ciencias Naturales, Madrid, Spain) and Dr. Mark J. Grygier (Lake Biwa Museum, Kusatsu) for helpful comments for the nomenclature and Dr. Thomas H. Cribb (School of Biological Sciences, The University of Queensland, Brisbane, Australia) for reviewing a draft of the manuscript.

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