

## Digeneans Parasitic in Freshwater Fishes (Osteichthyes) of Japan. II. Gorgoderidae and Orientocreadiidae

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**Abstract** Digeneans (Trematoda) parasitic in freshwater fishes of Japan are reviewed: four known species of *Phyllodistomum* Braun, 1899 and one known species of *Pseudophyllodistomum* Cribb, 1987 (Gorgoderoidea, Gorgoderidae) and two known species of *Orientocreadium* Tubanguui, 1931 (Plagiorchioidea, Orientocreadiidae). Each species is described and figured with a summarized life cycle where known. Questions relating to putative cercariae of species of *Phyllodistomum* and *Pseudophyllodistomum* and the excretory organs of *Orientocreadium* are discussed. Keys to the Japanese species of the Gorgoderidae of freshwater fishes and *Orientocreadium* are presented.

**Key words:** Digeneans, *Phyllodistomum*, *Pseudophyllodistomum*, *Orientocreadium*, freshwater fishes, Japan, review.

### Introduction

This is the second paper of a series that reviews adult digeneans (Trematoda) parasitic in freshwater fishes (Osteichthyes) of Japan (Shimazu, 2013). This contribution deals with the family Gorgoderidae Looss, 1899 *sensu* Campbell (2008) in the superfamily Gorgoderoidea Looss, 1899 *sensu* Bray and Blair (2008) and the family Orientocreadiidae Yamaguti, 1958 *sensu* Jones and Bray (2008) in the superfamily Plagiorchioidea Lühe, 1901 *sensu* Bray (2008). Shimazu (2013) gave the Introduction and Materials and Methods for the review.

*Abbreviations used in the figures.* cp, cirrus pouch; csd, common sperm duct; cvd, common vitelline duct; e, esophagus; ed, ejaculatory duct; egg, egg; ep, excretory pore; esv, external seminal vesicle; ev, excretory vesicle; fc, flame cell; ga, genital atrium; gp, genital pore; ic, intestinal cecum; isv, internal seminal vesicle; Lc, Laurer's canal; m, metraterm; Mg, Mehlis' gland; o, ovary; od, oviduct; os, oral sucker; ot, ootype; ovd, ovovitelline duct; p, pharynx; pc, prostatic cells; pp,

pars prostatica; prp, prepharynx; sd, sperm duct; sv, seminal vesicle; t, testis; tnc, transverse nerve commissure; u, uterus; v, vitellarium; vd, vitelline duct; vf, vitelline follicles; vs, ventral sucker.

Superfamily Gorgoderoidea Looss, 1899

Family Gorgoderidae Looss, 1899

Genus *Phyllodistomum* Braun, 1899

***Phyllodistomum biringo* Shimazu, 2005**

(Figs. 1–4)

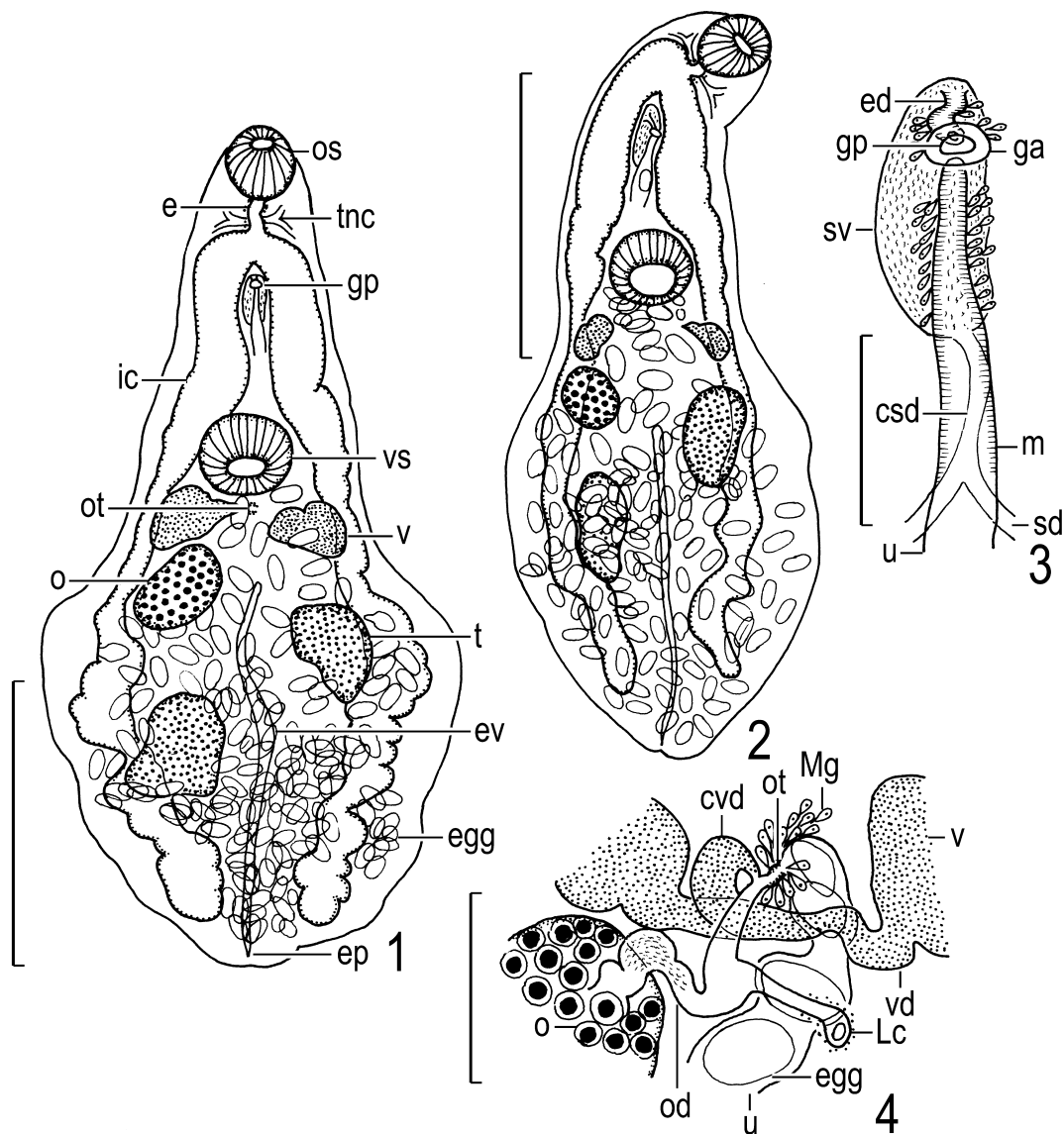
*Phyllodistomum biringo* Shimazu, 2005: 144–145, figs. 10–14.

*Hosts in Japan.* *Gymnogobius breunigii* (Steindachner, 1879) (Gobiidae) (type host) and *Silurus asotus* Linnaeus, 1758 (Siluridae) (accidental) (Shimazu, 2005).

*Sites of infection.* Urinary bladder and rectum (accidental).

*Geographical distribution.* Aomori Prefecture: Lake Ogawara (type locality) at Kamikita-kita, Tohoku Town (Shimazu, 2005; this paper).

*Material examined.* (1) 12 specimens (NSMT-



Figs. 1–4. *Phyllodistomum biringo*, adults (NSMT-PI 5239) found in urinary bladder of *Gymnogobius breunigii*. — 1, holotype, entire body, ventral view; 2, paratype, entire body, ventral view; 3, holotype, terminal genitalia, ventral view; 4, paratype, ovarian complex, dorsal view. Scale bars: 0.5 mm in Figs. 1–2; 0.1 mm in Figs. 3–4.

PI 5239, type series, holotype and 11 [not 12] paratypes) of *Phyllodistomum biringo*, 1 immature, 11 adult, whole-mounted, ex urinary bladder of *Gymnogobius breunigii*, Lake Ogawara, Kamikita Town, now Kamikita-kita, Tohoku Town, Aomori Prefecture, 6–9 September 1997 (Shimazu, 2005). (2) 1 (NSMT-PI 5240) of *P. biringo*, adult, whole-mounted, ex rectum of *Silurus asotus*, Lake Ogawara, 4 September 1997

(Shimazu, 2005).

**Description.** After Shimazu (2005), slightly altered from the present study (Figs. 1–4). Body dorsoventrally flat, translucent, banjo-shaped (forebody narrower, tapering anteriorly; hindbody wider, foliate, forming shoulders at anterolateral ends), small, 0.70–1.44 by 0.37–0.71 (holotype adult, 1.44 by 0.71), not oculate; forebody 0.31–0.56 long, 35–48% of body length. Tegument

smooth. Transverse nerve commissure dorsal to esophagus. Oral sucker subglobular, 0.08–0.12 by 0.09–0.11, subterminal. Pharynx absent. Esophagus thick-walled, surrounded by small gland cells, 0.03–0.07 long, bifurcating at about border of anterior and second thirds of forebody. Intestinal ceca slightly sinuate, slightly diverticulate, ending near posterior extremity of body. Ventral sucker subglobular, 0.12–0.14 by 0.11–0.16, slightly pre-equatorial; sucker width ratio 1:1.2–1.4. Testes two, irregularly indented, 0.09–0.17 by 0.09–0.14, oblique, separate, intercecal and slightly overlapping intestinal ceca, in middle third of hindbody, some distance anterior to cecal ends. Sperm ducts long; common sperm duct short, anterior to ventral sucker. Seminal vesicle pyriform, 0.12–0.25 by 0.05–0.11, median, dorsal to metraterm and genital pore. Cirrus pouch absent. Prostatic complex absent. Ejaculatory duct short, running posteriorly, surrounded by small gland cells. Genital atrium large. Genital pore large, median, slightly postbifurcal. Ovary globular to elliptical, entire, 0.06–0.15 by 0.09–0.14, dextrally or sinistrally submedian, intercecal, slightly overlapping intestinal cecum, pretesticular, immediately posterior to vitellarium on ovarian side of body. Oviduct dilated before giving off Laurer's canal, dilatation (fertilization chamber) usually including sperm. Laurer's canal short, running transversely, opening dorsally near vitellarium on antiovarian side of body. Ootype median, between vitellaria, anterior to vitelline ducts. Mehlis' gland large. Canalicular seminal receptacle absent. Uterus much coiled in hindbody, intercecal and spreading out ventrally to intestinal ceca and then into extra- and post-cecal fields of body; metraterm well developed, surrounded by small gland cells; uterine seminal receptacle not seen. Uterine eggs numerous, elongate elliptical, slightly curved, light yellow, not operculate, fully embryonated; eggs formed with no ovum 33–48 by 21–24  $\mu\text{m}$ , eggs in 1-cell stage of development 53–56 by 29–33  $\mu\text{m}$ , fully embryonated eggs (intact, daughter sporocyst seen in miracidium) 59–65 by 29–35  $\mu\text{m}$ , no hatched miracidia seen in uterus. Vitellaria two,

compact, elliptical or slightly indented, 0.05–0.06 by 0.09–0.11, symmetrical, posterolateral to ventral sucker, intercecal and overlapping intestinal ceca. Excretory vesicle I-shaped, extending anteriorly to ovarian level; excretory pore posteroterminal or posterodorsal [not posteroventral].

**Remarks.** Shimazu (2005) described *Phyllodistomum biringo* Shimazu, 2005 from *Gymnogobius breunigii*. In the species, the ejaculatory duct is short and not everted; the uterus spreads out ventrally to the intestinal ceca and then into the extra- and post-cecal fields of the body; and fully embryonated eggs are large, 59–65 by 29–35  $\mu\text{m}$ . It seems from these characteristics that the species is in an intermediate position between *Phyllodistomum* and *Pseudophyllodistomum* Cribb, 1987 as discussed below. I prefer to retain it in *Phyllodistomum* for the time being until its life cycle is elucidated.

Shimazu (2005) considered that *Silurus asotus* was an accidental host, which had acquired infection with the worm by ingesting the true final host *Gymnogobius breunigii* harboring it in the urinary bladder, and the rectum was an accidental site of infection.

**Life cycle.** Not known. The host fish *G. breunigii* lives in brackish-water to freshwater regions (Nakabo (ed.), 2013). Toyooka (1965a, b) found a new cercaria, Cercaria B, with a simple short tail, in *Corbicula japonica* Prime, 1864 (Japanese name: Yamato-shijimi) from Tokushima Prefecture (see the Discussion on *Phyllodistomum* and *Pseudophyllodistomum* below). Because *C. japonica* is a brackish-water species (Habe, 1973), it is possible that Cercaria B is the cercaria of *P. biringo*.

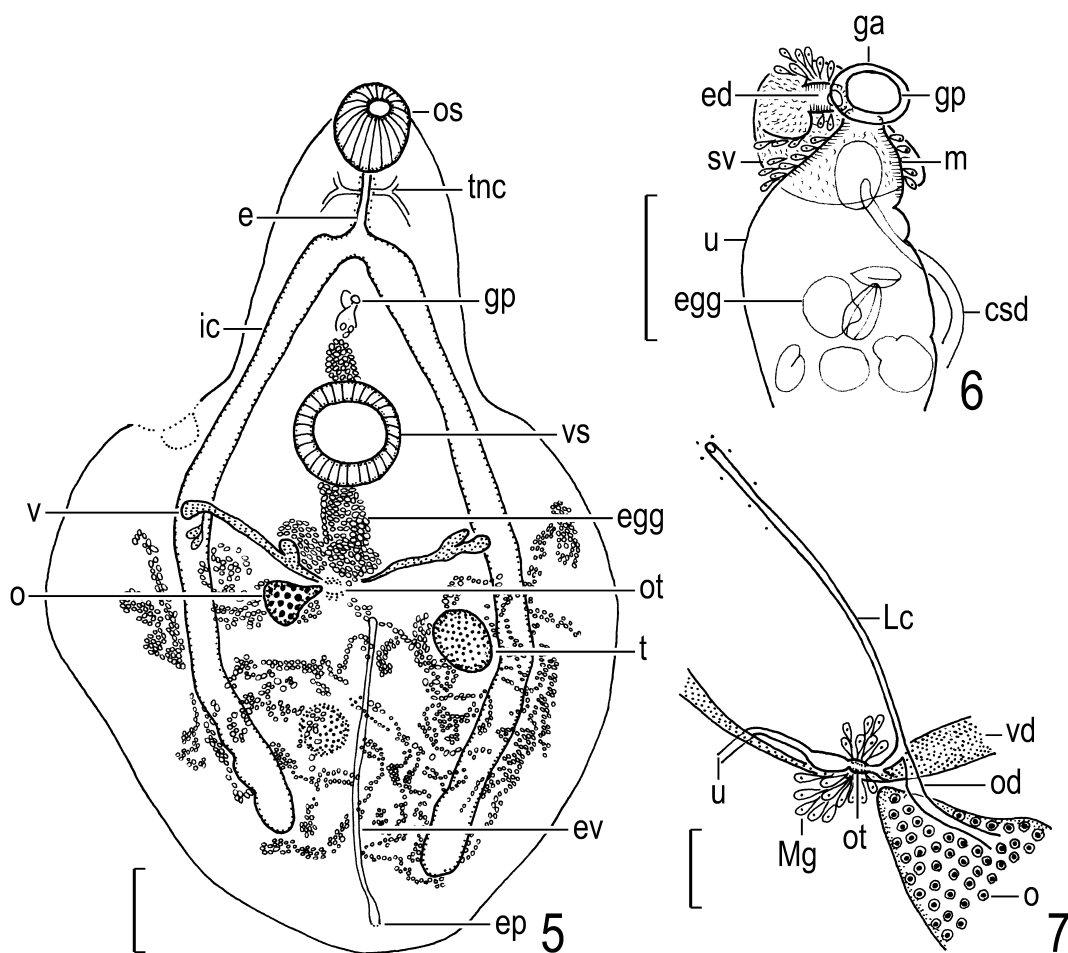
### *Phyllodistomum carassii* Long and Wai, 1958

(Figs. 5–7)

*Phyllodistomum (Catoptroides) carassii* Long and Wai, 1958: 356–357, fig. 8.

*Phyllodistomum carassii*: Shimazu *et al.*, 2011: 61–63, figs. 86–88.

**Host in Japan.** *Carassius auratus grandoculis* Temminck and Schlegel, 1846 (Cyprinidae)



Figs. 5–7. *Phyllodistomum carassii*, adult (NSMT-PI 5715) found in ureter of *Carassius auratus grandoculis*. — 5, entire body, ventral view; 6, terminal genitalia, ventral view; 7, ovarian complex, dorsal view. Scale bars: 0.5 mm in Fig. 5; 0.1 mm in Figs. 6–7.

(Shimazu *et al.*, 2011).

*Site of infection.* Ureters.

*Geographical distribution.* Shiga Prefecture: Lake Biwa at Onoe, Kohoku-cho, Nagahama City (Shimazu *et al.*, 2011; this paper).

In China: Lake Tai and Shanghai (e.g. Long and Wai, 1958).

*Material examined.* 2 specimens (NSMT-PI 5715) of *Phyllodistomum carassii*, adult, whole-mounted, ex ureters of *Carassius auratus grandoculis*, Lake Biwa, Onoe, Kohoku Town, now Kohoku-cho, Nagahama City, 3 June 1980 (Shimazu *et al.*, 2011).

*Description.* After Shimazu *et al.* (2011),

slightly altered from the present study (Figs. 5–7). 1 of 2 specimens slightly damaged, the other artificially cut transversely into 2 pieces representing anterior and posterior parts of body. Similar to *Phyllodistomum biringo* (this paper) in general morphology. Body banjo-shaped, fairly small, 3.92–5.37 by 2.96–3.42; forebody 2.08–2.21 long, occupying 41–53% of body length. Oral sucker 0.56 by 0.45–0.48. Esophagus 0.35–0.53 long, bifurcating at about midlevel of forebody. Intestinal caeca not sinuate, not diverticulate, ending some distance anterior to posterior extremity of body. Ventral sucker 0.61–0.62 by 0.53–0.64, about equatorial; sucker width ratio

1:1.1–1.4. Testes apparently atrophied, elliptical, medial to ceca, in middle third of hindbody; anterior testis 0.35 by 0.27, posterior 0.19–0.29 by 0.10–0.24. Seminal vesicle 0.12–0.14 by 0.07–0.08. Ejaculatory duct short. Genital atrium large. Genital pore large, slightly postbifurcal. Ovary 0.24 by 0.19 (damaged in 1 specimen), dextrally submedian, intercecal, slightly pretesticular. Laurer's canal long, opening dorsally between ventral sucker and left vitellarium. Uterus much folded in postvitelline field of hindbody, absent in lateral and posterior marginal fields of body; metraterm 0.10–0.16 long; uterine seminal receptacle not seen. Uterine eggs numerous, elliptical, light brown, fully embryonated; eggs formed with no ovum 17–19 by 13  $\mu\text{m}$ , eggs in 1-cell stage of development 25–27 by 19–21  $\mu\text{m}$ , intact large embryonated eggs (large embryonated eggs in Shimazu *et al.* (2011)) 33–38 by 24–29  $\mu\text{m}$ , miracidia (each tightly enclosed by thin torn eggshell) 48–64 by 32–41  $\mu\text{m}$ . Vitellaria transversely elongated, each with at least 2 long transverse branches, 0.48–0.75 by 0.05–0.12, intercecal and slightly overlapping ceca. Excretory vesicle extending to ovarian level; excretory pore posterodorsal.

**Remarks.** Long and Wai (1958) described *Phyllodistomum* (*Catoptroides*) *carassii* Long and Wai, 1958 on the basis of adult specimens found in the urinary bladder and ureters of *Carassius auratus* (Linnaeus) from Tai Hu (Lake Tai) and Shanghai, China. Shimazu *et al.* (2011) identified the present specimens as *P. carassii*, because they agreed well in morphology with the original description of this species by Long and Wai (1958) except for the egg size. The intact large embryonated eggs were 33–38 by 24–29  $\mu\text{m}$  in the present specimens. The egg size in Chinese materials was given as 25–26 by 17–20  $\mu\text{m}$  by Long and Wai (1958), as 15–25 by 11–17  $\mu\text{m}$  by Institute of Hydrobiology, Hubei Province, China (chief ed.) (1973), and as 17–26 by 10–16  $\mu\text{m}$  by Sun and Jiang (1991). These three descriptions did not indicate the developmental stage of the embryo. None of these three materials has been made available to me for reexamination.

**Life cycle.** Not known.

### *Phyllodistomum mogurndae* Yamaguti, 1934

(Figs. 8–11)

*Phyllodistomum mogurndae* Yamaguti, 1934: 425–427, figs. 87–88; Shimazu, 2007: 13, figs. 20–22; Shimazu *et al.*, 2011: 63–65, figs. 89–90.

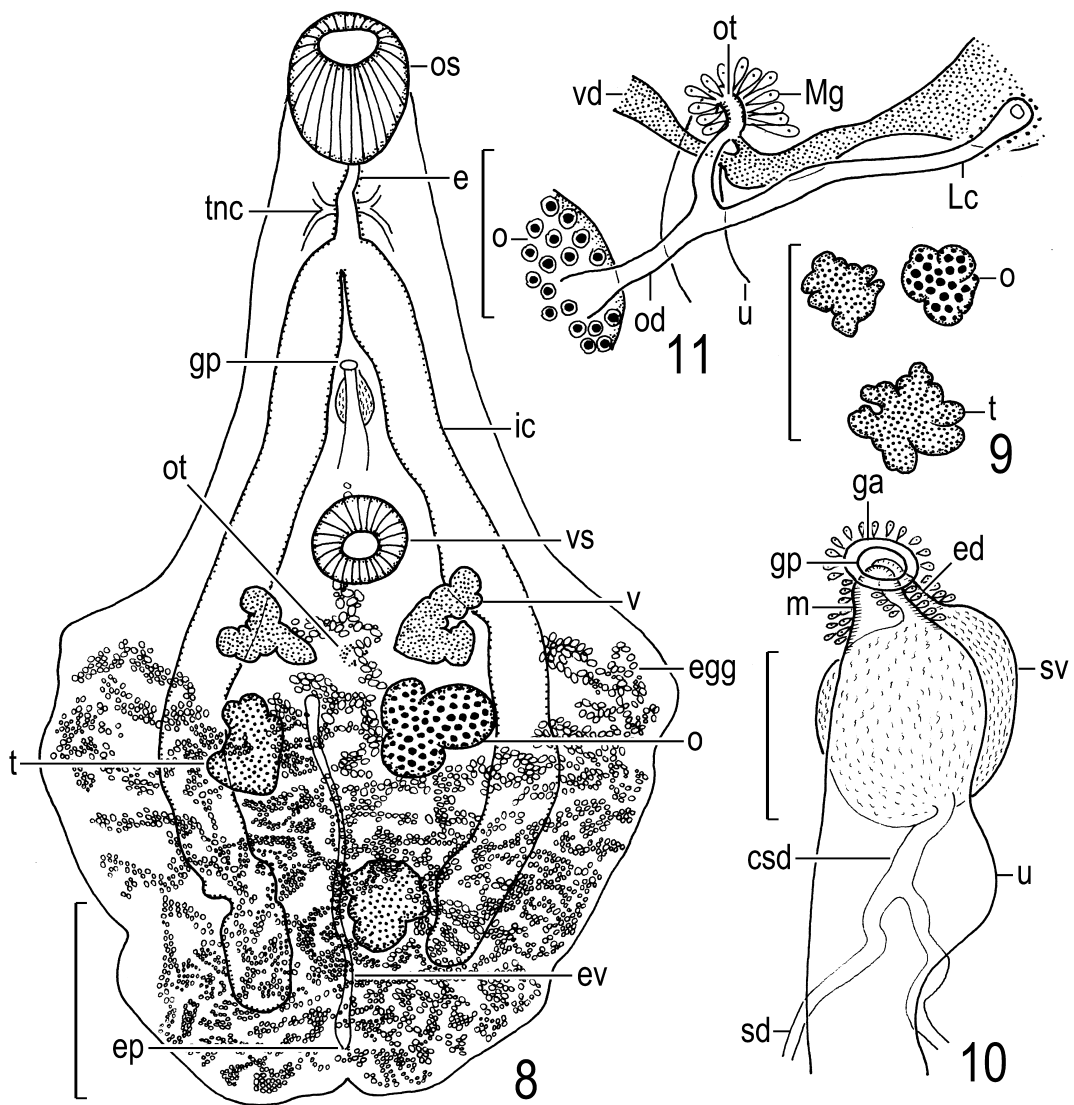
**Hosts in Japan.** *Odontobutis obscura* (Temminck and Schlegel, 1845) (Odontobutidae) (type host), *Gymnogobius urotaenia* (Hilgendorf, 1879) (Gobiidae), *Rhinogobius* sp. [most likely referring to *Rhinogobius kurodai* (Tanaka, 1908)] (Gobiidae) and *Tachysurus nudiceps* (Sauvage, 1883) (Bagridae) (Yamaguti, 1934; Shimazu, 2007; Shimazu *et al.*, 2011; this paper).

**Site of infection.** Urinary bladder.

**Geographical distribution.** (1) Nagano Prefecture: Lake Suwa at Suwa City (Shimazu, 2007). (2) Fukui Prefecture: Obama (Shimazu, 2007). (3) Shiga Prefecture: Lake Biwa (locality not specified) (Shimazu, 2007; Shimazu *et al.*, 2011). (4) Kyoto Prefecture: Lake Ogura (type locality) (Yamaguti, 1934; Shimazu, 2007); Katsura (probably Katsura River) (Shimazu, 2007); and Kyoto (locality not specified) (Shimazu, 2007). (5) Osaka Prefecture (?): Yodo River (locality not specified) (Shimazu, 2007). (6) Hiroshima Prefecture: Saijo-cho and Nukui River at Hara, Hachihonmatsu-cho, Higashihiroshima City (this paper).

**Material examined.** (1) 4 specimens (Yamaguti's Collection, MPM Coll. No. 22539, type series, holotype and 3 paratypes) of *Phyllodistomum mogurndae*, adult, whole-mounted, ex urinary bladder of *Odontobutis obscura* (syn. *Mogurnda obscura* (Temminck and Schlegel, 1845)), Lake Ogura, 20 November 1931, 4 May 1932 (Yamaguti, 1934; Shimazu, 2007). (2) 1 (Yamaguti's Collection, MPM Coll. No. 22540) of *P. mogurndae*, adult, whole-mounted, ex urinary bladder of *O. obscura*, Lake Ogura, 9 December 1931 (Shimazu, 2007). (3) 1 slide (Yamaguti's Collection, MPM Coll. No. 22260) of *P. mogurndae*, eggs, miracidia, whole-mounted, ex urinary bladder of *O. obscura*, Lake





Figs. 8–11. *Phyllodistomum mogurndae*, adults found in urinary bladder of *Odontobutis obscura*. — 8, holotype (MPM Coll. No. 22539), entire body, ventral view; 9, MPM Coll. No. 22541, testes and ovary, ventral view; 10, holotype, terminal genitalia, ventral view; 11, holotype, ovarian complex, dorsal view. Scale bars: 0.5 mm in Figs. 8–9; 0.1 mm in Figs. 10–11.

Ogura, 4 May 1932. (4) 2 (Yamaguti's Collection, MPM Coll. No. 22019) of *P. mogurndae*, adult, whole-mounted, ex urinary bladder of *O. obscura*, Obama, 26 March 1935 (Shimazu, 2007). (5) 3 (Yamaguti's Collection, MPM Coll. No. 22541) of *P. mogurndae*, adult, whole-mounted, ex urinary bladder of *O. obscura*, Katsura, 15 December 1938 (Shimazu, 2007). (6) 3 (Yamaguti's Collection, MPM Coll. No. 22268) of *P. mogurndae*,

adult, whole-mounted, ex urinary bladder of *O. obscura*, Yodo River (locality not specified, date not given) (Shimazu, 2007). (7) 49 (Ozaki's Collection, MPM Coll. No. 30212-a, labeled "*Phyllodistomum mog. SAIJO*," unpublished), 1 immature, 48 adult, now Saijo-cho, Higashihiroshima City, Hiroshima Prefecture (other data not given). (8) 1 (NSMT-PI 5781), adult, whole-mounted, ex urinary bladder of *O. obscura*,

Nukui River, Hara, Hachihonmatsu-cho, Higashihiroshima City, 30 October 2008. (9) 1 (Yamaguti's Collection, MPM Coll. No. 22542) of *P. mogurndae*, adult, whole-mounted, ex urinary bladder of *Tachysurus nudiceps* (syn. *Pseudobagrus nudiceps* Sauvage, 1883), Lake Biwa (locality not specified), 7 December 1938 (Shimazu, 2007; Shimazu *et al.*, 2011). (10) 1 (Yamaguti's Collection, MPM Coll. No. 22267) of *P. mogurndae*, adult, whole-mounted, ex urinary bladder of *T. nudiceps*, Kyoto (locality not specified), 29 October 1940 (Shimazu, 2007). (11) 1 (NSMT-PI 5326) of *P. mogurndae*, adult, whole-mounted, ex urinary bladder of *Rhinogobius* sp. [most likely referring to *Rhinogobius kurodai* (Tanaka, 1908)], Lake Suwa, 20 November 1993 (Shimazu, 2007). (12) 1 (NSMT-PI 5327) of *P. mogurndae*, adult, whole-mounted, ex urinary bladder of *Gymnogobius urotaenia* from Lake Suwa on 19 August 1995 (Shimazu, 2007).

**Description.** Based on 8 adult specimens (MPM Coll. Nos. 22539, type series, 4 specimens; 22019, 2; and 22541, 2) (Figs. 8–11). Similar to *Phyllodistomum biringo* (this paper) in general morphology. Body fairly small, 1.74–4.01 by 1.03–1.98 (holotype adult, 2.71 by 1.62); forebody 0.68–1.36 long, occupying 34–48% of body length. Oral sucker elliptical, longer than wide, 0.25–0.43 by 0.20–0.41. Esophagus 0.06–0.19 long, bifurcating at about border of anterior and second thirds of forebody. Intestinal ceca slightly sinuate, slightly diverticulate, ending some distance anterior to posterior extremity of body. Ventral sucker 0.19–0.33 by 0.21–0.34, pre-equatorial; sucker width ratio 1:0.7–1.1, sucker length ratio 1:0.6–0.9. Testes usually atrophied and slightly indented irregularly (Fig. 8), rarely normal and lobed irregularly (up to 11 lobes) (Fig. 9); anterior testis at ovarian level, 0.18–0.41 by 0.17–0.28, posterior slightly anterior to cecal ends, 0.20–0.34 by 0.16–0.41. Seminal vesicle globular to elliptical, 0.08–0.14 by 0.04–0.11. Ejaculatory duct short, 0.03–0.08 long. Genital pore midway between bifurcal level and ventral sucker. Ovary broad elliptical, 3-, 4-, or 6-lobed,

0.13–0.43 by 0.19–0.35, dextrally or sinistrally submedian. Uterus much folded throughout in postvitelline fields of hindbody, rarely extending into spaces anterolateral to vitellaria; metraterm well developed. Sperm scattered in uterus. Uterine eggs numerous, elliptical; eggs formed without ovum 16–17 by 11–13  $\mu\text{m}$ , eggs in 1-cell stage of development 21–24 by 14–16  $\mu\text{m}$ , intact large embryonated eggs 29–37 by 21–32  $\mu\text{m}$  (28–35 by 19–25  $\mu\text{m}$  in holotype), miracidia (each enclosed tightly by thin torn eggshell) 36–52 by 29–32  $\mu\text{m}$  (not seen in holotype). Vitellaria elliptical or 2-lobed, each lobe irregularly indented, 0.09–0.25 by 0.11–0.25. Excretory vesicle extending to near ootype; excretory pore posterodorsal.

2) In slide (MPM Coll. No. 22260), intact large embryonated eggs 32–35 by 24–29  $\mu\text{m}$  and hatched miracidia 38–40 by 29–32  $\mu\text{m}$  in Canada balsam.

**Remarks.** Yamaguti (1934) described *Phyllodistomum mogurndae* Yamaguti, 1934 on the basis on the type series found in the urinary bladder of *Odontobutis obscura* (syn. *Mogurnda obscura*) from Lake Ogura (type locality) in Kyoto Prefecture. This lake disappeared by 1941 owing to land reclamation.

Ozaki's Collection included a set of 14 old slides of an immature and 48 adult specimens (MPM Coll. No. 30212-a) of *P. mogurndae* and an adult specimen (MPM Coll. No. 30212-b) of *Coitocaecum plagiorchis* Ozaki, 1926 from Saijo. I consider that the host of these specimens was *Odontobutis obscura*, because Ozaki (1926) found *C. plagiorchis* in *O. obscura* from Saijo, and because I also found *P. mogurndae* in *O. obscura* from the Nukui River at Hara, Hachihonmatsu-cho, Higashihiroshima City (this paper).

Yamaguti (1934) described the egg size as 42–48 by 30–37  $\mu\text{m}$  in life. The two paratypes were labeled "Egg embryonated (0.045–0.048)  $\times$  (0.036–0.037)." The slide (MPM Coll. No. 22260) contained intact large embryonated eggs (32–35 by 24–29  $\mu\text{m}$ ) and hatched miracidia (38–40 by 29–32  $\mu\text{m}$ ) in Canada balsam. In the present specimens, intact large embryonated eggs were 29–37 by 21–32  $\mu\text{m}$ , and miracidia enclosed

tightly by torn eggshells were 36–52 by 29–32  $\mu\text{m}$ .  
*Life cycle.* Not known.

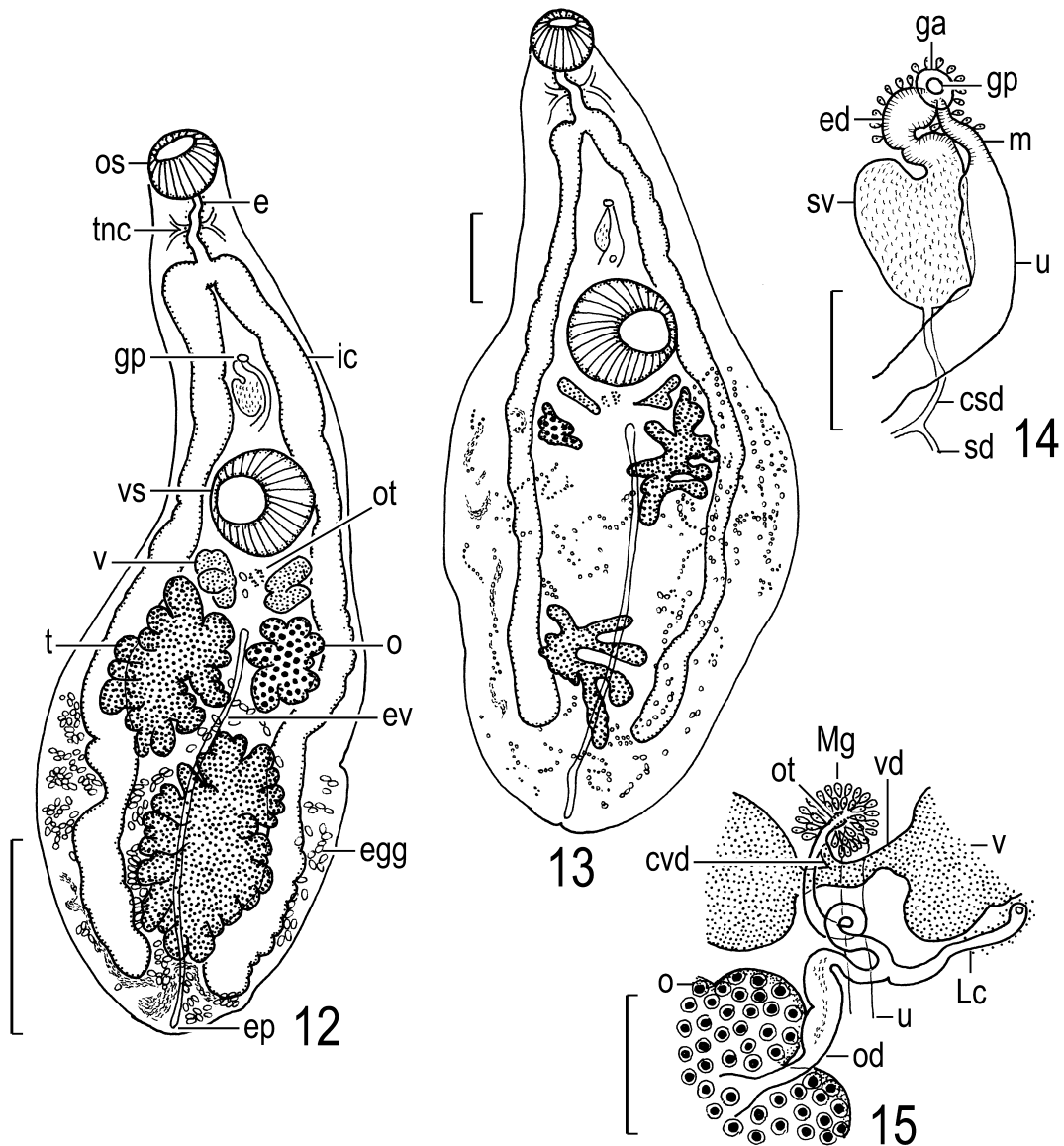
***Phyllodistomum parasiluri* Yamaguti, 1934**

(Figs. 12–15)

*Phyllodistomum parasiluri* Yamaguti, 1934: 423–425, fig.

86; Shimazu, 2007: 15–16, figs. 23–26; Shimazu *et al.*, 2011: 67, figs. 91–94.

*Hosts in Japan.* *Silurus asotus* (Siluridae) (type host), *Silurus lithophilus* (Tomoda, 1961) and *Tachysurus nudiceps* (Bagridae) (Yamaguti, 1934; Shimazu, 2007; Shimazu *et al.*, 2011; this paper).



Figs. 12–15. *Phyllodistomum parasiluri*, adults found in urinary bladder of *Silurus asotus*. — 12, holotype (MPM Coll. No. 22537), weakly matured, entire body, ventral view; 13, senile specimen (NSMT-PI 5333), entire body, ventral view; 14, holotype, terminal genitalia, ventral view; 15, holotype, ovarian complex, dorsal view. Scale bars: 0.5 mm in Figs. 12–13; 0.1 mm in Figs. 14–15.



*Sites of infection.* Urinary bladder and ureters.

*Geographical distribution.* (1) Nagano Prefecture: Lake Kizaki in Oomachi City and Lake Suwa at Suwa City (Shimazu, 2007; this paper). (2) Shiga Prefecture: Lake Biwa basin (Shimazu, 2007; Shimazu *et al.*, 2011). (3) Kyoto Prefecture: Lake Ogura (type locality) and Kyoto (locality not specified) (Yamaguti, 1934; Shimazu, 2007). (4) Osaka Prefecture (?): Yodo (probably Yodo River) (Shimazu, 2007). (5) Okayama Prefecture: Lake Kojima; irrigation canal at Mino, Kita-ku, Okayama City; and Sasagase River at Hirata, Kita-ku, Okayama City (this paper). (6) Fukuoka Prefecture: Okinohata-machi, Yanagawa City (Shimazu, 2007; this paper).

In China (e.g. Sun and Jiang, 1991).

*Material examined.* (1) 5 specimens (Yamaguti's Collection, MPM Coll. No. 22537, type series, holotype and 3 paratypes, adult, whole-mounted; and 1 voucher, immature, whole-mounted) of *Phyllodistomum parasiluri*, ex urinary bladder of *Silurus asotus* (syn. *Parasilurus asotus*), Lake Ogura, 9, 14 and 21 November 1931 (Yamaguti, 1934; Shimazu, 2007). (2) 1 (Yamaguti's Collection, MPM Coll. No. 22538) of *P. parasiluri*, immature, whole-mounted, ex urinary bladder of *S. asotus*, Lake Biwa (locality not specified), 1 November 1931 (Shimazu, 2007; Shimazu *et al.*, 2011). (3) 14 (NSMT-PI 5716, LBM 8-27 to -31) of *P. parasiluri*, adult, whole-mounted, ex urinary bladder of *S. asotus*, Lake Biwa basin, 4 May 1992, 19 and 24 April 2007, 24 November 2007 (Shimazu *et al.*, 2011). (4) 2 (Yamaguti's Collection, MPM Coll. No. 22018) of *P. parasiluri*, adult, whole-mounted, ex urinary bladder of *S. asotus*, Okinohata, now Okinohata-machi, Yanagawa City, Fukuoka Prefecture, 23 April 1935 (Shimazu, 2007). (5) 1 (Yamaguti's Collection, MPM Coll. No. 22263) of *P. parasiluri*, adult, whole-mounted, urinary bladder of *S. asotus*, Yodo (probably Yodo River in Osaka Prefecture), 12 December 1939 (Shimazu, 2007). (6) 1 (Yamaguti's Collection, MPM Coll. No. 22266) of *P. parasiluri*, adult, whole-mounted, ex urinary bladder of *S. asotus*, Kyoto (locality not specified), 2 November 1940

(Shimazu, 2007). (7) 3 (Yamaguti's Collection, MPM Coll. No. 22017) of *P. parasiluri*, adult (1 entire, 2 lacking posterior part of body), whole-mounted, ex urinary bladder of *S. asotus*, Lake Suwa, 16 May 1935 (Shimazu, 2007). (8) Many (NSMT-PI 5331–5333) of *P. parasiluri*, immature, adult, whole-mounted, ex urinary bladder of *S. asotus*, Lake Suwa, 2 October 1993, 12 May 1994, 9 June 1994 (Shimazu, 2007). (9) 7 (NSMT-PI 5782–5783), adult, whole-mounted, ex urinary bladder of *S. asotus*, Lake Suwa, 22 and 26 June 2007. (10) Many (NSMT-PI 5328–5330) of *P. parasiluri*, immature, adult, whole-mounted, ex urinary bladder of *S. asotus*, Lake Kizaki, 5 October 1976, 28 August 1981, 8 September 1981 (Shimazu, 2007). (11) 2 (NSMT-PI 5784, 5896), adult, whole-mounted, ex urinary bladder of *S. asotus*, Lake Kojima, Minami-ku, Okayama Prefecture, 4 July 2010, 12 May 2012. (12) 2 (NSMT-PI 5895), adult, whole-mounted, ex urinary bladder of *S. asotus*, irrigation canal at Mino, Kita-ku, Okayama City, Okayama Prefecture, 22 April 2012. (13) 2 (NSMT-PI 5897), adult, whole-mounted, ex urinary bladder of *S. asotus*, Sasagase River at Hirata, Kita-ku, Okayama City, 8 September 2013. (14) 13 (NSMT-PI 5717–5718) of *P. parasiluri*, 1 immature, 12 adult, whole-mounted, ex urinary bladder and ureter of *Silurus lithophilus*, Lake Biwa basin, 4 May 1979, 3 June 1980 (Shimazu *et al.*, 2011). (15) 3 (NSMT-PI 5719) of *P. parasiluri*, adult, whole-mounted, ex urinary bladder of *Tachysurus nudiceps* (syn. *Pelteobagrus nudiceps*), Lake Biwa basin, 4 May 1979 (Shimazu *et al.*, 2011).

*Description.* Based on 7 adult specimens (barely matured young and fully matured adults) of Yamaguti (MPM Coll. No. 22537, 4 specimens; 22018, 2; 22263, 1) (Figs. 12–15). Similar to *Phyllodistomum biringo* (this paper) in general morphology. Body lanceolate-oblong rather than banjo-shaped, small, 1.13–3.49 by 0.41–1.04 (holotype adult, 2.38 by 0.81); forebody 0.47–1.59 long, occupying 40–48% of body length. Oral sucker 0.09–0.25 by 0.11–0.24. Esophagus fairly long, 0.07–0.23 long, bifurcating at about

border of anterior and second thirds of forebody. Intestinal ceca sinuate, weakly diverticulate, ending some distance from posterior extremity of body. Ventral sucker 0.16–0.35 by 0.16–0.33, located at about border of anterior and middle thirds of body; sucker width ratio 1 : 1.4–1.9. Testes large, lobed irregularly in young adults, some lobes further lobulated irregularly (Fig. 12); anterior testis slightly posterior to or level with ovary, 0.22–0.63 by 0.12–0.41; posterior slightly anterior to cecal ends, 0.25–0.82 by 0.14–0.51; atrophied, more deeply lobed in senile adults (Fig. 13). Seminal vesicle 0.09–0.20 by 0.05–0.09. Ejaculatory duct short, usually constricted once, 0.03–0.05 by 0.02–0.04. Genital pore midway between intestinal bifurcation and ventral sucker. Ovary globular or 3- to 6-lobed, 0.09–0.32 by 0.09–0.25 in young adults; atrophied, smaller in senile adults. Laurer's canal opening dorsally to vitellarium. Ootype anterior to vitelline ducts; Mehlis' gland well developed. Uterus much folded in all available space of postvitelline field of hindbody; metraterm small; uterine seminal receptacle present. Uterine eggs numerous, elliptical; eggs formed without ovum 11–22 by 8–16  $\mu\text{m}$ , eggs in 1-cell stage of development 27–34 by 14–19  $\mu\text{m}$ , intact large embryonated eggs 30–36 by 16–27  $\mu\text{m}$ , no miracidia seen. Vitellaria globular, elliptical, or 2- or 5-lobed, 0.04–0.16 by 0.09–0.22. Excretory vesicle extending to ovarian level; excretory pore postero-dorsal or -terminal.

**Remarks.** Yamaguti (1934) described *Phyllodistomum parasiluri* Yamaguti, 1934 on the basis of the type series found in the urinary bladder of *Silurus asotus* from Lake Ogura. This lake disappeared by 1941 owing to land reclamation. Shimazu (2007) and Shimazu *et al.* (2011) described this species based on adults from Lakes Kizaki and Suwa and the Lake Biwa basin, respectively.

**Life cycle.** Not known.

Genus *Pseudophyllodistomum* Cribb, 1987

***Pseudophyllodistomum macrobrachicola***  
(Yamaguti, 1934)

(Figs. 16–21)

*Phyllodistomum macrobrachicola* Yamaguti, 1934: 430–431, fig. 90.

*Pseudophyllodistomum macrobrachicola*: Cribb, 1987: 1131; Shimazu *et al.*, 2011: 68, 70, figs. 95–99.

*Phyllodistomum anguilae* [sic]: Shimazu, 2005, not Long and Wai, 1958: 142–143, figs. 7–9; Shimazu, 2007: 11–12, figs. 16–19; Shimazu, 2008: 49, fig. 6.

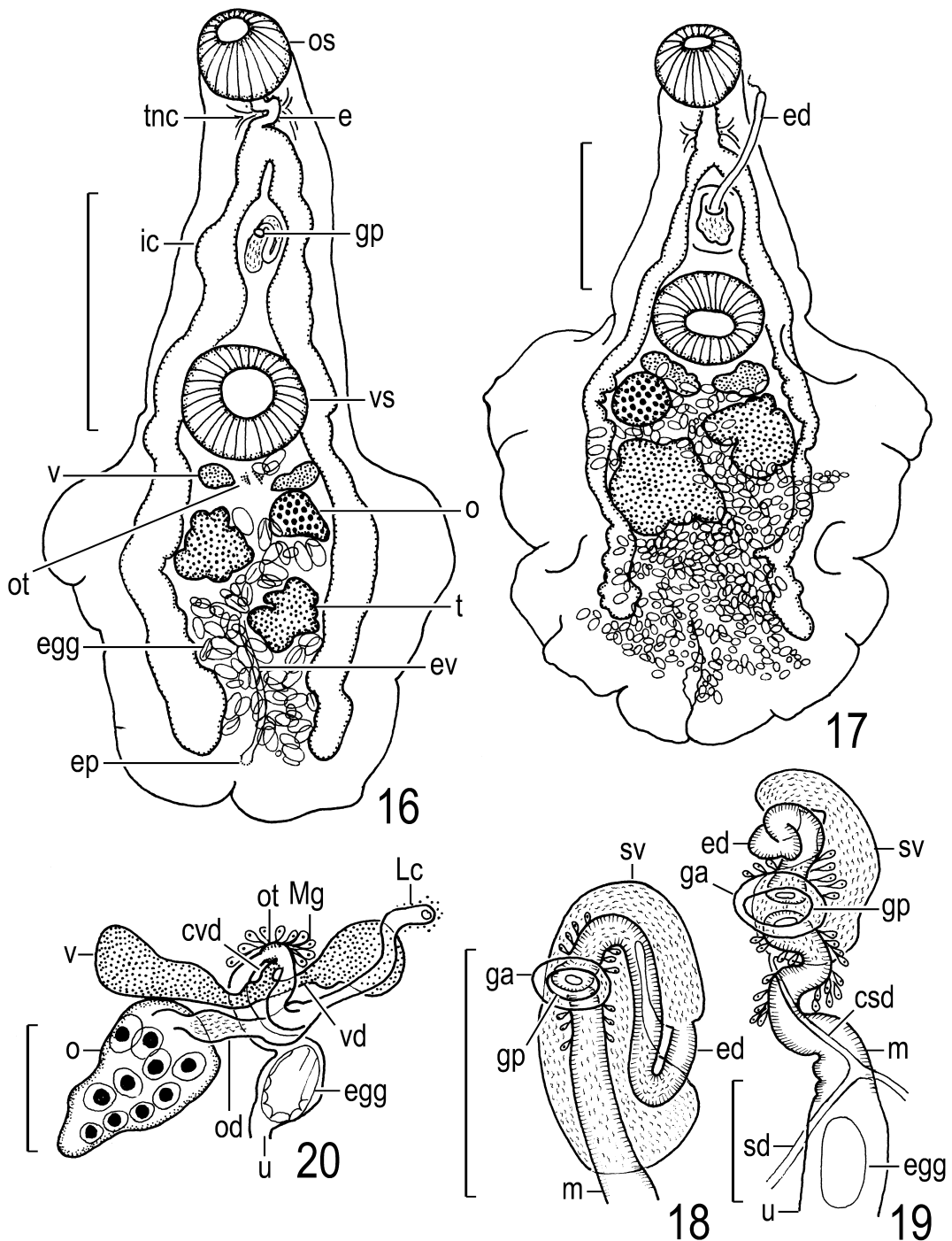
**Hosts in Japan.** *Odontobutis obscura* (Odon-  
tobutidae) (type host), *Anguilla japonica* Tem-  
minck and Schlegel, 1846 (Anguillidae), *Cottus*  
*reinii* Hilgendorf, 1879 (Cottidae), a fish (Japa-  
nese name: Kajika) [most likely referring to *Cot-  
tus reinii*], *Gymnogobius urotaenia* (Gobiidae),  
*Tachysurus nudiceps* (Bagridae) and *Silurus aso-  
tus* (Siluridae) (Yamaguti, 1934; Shimazu, 2005,  
2007, 2008; Shimazu *et al.*, 2011; this paper).

**Site of infection.** Urinary bladder.

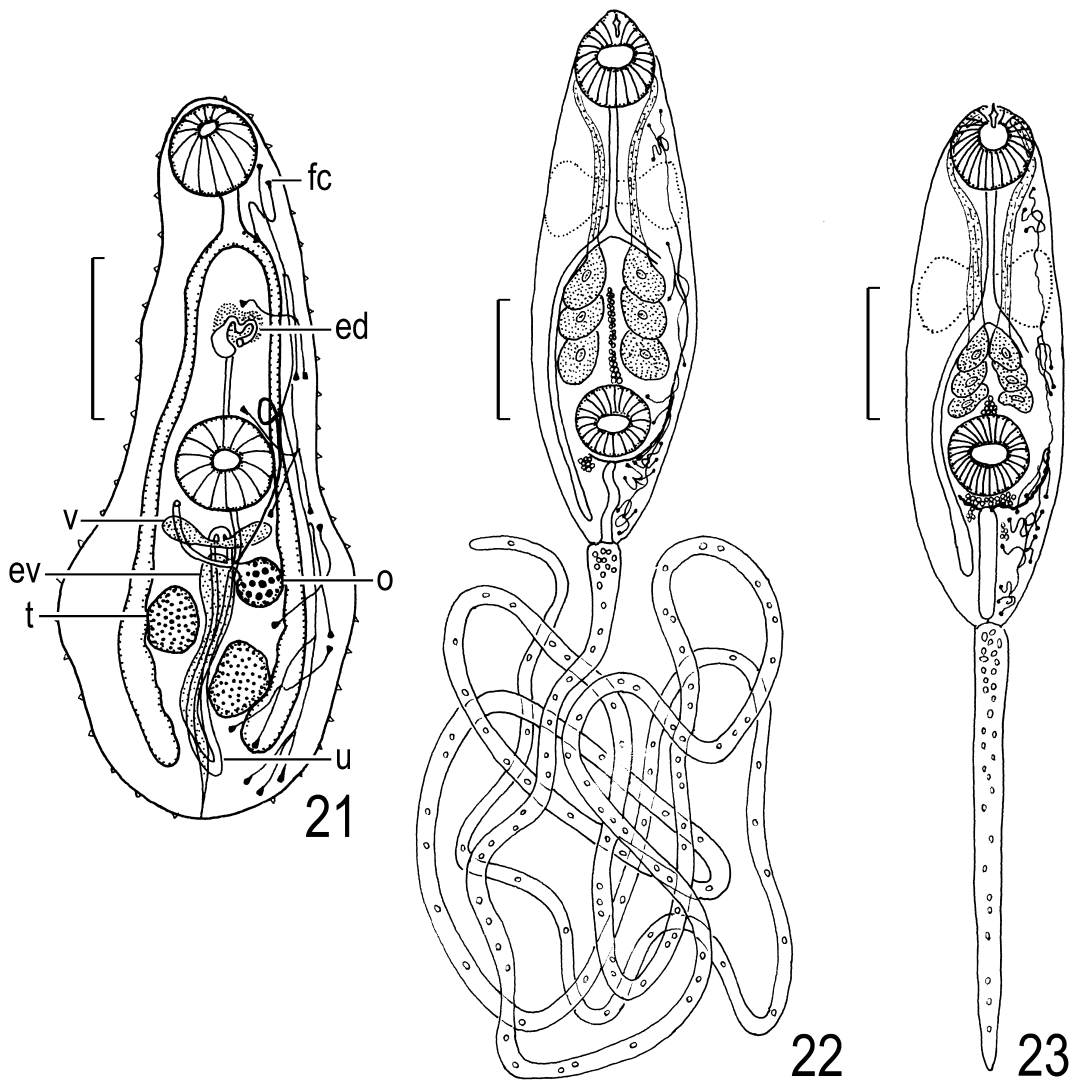
**Geographical distribution.** (1) Aomori Pre-  
fecture: Lake Ogawara at Kamikita-kita, Tohoku  
Town (Shimazu, 2005; this paper). (2) Ibaraki  
Prefecture: Tsuchiura (Shimazu, 2005; Shimazu  
*et al.*, 2011). (3) Nagano Prefecture: Lake Suwa  
at Suwa City (Shimazu, 2007). (4) Shiga Prefec-  
ture: Lake Biwa basin (Shimazu *et al.*, 2011). (5)  
Tokushima Prefecture: Kaifu River at Ooi, Kaiyo  
Town (Shimazu, 2008).

In Russia: Amur River basin, Primorskiy Kray  
(Akhmerov, [1961]).

**Material examined.** (1) 1 specimen (Yama-  
guti's Collection, MPM Coll. No. 22543, holo-  
type of *Phyllodistomum macrobrachicola*) of  
*Pseudophyllodistomum macrobrachicola*, adult  
(78 days after experimental infection), whole-  
mounted, ex urinary bladder of *Odontobutis*  
*obscura* (syn. *Mogurnda obscura*), 13 January  
1932 (Yamaguti, 1934; Shimazu *et al.*, 2011). (2)  
1 (Yamaguti's Collection, MPM Coll. No. 22265)  
of *Ps. macrobrachicola*, adult (20 days after  
experimental infection), whole-mounted, ex uri-  
nary bladder of *O. obscura*, 20 July 1940  
(Shimazu *et al.*, 2011). (3) 6 (Ozaki's Collection,  
MPM Coll. No. 30213) of *Ps. macrobrachicola*,



Figs. 16–20. *Pseudophyllodistomum macrobrachicola*, adults. — 16, holotype (MPM Coll. No. 22543) found in urinary bladder of *Odontobutis obscura*, barely matured, 78 days after experimental infection, entire body, ventral view; 17, MPM Coll. No. 30213 found in [presumably urinary bladder of] *O. obscura*, fully matured, entire body, ventral view; 18, holotype, terminal genitalia, ventral view; 19, LBM 8–19 found in urinary bladder of *Anguilla japonica*, ventral view; 20, holotype, ovarian complex, dorsal view. Figs. 17–19 redrawn from Shimazu *et al.* (2011), slightly altered. Scale bars: 0.5 mm in Figs. 16–17; 0.1 mm in Figs. 18–20.



Figs. 21. *Pseudophyllodistomum macrobrachicola*, life cycle, metacercaria found in *Neocaridina denticulata*, ventral view. Redrawn from Shibue (1954). Scale bar: 0.5 mm.

Figs. 22–23. Cercariae of gorgoderid species. — 22, a longicercous-noncystocercous-apharyngeate-stylet cercaria, most likely referring to *Ps. macrobrachicola*, found in either *Corbicula japonica* or *Co. leana*, ventral view; 23, a brevicercous-noncystocercous-apharyngeate-stylet cercaria, found in *Co. japonica*, ventral view. Redrawn from Toyooka (1965b), intestinal cecum on left side of body and excretory organs on right side omitted, respectively. Scale bars: 0.05 mm.

adult, whole-mounted, ex [most likely urinary bladder of] *O. obscura*, Lake Biwa (other data not given) (Shimazu *et al.*, 2011). (4) 2 (Yamaguti's Collection, MPM Coll. No. 22261) of *Ps. macrobrachicola*, immature, whole-mounted, ex small intestine [sic] of *Anguilla japonica* (wild), Tsuchiura, 16 April 1929 (Shimazu, 2005;

Shimazu *et al.*, 2011). (5) 2 (NSMT-PI 5247) of *Ps. macrobrachicola*, adult, whole-mounted, ex urinary bladder of *A. japonica*, Lake Ogawara at Kamikita Town, now Kamikita-kita, Tohoku Town, 7 and 9 September 1997 (Shimazu, 2005; Shimazu *et al.*, 2011). (6) 32 (NSMT-PI 5322–5325) of *Ps. macrobrachicola*, 11 immature, 21

adult, whole-mounted, ex urinary bladder of *A. japonica*, Lake Suwa, 10 September 1976, 30 June 1994, 9 July 1994 (Shimazu, 2007; Shimazu *et al.*, 2011). (7) 1 (NSMT-PI 5526) of *Ps. macrobrachicola*, adult, whole-mounted, ex urinary bladder of *A. japonica*, Kaifu River at Ooi, Kaiyo Town (Shimazu, 2008; Shimazu *et al.*, 2011). (8) 11 (LBM 8-19 to -20) of *Ps. macrobrachicola*, 1 immature, 10 adult, whole-mounted, ex urinary bladder of *A. japonica*, Lake Biwa basin, 19 May 1998, 1 May 2001 (Shimazu *et al.*, 2011). (9) 1 (Yamaguti's Collection, MPM Coll. No. 22264) of *Ps. macrobrachicola*, immature, whole-mounted, ex urinary bladder of *Gymnogobius urotaenia*, Tsuchiura, 4 April 1940 (Shimazu *et al.*, 2011). (10) 2 (NSMT-PI 5722) of *Ps. macrobrachicola*, 1 immature, 1 adult, ex urinary bladder of *G. urotaenia*, Lake Biwa basin, 3 June 1980 (Shimazu *et al.*, 2011). (11) 40 (NSMT-PI 5725–5727 and LBM 8-22) of *Ps. macrobrachicola*, immature, whole-mounted, ex urinary bladder of *Cottus reinii*, Lake Biwa basin, 3 June 1980, 4 and 6 May 1992, 24 November 2007 (Shimazu *et al.*, 2011). (12) 1 (Yamaguti's Collection, MPM Coll. No. 22549) of *Ps. macrobrachicola*, adult, whole-mounted, ex urinary bladder of a fish (Japanese name: Kajika) [most likely referring to *Cottus reinii*], Lake Biwa basin, 26 March 1928 (Shimazu *et al.*, 2011). (13) 1 (LBM 8-29) of *Ps. macrobrachicola*, adult, whole-mounted, ex urinary bladder of *Silurus asotus*, Lake Biwa basin, 24 April 2007 (Shimazu *et al.*, 2011). (14) 3 (NSMT-PI 5723–5724) of *Ps. macrobrachicola*, immature, whole-mounted, ex urinary bladder of *Tachysurus nudiceps* (syn. *Pelteobagrus nudiceps*), Lake Biwa basin, 5 May 1979, 3 June 1980 (Shimazu *et al.*, 2011).

**Description.** Based on the holotype and adult specimens of Shimazu (2005, 2007, 2008) and Shimazu *et al.* (2011); 31 (including holotype) measured (Figs. 16–20). Similar to *Phyllodistomum biringo* (this paper) in general morphology except in long ejaculatory duct and uterine distribution. Barely matured young and fully matured adults. Body small, 1.67–3.84 by 0.56–1.92

(holotype weakly matured young adult, 1.67 by 0.82), rarely depressed ventrally in region of seminal vesicle; forebody 0.72–1.49 long, occupying 30–53% of body length. Oral sucker 0.15–0.38 by 0.13–0.39. Esophagus short, 0.12–0.34 long, bifurcating at about junction of anterior and second thirds of forebody. Intestinal ceca slightly sinuate, slightly diverticulate. Ventral sucker 0.18–0.42 by 0.18–0.42, slightly pre-equatorial; sucker width ratio 1:1.0–1.5. Testes indented irregularly, usually diagonal but rarely almost symmetrical, usually separate but rarely contiguous, submedian, intercecal, in middle third of hindbody; anterior testis 0.12–0.44 by 0.11–0.37, posterior considerably anterior to cecal ends, 0.13–0.50 by 0.13–0.42. Seminal vesicle pyriform or retort-shaped, 0.10–0.23 by 0.05–0.18. Ejaculatory duct long, convoluted, rarely everted through genital pore (0.44 long in Fig. 17). Genital pore halfway between intestinal bifurcation and ventral sucker, with well-developed radial muscle fibers (not illustrated). Ovary globular, cordate or slightly indented, 0.10–0.28 by 0.09–0.20, dextrally or sinistrally submedian, intercecal, slightly pretesticular, on opposite side of anterior testis. Laurer's canal long, opening dorsally on anterolateral side of vitellarium located on opposite side of ovary. Ootype median, slightly anterior to vitelline ducts. Mehli's gland weakly developed. Uterus much folded in postvitelline region of hindbody, inter- and post-cecal, rarely slightly overlapping ceca and entering extracecal fields of body only slightly; metraterm well developed, anterior to ventral sucker; uterine seminal receptacle sometimes seen. Eggs numerous, elongate elliptical, slightly curved, brown, fully embryonated (not numerous, not embryonated in holotype); eggs formed with no ovum 32–50 by 17–24  $\mu\text{m}$ , eggs in 1-cell stage of development 48–73 by 27–37  $\mu\text{m}$ ; fully embryonated eggs (intact) 64–88 by 32–56  $\mu\text{m}$ ; miracidia in uterus 88–96 by 72–93  $\mu\text{m}$ . Vitellaria usually elliptical or weakly indented, 0.07–0.23 by 0.05–0.25, extracecal and slightly overlapping ceca. Excretory vesicle extending anteriorly to ovarian level; excretory pore posteroterminal.



Most specimens from *A. japonica*, *O. obscura* and *S. asotus* apparently fully matured adults with numerous uterine eggs; those from *G. urotaenia* and Kajika barely matured adults with a few uterine eggs; and those from *T. nudiceps* and *C. reinii* sexually immature.

**Remarks.** Yamaguti (1934) found metacercariae (fig. 89) “encysted” [correctly encapsulated] in the ovary of the shrimp *Macrobrachium nipponense* (de Haan, 1849) (Decapoda, Pleocyemata, Palaemonidae) (Japanese name: Tenagebi) from Lake Kasumigaura in Ibaraki Prefecture and the Yodo River (locality not specified). He fed them to *Odontobutis obscura* (syn. *Mogurnda obscura*) and *Silurus asotus* (syn. *Parasilurus asotus*) and subsequently recovered a weakly matured adult (MPM Coll. No. 22543) from the urinary bladder of *O. obscura* 78 days after feeding (see also Shimazu *et al.*, 2011). Yamaguti (1934) described *Phyllodistomum macrobrachicola* Yamaguti, 1934 on the basis of this adult specimen, or the holotype. Cribb (1987) transferred this species from *Phyllodistomum* to *Pseudophyllodistomum* as *Pseudophyllodistomum macrobrachicola* (Yamaguti, 1934). Neither Yamaguti (1934) nor the slide label (MPM Coll. No. 22543) said anything about the locality of the metacercaria of the holotype, so that the type locality of the species has not yet been determined. Even in metacercariae, the reproductive organs are well differentiated, and the ejaculatory duct (or cirrus) is long, convoluted and eversible (Yamaguti, 1934, figs. 89–90; Shibue, 1954, figs. 1–2; Shimazu, 2005, 2007, 2008; Shimazu *et al.*, 2011; this paper, Figs. 17–19).

Yamaguti's Collection also included immature worms (MPM Coll. Nos. 22543–22544) recovered in his above-mentioned experimental infection from the intestine and rectum of *O. obscura* and *S. asotus* 3–13 days after infection, from the rectum and urinary bladder of *O. obscura* 10 days, and from the urinary bladder of *O. obscura* and *S. asotus* 22–85 days; and a barely matured adult (MPM Coll. No. 22265) recovered from the urinary bladder of *O. obscura* 20 days after feeding in his second experimental infection. Shibue

(1954) fed metacercariae from *Neocaridina denticulata* (de Haan, 1844) (Atyidae) (Japanese name: Minami-numa-ebi) to *O. obscura* and recovered barely matured adults from the urinary bladder of the fish 27–32 days after feeding. The worms attained sexual maturity in *O. obscura* within much shorter time (20–32 day after feeding) in Yamaguti's second and Shibue's experimental infections than those in Yamaguti's first experimental infection (78 days). A well-controlled infection experiment should be carried out in the future to confirm the period in which worms attain sexual maturity in the final host.

Long and Wai (1958) described *Phyllodistomum* (*Catoptroides*) *anguilae* [sic, original spelling] Long and Wai, 1958, now *Phyllodistomum anguilae* (see Campbell, 2008), based on adult specimens found in the urinary bladder of *Anguilla japonica* and *Siniperca chuatsi* (Basilewsky) (Percichthyidae) collected in Tai Hu (Lake Tai), Zhejiang Province, China. This species is so similar to *Ps. macrobrachicola*, so that Shimazu (2005, 2007, 2008) misidentified his adult specimens found in *A. japonica* as *P. anguilae* (Shimazu *et al.*, 2011). The description by Long and Wai (1958) for *P. anguilae* lacks details of the terminal genitalia. If the species proves to have a long ejaculatory duct, the species would be considered to be a junior synonym of *Ps. macrobrachicola* (Shimazu *et al.*, 2011; see also Cribb, 1987). Komiya and Tajimi (1943, fig. 3) reported a metacercaria (small type) found “encysted” in the ovary of *M. nipponense* from Shanghai, China, stating that it closely resembled *Phyllodistomum folium* (Olfers, 1816) and *P. macrobrachicola*. Since it has a long ejaculatory duct (cirrus pouch interpreted by them), it more closely resembles the latter species.

**Life cycle.** Eggs become fully embryonated in the uterus of adults. Miracidia hatch in the water. Fully formed miracidia have two flame cells in the formula of  $2[(1)]=2$  and include a daughter sporocyst with four flame cells of the formula of  $2[(1+1)]=4$  (Shimazu, 2007). The first intermediate host has not yet been determined definitively (see the Discussion on *Phyllodisto-*

*mum* and *Pseudophyllodistomum* below).

Natural second intermediate hosts in Japan are freshwater shrimps, *Macrobrachium nipponense* (syn. *Palaemon nipponensis*) and *Palaemon paucidens* de Haan, 1844 (syn. *Leander paucidens*) (Palaemonidae) (Japanese name: Suji-ebi), *Neocaridina denticulata* (Atyidae) and [Kawa-ebi] (scientific name not given), in the gonads and sperm duct of which metacercariae are found either “encysted” or “unencysted” (Yamaguti, 1934; Okabe and Shibue, 1952; Shibue, 1953, 1954; Toyooka, 1965a; Shimazu, 2003, 2005; Shimazu *et al.*, 2011). The “cysts” are not true cysts of parasite origin but capsules of host origin (Cribb, 1987; Shimazu *et al.*, 2011). The genitalia are well differentiated in the metacercarial stage as discussed above. The flame cell formula is  $2[(3+3+3)+(3+3+3)]=36$  in the metacercaria (Shibue, 1954, fig. 1; this paper, Fig. 21).

Yamaguti's Collection included metacercariae of *P. macrobrachicola*: 1 each (MPM Coll. No. 22545), ex *Ma. nipponense* (Yamaguti, 1934, fig.

89) and *Pa. paucidens*, Tuchiura, 16 April 1929; 1 (MPM Coll. No. 22546) (unidentified, unpublished), ex *Ma. nipponense*, Katsura River, Kyoto Prefecture, 21 September 1929; 1 (MPM Coll. No. 22548) (unidentified, unpublished), ex [Kawa-ebi], Lake Ogura, Kyoto Prefecture, 9 December 1931; and many (MPM Coll. Nos. 22023, unidentified, unpublished, date not given) and many (MPM Coll. No. 22547, unpublished, 25 October 1931), ex *Ma. nipponense*, Lake Biwa.

Final hosts in Japan are *Odontobutis obscura* (natural and experimental), *Anguilla japonica*, *Silurus asotus*, *Gymnogobius urotaenia*, *Tachysurus nudiceps*, *Cottus reinii* and Kajika, in the urinary bladder of which adults develop. The degree of sexual maturity of the worms mentioned above suggests that the first three fishes are suitable as the final host; but the others, unsuitable. Adults were recorded from *S. asotus* and *Silurus soldatovi* Nikolskii and Soin, 1948 in the Amur River basin, Primorskiy Kray, Russia (Akhmerov, [1961]).

### Key to the genera and species of the Gorgoderidae of freshwater fishes in Japan

- 1.1. Uterus confined to inter- and post-cecal fields of postvitelline region of hindbody; ejaculatory duct long, eversible; fully embryonated eggs over 60  $\mu$ m long ..... *Pseudophyllodistomum macrobrachicola*
- 1.2. Uterus spreading into extra- and post-cecal fields of hindbody; ejaculatory duct short, not eversible ..... *Phyllodistomum*
  - 1.2.1. Uterus slightly spreading into extra- and post-cecal fields of postvitelline region; fully embryonated eggs over 60  $\mu$ m long ..... *P. biringo*
  - 1.2.2. Uterus coiling in all available space in postvitelline region; fully embryonated eggs less than 60  $\mu$ m long ..... 2
- 2.1. Oral sucker smaller than ventral sucker; testes small, globular; vitellaria branched .... *P. carassii*
- 2.2. Oral sucker smaller than ventral sucker; testes large, lobed; vitellaria entire or lobed ..... *P. parasiluri*
- 2.3. Oral sucker larger than ventral sucker; testes small, lobed; vitellaria lobed ..... *P. mogurndae*

### Discussion on *Phyllodistomum* and *Pseudophyllodistomum*

The genus *Phyllodistomum* Braun, 1899 is large and composed of species from freshwater and marine fishes and amphibians (Campbell, 2008).

According to an extensive phylogenetic study of the Gorgoderidae Looss, 1899 (Cutmore *et al.*, 2013), *Phyllodistomum* is paraphyletic. The adult morphology and life cycle of the type species *P. folium* (a freshwater species) have not yet been well understood (Cribb, 1987). It seems to me

that it is necessary to redefine *P. folium* on the basis of either the type material or a new material including a neotype from the type host in the type locality and then *Phyllodistomum* needs a revision from morphological, molecular and life cycle data first of all. I follow the classification of the Gorgoderinae Looss, 1899 in the Gorgoderidae by Campbell (2008) for the time being.

Cribb (1987) established a new genus, *Pseudophyllodistomum*, with *Pseudophyllodistomum johnstoni* Cribb, 1987 as the type species, distinguishing it from *Phyllodistomum* primarily by having a simple uterus (restricted to the inter- and post-cecal fields of the hindbody), fully embryonated eggs reaching over 60  $\mu$ m long, a saccular excretory vesicle, the flame cell formula of  $2[(3+3+3)+(3+3+3)]=36$ , the cercaria with a long noncystocercous tail, genitalia fully developed in the metacercaria, and using corbiculid bivalves as the first intermediate host and decapod crustaceans as the second intermediate host. Campbell (2008) followed him. I add a long eversible ejaculatory duct as a diagnostic feature of the genus, because such a long ejaculatory duct is seen in *Ps. johnstoni*, *Pseudophyllodistomum murrayense* Cribb, 1987, *Pseudophyllodistomum mingense* (Tang, 1985) (the original spelling *mingensis* changed) and *Ps. macrobrachicola* (Tang, 1985; Cribb, 1987; Shimazu *et al.*, 2011; this paper). *Phyllodistomum biringo* appears to be in an intermediate position between *Phyllodistomum* and *Pseudophyllodistomum* in morphology.

Cribb (1987) described the cercaria of *Ps. johnstoni* (a longicercous-noncystocercous-apharyngeate-stylet cercaria) produced in a [daughter (?)] sporocyst in *Corbicula* sp. (Bivalvia, Cyrenidae) collected in Australia. The cercaria had a 1-pointed stylet, three pairs of penetration glands, the flame cell formula of  $2[(3+3+3)+(3+3+3)]=36$ , a very long noncystocercous tail and no "cystogenous" cells (columnar large gland cells surrounding the excretory vesicle). The cercariae of *Ps. johnstoni* (Cribb, 1987) and *Ps. mingense* (Tang, 1985) morphologically differ from those previously known for species of *Phyllodistomum*.

The latter have a microcercous, cystocercous or rhopalocercous tail and the "cystogenous" cells (Yamaguti, 1975; Cribb, 1987).

No cercariae have been definitively determined for any of the Japanese species. Cercariae similar to those of *Ps. johnstoni* and *Ps. mingense* have been reported in Japan: (1) *Cercaria longicrura* Faust, 1924 (syn. [Long-tailed cercaria A] of Kobayashi, 1922) (Kobayashi, 1922; Faust, 1924), which was originally described by Osafune (1898) from a species of *Corbicula* (Japanese name: Shijimi) collected in Mishima-son, now Mishima, Minami-ku, Okayama City, Okayama Prefecture; (2) "Apharyngeal stylet cercaria" of Yamaguti, [1954] in *Corbicula sandai* Reinhardt, 1878 (Japanese name: Seta-shijimi) collected in Lake Biwa (Yamaguti, [1954]); and (3) Cercaria A of Toyooka, 1965b in *Corbicula japonica* Prime, 1864 (Japanese name: Yamato-shijimi) and *Corbicula leana* (Prime, 1864) (Japanese name: Ma-shijimi) collected in the Yoshino River and the rivulets in the vicinity of Komatsushima, near Tokushima City, Tokushima Prefecture (Toyooka, 1954, [Cercaria] B; 1962, fig. 8; 1965a, b, Cercaria A, fig. 2). The flame cell formula was  $2[(3+3+3)+(3+3+3)]=36$  in this cercaria. Reexamination of Yamaguti's [1954] specimens (Yamaguti's Collection, MPM Coll. No. 22269, 18 October 1931) and the description by Toyooka (1965b, fig. 2) for Cercaria A (this paper, Fig. 22) suggest that Osafune (1898, fig. IIb) erroneously described the pharynx as present and the stylet as absent (see also Cribb, 1987) and that the three cercariae are the same species. I agree with Toyooka (1965b) that Cercaria A is considered to be the cercaria of *Phyllodistomum macrobrachicola* (see also Cribb, 1987). If this proves to be true, the specific name *longicrura* Faust, 1924 preoccupies the specific name *macrobrachicola* Yamaguti, 1934. In Tokushima Prefecture, the metacercaria and adult of *Ps. macrobrachicola* have been recorded from *Macrobrachium nipponense* and *Anguilla japonica*, respectively (Toyooka, 1965a; this paper).

In addition, Toyooka (1954, [Cercaria] B; 1962, fig. 9; 1965a, b, Cercaria B, fig. 4) described a

new cercaria (a brevicercous-noncystocercous-apharyngeate-stylet cercaria) produced in a daughter sporocyst in *Co. japonica* collected at the same localities. A metacercaria was found free in a sporocyst (Toyooka, 1965b, fig. 5). This cercaria (this paper, Fig. 23) resembles Cercaria A (this paper, Fig. 22) in morphology of the body proper but differs from it in having a much shorter tail. In Toyooka's descriptions and figures (Toyooka, 1965b, figs. 2–4) for Cercariae A and B, the collecting tubules, secondary collecting tubules and terminal capillaries are difficult to trace accurately, but the figures clearly indicate that the flame cell formula was  $2[(3+3+3)+(3+3+3)]=36$  in both cercariae.

Yamaguti [1954] recorded a *Phyllodistomum* larva "encysted" in the coelom of *Hemiclepsis kasmiana* Oka, 1910 (Hirudinea) (Japanese name: Kai-biru), which lives in the mantle cavity of the bivalve *Cristaria plicata* (Leach, 1815) (Japanese name: Karasu-gai), collected in Kyoto. I have not found this specimen in Yamaguti's Collection.

Toyooka (1962, 1965a) recorded specimens of *Phyllodistomum* from the urinary bladder of the fish [Funa] [a species of *Carassius*] from Tokushima Prefecture: immature worms in Shiobara and Hamazaki [now both in Oozato, Kaiyo Town (?)]; adults in Tomioka-cho, Anan City; and immature worms in Ookubo, Satoura-cho, Naruto City. He presented photomicrographs (Toyooka, 1962, fig. 7; Toyooka, 1965a, fig. 28) of the specimens, but they are out of focus and lack details of the morphology. Toyooka's materials have not been described. None of them was made available to me for the present study. The present study shows that four nominal species of *Phyllodistomum* and one nominal species of *Pseudophyllodistomum* occur in Japan. Gorgoderids in Japanese freshwater fishes and their life cycles need further studies.

Some workers in Japan assigned their materials to *Phyllodistomum folium*, a European species (Shimazu *et al.*, 2011). Kobayashi (1915, 1921) stated that he found worms of *P. folium* in the urinary bladder of *Pseudobagrus aurantiacus*

(Japanese name: Gigi of Kobayashi) collected in Lake Biwa [*Tachysurus nudiceps* in this lake] and Lake Kasumigaura [*Tachysurus tokiensis* Döderlein, 1887 in this lake], but he did not describe gross morphology of his material at all. Unencapsulated and encapsulated metacercariae of *P. folium* were reported from the gonads and sperm duct of *Macrobrachium nipponense* collected in Yamaguchi, Nagasaki and Shiga Prefectures (Yokota, 1924; Hirata, 1928; Asada, 1928; Kurokawa, 1934a, b; Shimazu, 2003). Although these metacercariae were said to bear the tegumental spines [presumably the sensory papillae] (Hirata, 1928; Kurokawa, 1934a; 1934b, figs. 6–7), to have a very weakly developed pharynx (Yokota, 1924; Kurokawa, 1934a, b) and to have the genital pore located posterior to the ventral sucker (Hirata, 1928), it is almost certain from the present study that the metacercariae refer to *Ps. macrobrachicola*. Kurokawa (1934a, b) claimed that, experimentally keeping metacercariae and *Pseudobagrus aurantiacus*, *Silurus asotus* (syn. *Parasilurus asotus*), *Cynops pyrrhogaster* (Boie, 1826) (syn. *Diemyctylus pyrrhogaster*) (Amphibia) together in the water, he identified, as *P. folium*, immature worms recovered from the urinary bladder of these three animals and adult worms recovered from the urinary bladder of *Pseudobagrus aurantiacus* and *Cynops pyrrhogaster* 14–30 days after infection; and that he found adults of *P. folium* of natural infection in *Cynops pyrrhogaster* and *Andrias japonicus* (Temminck, 1836) (syn. *Megalobatrachus japonicus*) (Amphibia). It seems impossible that the metacercariae were swallowed by these three animals or they invaded the animals through the cloaca to migrate into the urinary bladder. As mentioned above, the metacercariae (Kurokawa, 1934b, figs. 3–5) are considered to have belonged to *Ps. macrobrachicola*. However, as pointed out by Cribb (1987), Kurokawa (1934a, b) should have misidentified the adults as *P. folium*, because the uterine loops were present in all available space of the postvitelline region of the body (Kurokawa, 1934b, figs. 6–7), instead of restricted to the inter- and post-cecal fields in



the adult of *P. folium* as in Yamaguti (1971, fig. 189) after Looss (1894). Campbell (2008, fig. 10.15) presented a figure of an adult of *P. folium* after Yamaguti (1971), but this figure is not a redrawing of Yamaguti's. Later, fully matured adults, the uterus of which was folded much more widely in all available space of the postvitelline region, were also included in the diagnosis of *P. folium* (Pigulewsky, 1953; Bykhovskaya-Pavlovskaya and Kulakova, 1987). However, Kurokawa (1934a, b) cannot have known such adults from Europe at that time. Yamaguti (1936) reported *Phyllodistomum patellare* (Sturges, 1897) from *Cynops pyrrhogaster* in Kyoto. Kurokawa's adult resembles *P. patellare* as described by Yamaguti (1971, fig. 811) rather than *P. folium* as described by Pigulewsky (1953, fig. 62) and Bykhovskaya-Pavlovskaya and Kulakova (1987, fig. 155) in the shape of the body and distribution area of the uterus. Yamaguti (1971) doubted the occurrence of *P. folium* in Japan. I have not yet met with this species in Japan (this paper). I consider that Kurokawa (1934a, b) mistook the amphibian parasite of natural infection for the fish parasite of experimental infection.

Another difficult problem in the taxonomy of species of this family arises in describing the egg size. In general, the egg size is considered to be one of the most important diagnostic features in digeneans. In *Phyllodistomum* and *Pseudophyllodistomum*, the eggshell is thin and nonoperculate. Uterine eggs increase greatly in size with development of embryos inside. Further, eggs formed with no ovum are also often included in the uterus. In the present whole-mounts of *Ps. macrobrachicola*, eggs formed with no ovum were 32–50 by 17–24 µm, eggs in 1-cell stage of development were 48–73 by 27–37 µm, fully embryonated eggs (intact) were 64–88 by 32–56 µm, and miracidia in the uterus were 88–96 by 72–93 µm. Moreover, the eggshells of fully embryonated eggs each including a fully formed miracidium often tear longitudinally when worms are fixed or mounted in Canada balsam, so that the size of fully embryonated eggs cannot

be accurately measured on whole-mounts. Accordingly, the description of the egg size should be accompanied by specific mention of the developmental stage of the embryo.

Superfamily Plagiorchioidea Lühe, 1901

Family Orientocreadiidae Yamaguti, 1958

Genus *Orientocreadium* Tubangui, 1931

***Orientocreadium chaenogobii* Shimazu, 1990**

(Figs. 24–27)

*Orientocreadium chaenogobii* Shimazu, 1990: 934–935, figs. 1–3.

*Hosts in Japan.* *Gymnogobius castaneus* (O'Shaughnessy, 1880) (Gobiidae) (type host) and *Gymnogobius urotaenia* (Shimazu, 1990, 1994; this paper).

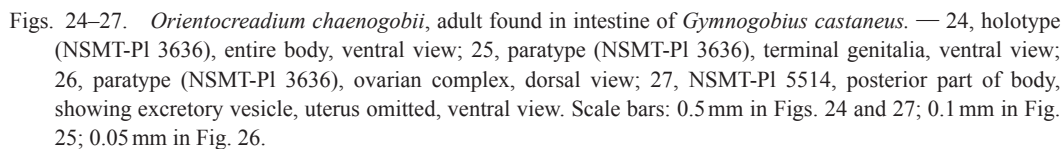
*Site of infection.* Rectum.

*Geographical distribution.* Hokkaido: Lake Toro (type locality) at Toro, Shibeche Town; and Chitose River at Ebetsu City (Shimazu, 1990, 1994; this paper).

*Material examined.* (1) Type series of *Orientocreadium chaenogobii*: holotype (NSMT-PI 3636) and 21 paratypes (NSMT-PI 3636–3637), adult, whole-mounted, ex rectum of *Gymnogobius castaneus* [now not *Chaenogobius laevis* (Steindachner, 1879)], Lake Toro, 10 October 1981, 22 July 1984; 58 paratypes (NSMT-PI 3064, 3638), adult, whole-mounted, ex rectum of *Gymnogobius urotaenia* (syn. *Chaenogobius urotaenia* (the freshwater type), *Chaenogobius annularis*, *Chaenogobius* sp. 1), Lake Toro, 28 June 1984; and 4 paratypes (NSMT-PI 3639), 1 immature, 3 adult, ex rectum of *G. castaneus*, Chitose River, 2 August 1984 (Shimazu, 1990, 1994). (2) Several (NSMT-PI 5514), adult, whole-mounted, serially sectioned, ex rectum of *G. castaneus*, Lake Toro, 5 September 1991.

*Description.* 1) Based on the type series, after Shimazu (1990), slightly altered from the present study (Figs. 24–26). Body elongate to fusiform, small, 0.99–2.13 by 0.46–0.63 (holotype 2.13 by 0.60); forebody 0.43–0.71 long, occupying 30–44% of body length. Tegument





protuberances. Esophagus short, 0.03–0.08 long, thick-walled, surrounded by small gland cells, bifurcating about midway between two suckers. Intestinal ceca thick, slightly sinuous, ending blindly some distance away from posterior extremity of body. Ventral sucker subglobular, 0.14–0.24 by 0.15–0.26, at about junction of anterior and middle thirds of body; sucker width

ratio 1:1.2–1.5. Testes two, entire, 0.08–0.27 by 0.12–0.24, oblique, usually separated by uterus but rarely contiguous, intercecal, pre-equatorial in hindbody. External seminal vesicle clavate, voluminous, thin-walled, folded once, posterior to ventral sucker. Cirrus pouch club-shaped, curved, thick-walled, outer longitudinal and inner circular muscle fibers well developed, 0.20–0.35 by 0.06–0.09, extending posteriorly between ventral sucker and ovary, but not beyond ovary. Internal seminal vesicle oval, small, thick-walled. Pars prostatica oblong, with well-developed sphincter at posterior end; prostatic cells numerous, densely surrounding pars prostatica and internal seminal vesicle. Ejaculatory duct long, slender, about half as long as cirrus pouch, lined with spines, sometimes slightly everted. Genital atrium wide, shallow. Genital pore median, between ventral sucker and intestinal bifurcation. Ovary globular, 0.08–0.19 by 0.14–0.20, dextrally submedian, pretesticular, closely facing ventral sucker across cirrus pouch. Oviduct dilated proximally, dilatation including sperm. Laurer's canal running posteriorly to open dorsally at anterior testis, usually dilated in proximal portion, dilatation containing sperm. Ovarian complex medial to ovary. Seminal receptacle absent. Ootype large; Mehlis' gland well developed. Uterus occupying all available space in hindbody, acting as uterine seminal vesicle; metratrum lined with spines, slightly shorter than cirrus pouch. Eggs numerous, oval to elliptical, light brown, 32–38 by 18–22  $\mu\text{m}$ , operculate, usually weakly embryonated. Vitellaria follicular, follicles various in shape and size, distributed contiguously on lateral and ventral sides of intes-

tinal ceca from midlevel of ventral sucker to a short distance anterior to cecal ends, separate. Excretory vesicle possibly extending to cecal ends; excretory pore posteroterminal.

2) In the specimens (NSMT-PI 5514), excretory vesicle I-shaped, ending slightly anteriorly to cecal ends, not reaching to posterior testis (Fig. 27).

**Remarks.** Shimazu (1990) described *Orientocreadium chaenogobii* Shimazu, 1990 on the basis of the type series. He failed to observe the excretory vesicle clearly. In the specimens (NSMT-PI 5514), it was I-shaped, ending slightly anteriorly to the cecal ends or about midway in the post-testicular region of the body, not reaching to the posterior testis (Fig. 27). Shimazu (1990) mentioned an adult specimen (NSMT-PI 3640) of *O. chaenogobii*. This specimen was originally labeled "[Intestine of *Moroco percunurus sachalinensis* from Lake Toro on 8 August 1981.]" It seems likely that the name of the fish host *Phoxinus phoxinus sachalinensis* (Berg, 1907) (syn. *M. percunurus sachalinensis*) (Cyprinidae) was erroneously labeled (Shimazu, 1990).

**Life cycle.** Not known.

### *Orientocreadium pseudobagri* Yamaguti, 1934

(Figs. 28–35)

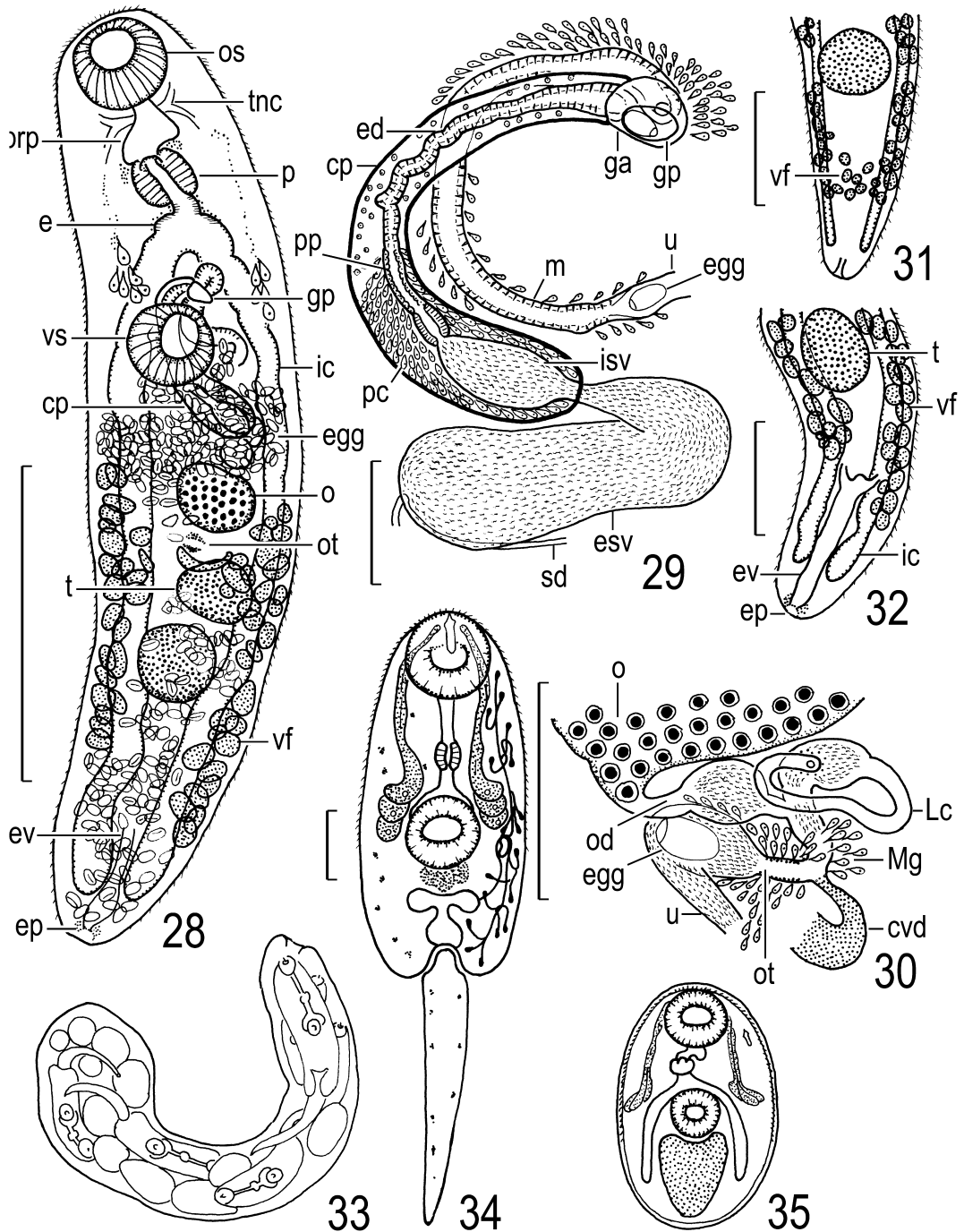
*Orientocreadium pseudobagri* Yamaguti, 1934: 334–335, fig. 39; Shimazu, 1990: 936–937, figs. 4–6; Shimazu *et al.*, 2011: 60–61, figs. 81–85.

*Macroderoides asiaticus* Belous in Skryabin and Antipin, 1958: 519–525, fig. 149.

**Hosts in Japan.** *Tachysurus nudiceps* (Bagridae) (type host) and *Silurus lithophilus* (Siluri-

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Figs. 28–35. *Orientocreadium pseudobagri*, adults found in intestine of *Tachysurus nudiceps* and life cycle. — 28, holotype (MPM Coll. No. 22290), entire body, ventral view; 29, NSMT-PI 3634, terminal genitalia, ventral view; 30, NSMT-PI 3633, ovarian complex, dorsal view; 31, NSMT-PI 3633, post-testicular region of body, showing confluent vitelline follicles, uterus and excretory vesicle omitted, ventral view; 32, NSMT-PI 5560, post-testicular region of body, showing excretory vesicle, uterus omitted, ventral view; 33, daughter sporocyst found in *Lymnaea amurensis*, scale not given; 34, cercaria found in *L. peregra coreana*, ventral view; 35, encysted metacercaria, 8 days after experimental infection, neither host nor scale given. Figs. 31–32 redrawn from Shimazu *et al.* (2011). Figs. 33 and 35 redrawn from Besprozvannykh (1984). Fig. 34 redrawn from Besprozvannykh *et al.* (2009). Scale bars: 0.5 mm in Fig. 28; 0.2 mm in Figs. 31–32; 0.1 mm in Figs. 29–30; 0.04 mm in Fig. 34.



dae) (Yamaguti, 1934; Shimazu, 1990; Shimazu *et al.*, 2011; this paper).

*Site of infection.* Intestine.

*Geographical distribution.* Shiga Prefecture: Lake Biwa (type locality) and Lake Biwa basin (Yamaguti, 1934; Shimazu, 1990; Shimazu *et al.*, 2011).

In Russia: Primorskiy Kray (e.g. Akhmerov, [1961]; Bykhovskaya-Pavlovskaya and Kulakova, 1987; Besprozvannykh *et al.*, 2009).

In China: Wuyishan, Fujian Province (Wang *et al.*, 1985).

*Material examined.* (1) Type series (Yamaguti's Collection, MPM Coll. No. 22290, holotype and 3 paratypes) of *Orientocreadium pseudobagri*, adult, ex small [sic] intestine of *Tachysurus nudiceps* (syn. *Pelteobagrus nudiceps*), Lake Biwa (locality not specified), 15 July 1927 (Yamaguti, 1934; Shimazu, 1990; Shimazu *et al.*, 2011). (2) 6 specimens (NSMT-PI 3633–3634, 5560) of *O. pseudobagri*, adult, ex intestine of *T. nudiceps*, Lake Biwa at Onoe, Kohokuchō, Nagahama City, 4 May 1979, 3 June 1980, 4 May 1992 (Shimazu, 1990; Shimazu *et al.*, 2011). (3) 1 (NSMT-PI 3635) of *O. pseudobagri*, adult, ex intestine of *Silurus lithophilus*, Lake Biwa at Onoe, 3 June 1980 (Shimazu, 1990; Shimazu *et al.*, 2011).

*Description.* 1) Based on the specimens (MPM Coll. No. 22290, NSMT-PI 3633–3635 and 5560), after Shimazu *et al.* (2011), slightly altered from the present study (Figs. 28–32). Two of the three paratypes lacked the anterior and posterior part of the body, respectively. Similar to *Orientocreadium chaenogobii* (this paper) in general morphology. Body elongate, small, 1.10–3.63 by 0.23–0.66 (holotype 1.48 by 0.34); forebody 0.43–1.13 long, occupying 28–39% of body length. Oral sucker 0.09–0.22 by 0.10–0.23. Prepharynx 0.05–0.24 long. Pharynx 0.06–0.14 by 0.05–0.14, with 4 anterior muscular protuberances. Esophagus 0.04–0.16 long, bifurcating at about junction between middle and posterior thirds of forebody. Intestinal ceca reaching to near posterior extremity of body. Ventral sucker usually smaller than oral sucker, 0.09–0.19 by

0.10–0.21, slightly posterior to junction between anterior and middle thirds of body; sucker width ratio 1:0.8–1.1. Testes 0.06–0.37 by 0.07–0.30, diagonal or almost tandem, usually slightly separated by uterus, intercecal, in middle third of hindbody. External seminal vesicle clavate, voluminous, folded once, 0.08–0.33 by 0.05–0.16, usually reaching to ovary. Cirrus pouch clavate, curved, fairly thick-walled, circular and longitudinal muscle fibers well developed especially in posterior part, 0.16–0.40 by 0.04–0.12, extending backward far beyond ventral sucker. Internal seminal vesicle oval, small, 0.04–0.14 by 0.02–0.08. Pars prostatica oblong, with well-developed sphincter at its posterior end; prostatic cells well developed. Ejaculatory duct long, slender, about two-thirds as long as cirrus pouch, lined with spines, sometimes slightly everted. Genital atrium shallow. Genital pore median or only slightly shifting to left, between ventral sucker and intestinal bifurcation. Ovary globular, 0.07–0.22 by 0.09–0.22, almost median, separated from ventral sucker by cirrus pouch and uterus. Ovarian complex between ovary and anterior testis. Oviduct dilated in proximal and distal portions, dilatations including sperm. Laurer's canal fairly long, running anteriorly or coiled, dilated in proximal portion, dilatation including sperm. Ootype postovarian; Mehlis' gland well developed. Uterus first descending from ootype to near posterior extremity of body and then ascending to ovary along median line of body, and then transversely folded tightly between ovary and metraterm (or ventral sucker), overlapping intestinal ceca; uterine seminal vesicle well developed; metraterm slightly shorter than cirrus pouch, lined with spines. Eggs numerous, elongate-elliptical, brown, 29–44 by 14–22  $\mu\text{m}$  (collapsed), operculate, weakly embryonated. Vitelline follicles various in shape and size, lateral and ventral to intestinal ceca, extending from ovarian level to some distance anterior to cecal ends, rarely interrupted at testicular level, separate anteriorly, usually separate (Figs. 28, 32) but rarely confluent posteriorly (Fig. 31). Excretory vesicle ending considerably anteriorly to cecal

ends or about midway in post-testicular region of body; excretory pore postero-dorsal to -terminal.

**Remarks.** Yamaguti (1934) described *Orientocreadium pseudobagri* Yamaguti, 1934 on the basis on the type series found in the small intestine of “*Pseudobagrus aurantiacus*” (Bagridae) from Lake Biwa. The current scientific name of the bagrid in Lake Biwa is *Tachysurus nudiceps* (syn. *Pelteobagus nudiceps*) (see also Shimazu, 1990; Shimazu *et al.*, 2011). In Japan, the distribution of *O. pseudobagri* is confined to the Lake Biwa basin (Yamaguti, 1934; Shimazu, 1990; Shimazu *et al.*, 2011).

*Orientocreadium pseudobagri* differs from *O. chaenogobii* (this paper) in that the oral sucker is larger than the ventral sucker, the sucker width ratio being 1:0.8–1.1 instead of 1:1.2–1.5; the ovary is median instead of dextrally submedian and largely separated from the ventral sucker by the cirrus pouch and uterus instead of far closer to the ventral sucker; and the vitelline follicles commence at the level of the ovary instead of the ventral sucker.

Yamaguti (1934) described that the excretory vesicle extended to the posterior testis. However, Shimazu *et al.* (2011, fig. 85) showed that the excretory vesicle was I-shaped, ending anteriorly considerably anteriorly to the cecal ends or about midway in the post-testicular region of the body (this paper, Fig. 32).

**Life cycle.** Not known in Japan. The life cycle was studied in the laboratory and field in Primorskiy Kray, Russia (Besprozvannykh, 1984; Ermolenko and Besprozvannykh, 1987; Ermolenko, 1992; Besprozvannykh *et al.*, 2009). First intermediate hosts were pulmonate snails, *Lymnaea schubinae*, *Lymnaea coreana*, *Lymnaea ussuriensis* and *Lymnaea amurensis* (Lymnaeidae). Xiphidiocercariae are produced in daughter sporocysts (this paper, Figs. 33–34). Second intermediate hosts are several species of fishes and mollusks (experimental), in which encysted metacercariae (this paper, Fig. 35) develop. Final hosts are *Perccottus glenii* (Odontobutidae) and *Pelteobagrus fulvidraco* (Bagridae) (Besprozvannykh *et al.*, 2009).

### Key to the species of the genus *Orientocreadium* in Japan

- 1.1. Oral sucker smaller than ventral sucker; ovary dextrally submedian, far closer to ventral sucker; vitelline follicles commencing at level of ventral sucker ..... *O. chaenogobii*
- 1.2. Oral sucker larger than ventral sucker; ovary median, largely separated from ventral sucker by cirrus pouch and uterus; vitelline follicles commencing at level of ovary ..... *O. pseudobagri*

### Discussion on *Orientocreadium*

The shape of the excretory vesicle in *Orientocreadium* has previously been undetermined definitively (Shimazu, 1990; Jones and Bray, 2008). The present study shows that the excretory vesicle is distinctly I-shaped in the adults of *O. chaenogobii* and *O. pseudobagri* (Figs. 27, 32). The excretory vesicle was misinterpreted as Y-shaped in *Orientocreadium batrachoides* Tubanguí, 1931 (Tang and Lin, 1973), in the cercaria of *O. pseudobagri* (Besprozvannykh, 1984; Shimazu, 1990), in the cercaria and metacercaria of *O. pseudobagri* (Besprozvannykh *et al.*, 2009)

and in *Orientocreadium elegans* Besprozvannykh *et al.*, 2009 (Besprozvannykh *et al.*, 2009). The excretory vesicle was saccular and large in the cercaria and metacercaria of *O. batrachoides* (Sophon Sirikantayakul, 1985) and in the metacercariae of *Orientocreadium siluri* (Bychovsky and Dubinina, 1954), *O. batrachoides* and *O. pseudobagri* (Zablotzky *et al.*, 1964; Tang and Lin, 1973; Besprozvannykh, 1984) (this paper, Fig. 35). I consider that the excretory vesicle is actually saccular, large and thin-walled in the cercaria and metacercaria (this paper, Fig. 35), and so it tends to transform from saccular into inverted hastate-shaped (or Y-shaped) (this paper,



Fig. 34).

Tang and Lin (1973) described 18 flame cells on either side of the body in the cercaria of *O. batrachoides* and the flame cell formula as  $2[(3+3+3)+(3+3+3)]=36$  in the Plagiorchiidae Lühe, 1901 (Plagiorchioidea). Accordingly, I gave the flame cell formula as  $2[(3+3+3)+(3+3+3)]=36$  for *Orientocreadium* (Shimazu, 1990). However, the flame cell formula was reported as  $2[(4+4+4)+(4+4+4)]=48$  in the cercaria of *O. batrachoides* (Sophon Sirikantayakul, 1985),  $2[(2+2+2)+(2+2+2)]=24$  in the metacercaria of *O. siluri* (Zablosky *et al.*, 1964) and  $2[(3+3+3)+(3+3+3)]=36$  in the cercaria of *O. pseudobagri* (Besprozvannykh *et al.*, 2009) (this paper, Fig. 34) and *O. elegans* (Besprozvannykh *et al.*, 2009). Further studies of the flame cell formula in *Orientocreadium* are required.

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