# Trematodes of the Genera Asymphylodora, Anapalaeorchis and Palaeorchis (Digenea: Lissorchiidae) from Freshwater Fishes of Japan

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### Abstract

Digenetic trematodes, Asymphylodora japonica Yamaguti, 1938, A. macrostoma Ozaki, 1925, Anapalaeorchis hamajimai Fujino and Kifune, 1991, and Palaeorchis diplorchis (Yamaguti, 1936) Szidat, 1943 (Lissorchiidae Magath, 1917), all parasitic in Japanese freshwater fishes are described and figured. Data on their museum specimens, hosts, localities and life cycles are given. Amended diagnoses of the family and genera concerned are proposed.

# Key words : Asymphylodora, Anapalaeorchis, Palaeorchis, Lissorchiidae, Digenea, freshwater fishes, Japan

This paper, the eighth in a series on the digenetic trematodes of Japanese freshwater fishes, deals with species of the genera *Asymphylodora* Looss, 1899, *Anapalaeorchis* Fujino and Kifune, 1991, and *Palaeorchis* Szidat, 1943, in the family Lissorchiidae Magath, 1917.

# Materials and Methods

The materials and methods were given in the first paper (Shimazu, 1988). Several lots of specimens have been obtained since then.

# Family Lissorchiidae Magath, 1917

Lissorchiinae Magath, 1917, p. 60 (type genus, *Lissorchis* Magath, 1917).

Lissorchidae: Poche, 1926, pp. 135-136 (type genus, Lissorchis Magath, 1917).

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Family diagnosis. Digenea. Body elongate, oval or fusiform, spinose. Oral sucker subterminal. Prepharynx short. Pharynx present. Esophagus short; intestinal ceca variable in length, usually terminating blindly in hindbody. Ventral sucker about bifurcal, in anterior half of body. Testes single or double, tandem, oblique or symmetrical, usually in posterior half of hindbody. Cirrus pouch posterolateral to ventral sucker, containing a seminal vesicle, prostatic complex, and spined cirrus. Genital atrium indistinct. Genital pore marginal or submarginal, sinistral or posterosinistral to ventral sucker. Ovary entire or lobed, median or submedian, pretesticular. Ootype complex usually preovarian. Seminal receptacle small, usually functionless. Laurer's canal present. Uterus long, folded several times to make descending and ascending limbs, usually confined to hindbody, serving as a uterine seminal receptacle in initial limbs; metraterm well developed, spinous or not, receiving uterus at its posterior end. Eggs numerous, operculate, with or without a filament, embryonated. Vitellaria aciniform, limited to hindbody, rarely extending slightly into forebody, mostly lateral, rarely confluent dorsally. Excretory vesicle tubular or saccular; excretory pore terminal.

Life cycles involving three or two hosts where known for freshwater members. Cercariaeum cercariae produced in rediae in freshwater prosobranch and pulmonate snails; usually tailless, exceptionally with a small spined tail; a vestigial stylet may be present; excretory system of stenostomate type, flame-cell formula probably 2[(4+4+4+4)+(4+4+4+4)] =64 or 2[(3+3+3)+(3+3+3)]=42. Metacercariae encysting in snails of the same species, planarians, oligochaetes and fish, or rarely brackish-water polychaetes, or while still staying in rediae, rarely attaining sexual maturity. Adults parasitic in intestine, exceptionally kidney, usually of freshwater, rarely marine, teleosts.

Discussion. This family diagnosis is based chiefly on Wallace (1941), Schell (1973, 1985), Anonym (1973), Yamaguti (1975) and the present study. At present this family consists of at least Asymphylodora Looss, 1899, Lissorchis Magath, 1917 (= Triganodistomum Simer, 1929), Palaeorchis Szidat, 1943, Neopaleorchis Schell, 1973, Asymphylotrema Dvoryadkin and Besprozvannykh, 1985, Anapalaeorchis Fujino and Kifune, 1991, Anarhichotrema Shimazu, 1973, and Neolissorchis Machida, 1985. The first genus has one testis, but the rest have two testes (Looss, 1899; Magath, 1917; Szidat, 1943; Schell, 1973; Dvoryadkin and Besprozvannykh, 1985; Fujino and Kifune, 1991; Shimazu, 1973; Machida, 1985). All are freshwater except for the last two, which are marine and possibly synonyms. Some writers (e.g., Yamaguti, 1971) place the genera Asymphylodora and Palaeorchis in the family Monorchiidae Odhner, 1911, but others (e.g., Schell, 1985) allocate them to the family Lissorchiidae. I accept this allocation in this paper, because (1) they comprise mostly freshwater (not exclusively marine) species; (2) they produce non-oculate cercariaea in rediae in snails (not oculate and tailed cercariae in sporocysts in clams); (3) the excretory system is of stenostomate type with a

flame-cell formula of 2[(4+4+4+4)+(4+4+4)]4+4]=64 or 2[(3+3+3)+(3+3+3)]=42 (not of mesostomate type with a flame-cell formula of 2[(2+2)+(2+2)]=16) in the cercarial stage; (4) the genital pore is marginal or submarginal at the level of the ventral sucker (not median in front of the ventral sucker); and (5) the metraterm is simple, without terminal organ, regardless of the presence or absence of spines (not complex, with a terminal organ). Magath (1917) found an armatae-xiphidiocercaria developing in a sporocyst in a planorbid snail collected in the field and associated it with L. fairporti Magath, 1917, the type species of the genus Lissorchis. Magath seems to have mistaken the cercaria of some plagiorchiid for that of this species. Subsequent studies of life cycles of lissorchiids have shown that they produce cercariaea in rediae in snails (see Yamaguti, 1975; Schell, 1985). The life cycle of L. fairporti has not yet been elucidated. Yamaguti (1971) erected the new subgenus Asymphylodoroides in the genus Asymphylodora for a marine species, Asymphylodora atherinopsidis Annereaux, 1947. This trematode has the genital pore posterosinistral to the ventral sucker and a welldifferentiated, spined terminal organ (Annereaux, 1947; Olson, 1977). In spite of the position of the genital pore, the presence of the terminal organ suggests that it is a member of the family Monorchiidae. Further review of the family Lissorchiidae may be needed, but such is beyond the scope of this paper.

At present Japanese species of the family are assigned to three genera: *Asymphylodora* with one testis, and *Anapalaeorchis* and *Palaeorchis* with two testes.

#### Genus Asymphylodora Looss, 1899

- Asymphylodora Looss, 1899, pp. 598-599 (type species, Distomum perlatum von Nordmann, 1832=Fasciola tincae Modeer, 1790).
- Parasymphylodora Szidat, 1943, p. 44 (type species, Asymphylodora macrostoma Ozaki, 1925, by subsequent designation).
- Orientotrema Tang, 1962, pp. 169 and 182-183 (type species, Asymphylodora japonica Yamaguti, 1938). N. syn.

Generic diagnosis. Lissorchiidae. Intestinal ceca short or half-long, may extend into posttesticular region. Testis single. Seminal vesicle saccular, oblong or bipartite. Genital pore sinistral, marginal or submarginal. Ovary entire. Uterus sometimes extending to bifurcal level; metraterm well differentiated, spined. Eggs not filamented. Vitellaria distributed in hindbody, sometimes sightly entering forebody. Excretory vesicle I-shaped or saccular.

Life cycles involving two or three hosts where known. Cercariaea developing in rediae in freshwater snails; flame-cell formula 2[(4+4+4+4)+(4+4+4+4)]=64 or 2[(3+3+5)+(3+3+4)]=42. Metacercariae encysting in snails of the same species, oligochaetes and fish, or brackish-water polychaetes, or while still staying in rediae, sometimes becoming sexually mature. Adults parasitic in intestine, exceptionally kidney, of freshwater, rarely brackish-water, fishes.

Discussion. This generic diagnosis is based on Tang (1962), Yamaguti (1971, 1975), Anonym (1973), Schell (1985) and the present study. Szidat (1943) erected a new genus, Parasymphylodora, for two East Asian species, Asymphylodora macrostoma Ozaki, 1925, and A. indica Srivastava, 1936, without designating the type species. He distinguished it from the genus Asymphylodora by (1) the extent of the vitellaria symmetrically anterior and posterior to the level of the ventral sucker; (2) the very short intestinal ceca ending at the midlevel of the ovary; (3) the very weakly developed cirrus pouch and metraterm; (4) the vitelline duct running far anterior to the ovary and just posterior to the ventral sucker; and (5) in the metacercaria, the tubular excretory vesicle reaching to a short distance in front of the testis. These features do not seem of generic importance. I agree with Sobolev (1955) that the genus *Parasymphylodora* is suppressed as a synonym of the genus Asymphylodora. Tang (1962) created a new genus, Orientotrema, for A. macrostoma and A. japonica Yamaguti, 1938 (type species) and others which possess a tubular excretory vesicle. Likewise this genus is suppressed. The following two species of the genus Asymphylodora are known from Japan.

Asymphylodora japonica Yamaguti, 1938 (Figs. 1-5)

- Asymphylodora japonica Yamaguti, 1938, pp. 87-88, fig. 47.
- Asymphylodora japonica: Yamaguti, 1942, p. 371.
- *Orientotrema japonica*: Tang, 1962, pp. 169 and 182-183. N. syn.

Material examined. 1) Lot 1. A large number of immature and mature whole-mounted specimens, the type series of A. japonica of Yamaguti (1938): 12 slides (MPM Coll. No. 22271) including the figured (fig. 47) or holotype specimen from the intestine of Cyprinus carpio (Cyprinidae) (type host) from [Fukuda-mura, now in Okayama-shi(?)] Okayama Prefecture (type locality) [on November 26, 1937]; and 1 slide (MPM Coll. No. 22272) from the intestine of C. carpio from Lake Biwa [Shiga Prefecture, on June 1, 1936].

2) Lot 2. Three gravid whole-mounts (MPM Coll. No. 22270) of *A. japonica* of Yamaguti (1942) from the intestine of *C. carpio* from Kasumigaura [at Tsuchiura, Ibaraki Prefecture], on April 4, 1940. (He obtained seven mature specimens then.)

Description. 1) From lot 1, 10 better adults including the holotype measured (Figs. 1-3). Body elliptical, 0.75-1.31 by 0.26-0.48; forebody containing numerous gland cells on either side of esophagus, 0.30-0.48 long, 35-42% of total body length. Tegumental spines covering whole body, becoming sparser posteriorly. (A cellular, possibly protozoan, mass seen to right of pharynx in parenchyma of the holotype.) Oral sucker round, subterminal, 0.12-0.17 by 0.13-0.19. Prepharynx short. Pharynx oval, 0.06-0.08 by 0.05-0.07. Esophagus 0.03-0.11 long, bifurcating usually anterodorsal to, sometimes just in front of, ventral sucker; intestinal ceca ending at about level of posterior border of testis, rarely extending to midlevel of post-testicular region. Ventral sucker rounded, located a little anterior to midlevel of body, 0.16-0.22 by 0.17-0.25; sucker width ratio 1: 1.2-1.6.

Testis single, longitudinally elongated, in posterior half of middle third of hindbody, 0.11-0.22 by 0.09-0.14. Sperm ducts two; common sperm duct absent. Cirrus pouch club-shaped,



Figs. 1-5. Asymphylodora japonica. 1-3: Holotype (lot 1) from Cyprinus carpio from Okayama Prefecture; 1, entire body, ventral view; 2, terminal genitalia, ventral view; 3, ovarian complex, dorsal view. 4-5: Redia and an excysted metacercaria from Bulimus striatulus japonicus [=Parafossarulus manchouricus] from Okayama Prefecture (Yamaguti, 1938). (Scale bars: 0.2 mm in Figs. 1 and 4-5; 0.05mm in Figs. 2-3.)

5

lving obliquely just behind ventral sucker, extending a little farther than median line of body, usually reaching ovary, 0.19-0.25 by 0.07-0.09; seminal vesicle bipartite; pars prostatica globular, small; cirrus fairly long, spinose, protrusible. Genital atrium small. Genital pore on left margin of body, a little anterior to posterior border of ventral sucker. Ovary globular or triangular, to right of median line, anterolateral to testis, 0.09-0.12 by 0.06-0.14. Ootype complex preovarian. Laurer's canal short. Seminal receptacle spherical, small, empty in specimens examined. Uterus with many ascending and descending limbs, occupying all available space in hindbody, sometimes extending to bifurcal level, serving as a uterine seminal receptacle; metraterm well developed. shorter than half length of cirrus pouch, spined, surrounded by gland cells. Eggs elongate-oval, symmetrical, thin-shelled, operculate, somewhat thickened at anopercular end, yellow, embryonated, 37-40 by 16-22 µm in balsam. Vitelline follicles 2 to 6 on each side of body, fairly large, lying lateral to intestinal ceca in gonadal zone. Excretory vesicle I-shaped, extending usually to anterior half of testis or sometimes to posterior half of ovary; excretory pore terminal.

2) Yamaguti (1942) briefly described lot 2. It was similar to lot 1: body elongate; esophagus bifurcating in front of ventral sucker; intestinal ceca extending to midlevel of post-testicular region; testis and ovary of irregular outline; and uterine eggs dark brown.

Discussion. Yamaguti (1938) fully described lot 1. He said the excretory vesicle to terminate anteriorly near the anterior end of the testis, but his figure (fig. 47) for the holotype shows the organ ending over the posterior half of the testis. My reexamination has shown that it extends to near the anterior border of the testis in the holotype (Fig. 1) and to the anterior half of the ovary in some others. He merely stated that this species very closely resembles A. tincae (Modeer, 1790) Lühe, 1909, but differs from it markedly in egg size. It is distinguished from A. tincae not only by egg size (37-40 by 16-22  $\mu$ m versus 23-27 by 12-14  $\mu$ m) but also by shape and size of the excretory vesicle (Ishaped and reaching to the ovary instead of sacciform and located behind the testis) (Looss, 1894; this paper).

Yamaguti (1938) found both cercariaea developing in rediae and encysted metacercariae in Bulimus striatulus japonicus [=Parafossarulus manchouricus, collected at Koujo, now in Okayama-shi] and referred them to A. japonica on morphological and ecological grounds. He briefly described the metacercariae. I reexamined mounted rediae and excysted metacercariae of his material (MPM Coll. Nos. 22744 and 22281). The rediae (Fig. 4) each contained one encysted metacercaria and several developing cercariae, one of which usually was fully formed: body 0.57-0.66 by 0.25-0.31; pharvnx 0.06-0.07 by 0.05-0.06; intestine small, 0.14-0.16 by 0.07-0.11; and metacercarial cyst 0.22-0.33 by 0.14-0.17. The excysted metacercariae (Fig. 5; pl. fig. 7 of Yamaguti) was closely similar to immature specimens of lot 1. I agree with his identification. He regarded as A. japonica Nagano's (1930) A. tincae obtained from snails of the same species (locality not given). Kurokawa's (1935) metacercaria (type 4) and Kuyama's (1938) cercaria (A) found in snails of the same species collected in Okayama Prefecture must have been A. japonica as well. Tang (1962) reported as A. *japonica*, the type species of his new genus Orientotrema, adults from Pseudorasbora parva and Cyprinus carpio and the daughter rediae, cercariae and metacercariae from *P. eximius* and *P. striatulus* [=P]. manchouricus] from ponds in Fuzhou, Fujian Province, China. These adults differ from A. *japonica* as described herein in having a small pharynx, short intestinal ceca ending posteriorly at the midlevel of the ovary and a semicircular seminal vesicle. Wang and Pan (1984) reported as A. japonica adults from fishes of three species and the rediae, cercariae and metacercariae from Cipangopaludina chinensis, Bellamya aeruginosa and Parafossarulus sp. from fish farming ponds in Huangpi, Hubei Province, China. These adults are smaller than those of A. japonica as described herein and also have an oblong seminal vesicle. Moreover, they are smaller than the cercariae. If the descriptions for these two Chinese trematodes are correct. the identifications of them as A. japonica are questionable. They need restudy.

The species has been recorded from *Cyprinus* carpio of Ibaraki, Shiga and Okayama Prefectures, Japan (Yamaguti, 1938, 1942). Kobayashi (1918) mentioned having found numerous flukes similar to *A. perlata* in the intestine of *C. carpio* from Lake Biwa in November, 1911. They must have been *A. japonica* because *A. japonica* is morphologically similar to *A. perlata* (=*A. tincae*), as mentioned above, but the latter has not been identified since in Japan. The species is said to occur as *O. japonica* in China (Tang, 1962; Anonym, 1973; Wang and Pan, 1984; Wang *et al.*, 1985).

# Asymphylodora macrostoma Ozaki, 1925 (Figs. 6-11)

Asymphylodora macrostoma Ozaki, 1925, pp. 104-107, fig. 4.

Asymphylodora macrostoma: Yamaguti, 1934, p. 393. Parasympylodora macrostoma: Szidat, 1943, p. 44.

Asymphylodora macrostoma: Tang, 1962, pp. 162-163, fig. 2.

Orientotrema macrostoma: Tang, 1962, pp. 169 and 182. N. syn.

*Material examined.* 1) Lot 1. Three gravid whole-mounts (MPM Coll. No. 30028) of Ozaki from "gori" (a Japanese name for small gobiid fishes of several species, its scientific name not given) (site of infection, locality and date not given).

2) Lot 2. One gravid whole-mount (MPM Coll. No. 22280) of *A. macrostoma* of Yamaguti (1934) from [the rectum of] *Mogurnda* [= *Odontobutis*] *obscura* (Gobiidae) from Lake Ogura [Kyoto Prefecture], on May 4, 1932.

3) Lot 3. Two gravid whole-mounts (NSMT-P1 3683) found in the rectum of a formalinpreserved *O. obscura* from the Matsuita River, Umaki, Saijo-cho, Higashihiroshima, Hiroshima Prefecture, on April 16, 1991.

4) Lot 4. Three gravid whole-mounts (MPM

Coll. No. 22746) of *A. macrostoma* of Yamaguti (1934) from [the intestine of] *Opsariichthys uncirostris* (Cyprinidae) from Lake Biwa [at Omatsu, Shiga Prefecture], on July 17, 1927.

5) Lot 5. Fifteen gravid whole-mounts (MPM Coll. No. 22284) of *A. macrostoma* of Yamaguti (1934) from [the intestine of] *O. uncirostris* of the Yodo River (locality not specified) on October 16, 1929.

6) Lot 6. Two gravid whole-mounts (MPM Coll. No. 22745) of *A. macrostoma* of Yamaguti (unpublished) from the intestine of *O. uncirostris* from the Yodo River (locality not specified) on September 27, 1928.

7) Lot 7. A large number of immature and mature whole-mounts (NSMT-Pl 3684-3686, 3700 and 3973) found in the intestine of *O. uncirostris* of Lake Biwa at Onoe (May 5, 1979; June 3, 1980; November 11, 1980), Omatsu (April 30, 1992) and Moriyama (May 2, 1992), Shiga Prefecture. The specimens (NSMT-Pl 3684 and 3686) were obtained by Nagasawa.

8) Lot 8. Two gravid whole-mounts (MPM Coll. No. 22278) of *A. macrostoma* of Yamaguti (1934) from [the intestine of] *Leuciscus* [= *Tribolodon*] *hakonensis* (Cyprinidae) of Namerikawa [Toyama Prefecture], on October 28, 1929.

9) Lot 9. One immature and 1 gravid wholemounts (MPM Coll. No. 22274) of *A. macrostoma* of Yamaguti (unpublished) from the intestine of *T. hakonensis* of Obama, Fukui Prefecture, on Mach 27, 1935.

10) Lot 10. Eight gravid whole-mounts (NSMT-Pl 3687) found in the intestine of *T. hakonensis* of the Eno River, Yosida, Hiroshima Prefecture, on October 30, 1976.

11) Lot 11. Nineteen gravid whole-mounts (NSMT-Pl 3688) found in the intestine of *T. hakonensis* of the Saijo River, Hibayama, Saijomachi, Hiroshima Prefecture, on October 31, 1976.

12) Lot 12. Six gravid whole-mounts (NSMT-

Figs. 6-11. Asymphylodora macrostoma. 6: Adult [holotype(?)] from Mogurnda [= Odontobutis] obscura from Saijo, Hiroshima Prefecture, entire body, ventral view, redrawn from Ozaki (1925). 7: Adult (lot 1) from "gori" (locality unknown), entire body, ventral view. 8: Adult (lot 7) from Opsariichthys uncirostris from Lake Biwa, Shiga Prefecture, entire body, ventral view. 9-11: Adults (lot 15) from Tribolodon hakonensis from the Torii River, Nagano Prefecture; 9, entire body, ventral view; 10, terminal genitalia, ventral view; 11, ovarian complex, dorsal view. (Scale bars: 0.1 mm in Figs. 10-11; 0.2 mm in Figs. 7-9.)

Lissorchiid digeneans of freshwater fishes



Pl 3689) found in the intestine of *T. hakonensis* of the Oppe River, Ogose-machi, Saitama Prefecture, on October 13, 1976.

13) Lot 13. Ten gravid whole-mounts (NSMT-Pl 3690 and 3974) found in the intestine of *T. hakonensis* of Lake Biwa at Onoe (February 4, 1980) and Omatsu (April 30, 1992). The specimens (NSMT-Pl 3690) were obtained by Nagasawa.

14) Lot 14. Thirty-seven gravid wholemounts (NSMT-Pl 3691) found in the intestine of *T. hakonensis* of the Nogu River, Oomachishi, Nagano Prefecture, on July 1, 1987.

15) Lot 15. Three immature and 106 gravid whole-mounts (NSMT-Pl 3692) found in the intestine of *T. hakonensis* of the Torii River, Mure-mura, Nagano Prefecture, on August 16, 1987.

16) Lot 16. Three immature and 66 gravid whole-mounts (NSMT-Pl 3693, 3694, 3975 and 3984) found in the intestine of *T. hakonensis* of Lake Suwa, Suwa, Nagano Prefecture, on October 5 and November 14, 1991, and May 19 and June 2, 1992.

17) Lot 17. Four gravid whole-mounts (MPM Coll. No. 22277 and 22743) of *A. macrostoma* of Yamaguti (1934) from [the intestine of] *Hemibarbus barbus* (Cyprinidae) of Kasumigaura [at Tsuchiura, Ibaraki Prefecture], on April 16, 1929.

18) Lot 18. One immature whole-mount (NSMT-Pl 3976) found in the intestine of H. *barbus* of Lake Biwa at Once on May 4, 1992.

19) Lot 19. Three gravid whole-mounts (MPM Coll. No. 22279) of *A. macrostoma* of Yamaguti (1934) from [the intestine of] *Moroco steindachneri* (Cyprinidae) of the Yura River [Ayabe, Kyoto Prefecture], on November 20, 1929.

20) Lot 20. Ten gravid whole-mounts (MPM Coll. No. 22774) of *A. macrostoma* of Yamaguti (unpublished) from the intestine of *M. steindachneri* of Obama, Fukui Prefecture (date not given).

21) Lot 21. One immature whole-mount (MPM Coll. No. 22747) of Asymphylodara of Yamaguti (unpublished) from "ukikamatsu-ka(?)" [= Hemibarbus longirostris(?)] (site of infection not given) of Lake Biwa (locality not specified) on December 4, 1938.

22) Lot 22. One immature whole-mount (MPM Coll. No. 22748) of Asymphylodora Yamaguti (unpublished) from "bote" (a Japanese name for cyprinid fishes of the subfamily Acheilognathinae, its scientific name not given) of Tsuchiura, Ibaraki Prefecture, on April 4, 1940.

23) Lot 23. Seven gravid whole-mounts (NSMT-Pl 3977) found in the intestine of *Chaenogobius isaza* (Gobiidae) of Lake Biwa at Omatsu on April 30, 1992.

24) Lot 24. One immature whole-mount (NSMT-Pl 3978) found in the intestine of *Tridentiger brevispinis* (Gobiidae) of Lake Biwa at Omatsu on May 5, 1992.

Description. 1) After Ozaki's (1925) original description for A. macrostoma (Fig. 6). Body conical, broadest at middle of body, bluntly pointed toward both ends, 0.70-0.95 by 0.27-0.33 in specimens fixed in sublimate solution, 1.00-1.31 by 0.37-0.52 in specimens mounted in toto under a slight pressure. Minute scale-like spines covering anterior half of body, closely set anteriorly, more sparsely set posteriorly and disappearing entirely at level of posterior border of ventral sucker. Oral sucker subterminal, large, 0.165 in average diameter. Prepharynx short but distinct, 0.023 long. Pharynx globular, 0.12 in diameter, slightly smaller than ventral sucker. Esophagus 0.10 long, becoming pretty wide at its posterior end; intestinal ceca beginning in front of ventral sucker, short, 0.08-0.108 in diameter, terminating with second one-third of body length or in front of testis. Ventral sucker located at junction of anterior and middle thirds of body, 0.138 in average diameter; relative size of both suckers very constant, 3: 2.5 [or 1: 0.8] in most specimens measured.

Testis single, oval, 0.215 by 0.179, lying at beginning of last third of body. Cirrus pouch slender, slightly curved, conical, 0.27 by 0.07, lying obliquely across left cecum, with its base between ventral sucker and anterior end of ovary, not quite reaching median line of body. Seminal vesicle slightly convoluted. Pars prostatica well set apart as a globose sac into which open the ducts of the numerous prostate cells. Terminal part of ejaculatory duct lined by a thick cuticula with spines, functioning as a protrusible cirrus. Genital atrium absent, or at

8

most represented merely by a shallow depression, in which the male and female ducts open separately but contiguously, the latter in front and to the left of the former. Genital pore situated on left margin of ventral surface at level of ventral sucker. Ovary rounded, somewhat triangular, lying some distance behind ventral sucker and just in front of testis, 0.14 by 0.12. Oviduct arising from anterior dorsal surface of ovary, giving off Laurer's canal after an anterior course of 0.03, soon joined by common vitelline duct coming from vitelline reservoir, immediately after this, surrounded by Mehlis' glands which are diffusely scattered in front of the ovary and have long ducts. Laurer's canal short, slightly curved, opening on dorsal surface opposite center of ovary, branching off a small functionless rudiment of seminal receptacle measuring 0.015 by 0.005. Uterus filled with spermatozoa and forming a uterine seminal receptacle in initial part, forming two or three, sometimes more, loops on either side of ovary and testis in posterior half of body; metraterm armed with spines, proceeding parallel with cirrus pouch, lateroventrally toward genital pore. Eggs of oval shape, without filament, 0.173-0.182 by 0.090-0.112 [sic]. Vitellaria consisting of a number of follicles, about 0.03-0.04 in diameter, lying outside of intestinal ceca, arranged in shape of grape bunches conterminous with intestinal ceca; transverse vitelline ducts lying at middle of body, opening into a small median reservoir lying anterodorsally to ovary. Excretory vesicle I-shaped, about 0.01 in diameter, stretching from posterior end of body to level of center of ovary.

2) Lot 1 was similar to the above description. One specimen (Fig. 7) measured: body elongate, 1.20 by 0.44; forebody 0.50 long; oral sucker 0.14 by 0.16; pharynx 0.10 by 0.11; esophagus 0.08 long; ventral sucker 0.18 by 0.16, with sucker with ratio of 1: 1.0; testis 0.34 by 0.16; cirrus pouch 0.22 by 0.07; seminal vesicle elliptical; ovary triangular, 0.17 by 0.12; and eggs 19 by 11  $\mu$ m (collapsed). The second lacked the oral sucker, and the third was found mounted laterally.

3) Yamaguti (1934) briefly described lots 2, 4, 5, 8, 17 and 19. The mature specimen of lot 2 was: body 0.98 by 0.33; forebody 0.31 long; oral sucker 0.11 by 0.15; pharynx 0.08 in diameter; esophagus 0.08 long; ventral sucker 0.13 by 0.19, with sucker width ratio of 1: 1.3; testis 0.28 by 0.10; cirrus pouch 0.20 by 0.05; ovary 0.15 by 0.09; and eggs 20-22 by 11-13  $\mu$ m (collapsed). The two mature specimens of lots 3 (shrunken) measured: oral sucker 0.11-0.12 by 0.17-0.18; and ventral sucker 0.14-0.15 by 0.14-0.16, with sucker width ratio of 1: 0.8-0.9.

4) From lot 7, 10 adults measured (Fig. 8). Similar to Ozaki's original description. Body oval, 0.85-1.45 by 0.47-0.69; forebody containing numerous gland cells, 0.31-0.63 long, 31-39% of total body length. Tegumental spines lanceolate, traceable to near posterior end of body. Oral sucker subterminal, 0.11-0.14 by 0.15-0.19. Pharynx globular, 0.07-0.12 in diameter. Esophagus 0.07-0.12 long, bifurcating dorsal to ventral sucker; intestinal ceca extending to about midlevel of hindbody or posterior border of ovary. Ventral sucker located at about junction of anterior and middle thirds of body, 0.10-0.17 by 0.13-0.18; sucker width ratio 1: 0.8-1.1.

Testis large, 0.28-0.59 by 0.22-0.37. Cirrus pouch 0.19-0.31 by 0.07-0.12. Seminal vesicle elliptical or tubular, slightly undulating. Ovary 0.14-0.29 by 0.12-0.21. Seminal receptacle small, spherical, containing a small number of spermatozoa. Laurer's canal short. Uterus usually confined posterior to bifurcal level, rarely extending a little more anteriorly to it; metraterm well differentiated, spinous, slightly shorter than half length of cirrus pouch, surrounded by gland cells. Eggs pyriform, symmetrical, rather thick-shelled, operculate at attenuated end, sometimes thickened at anopercular end, yellow or bright brown, embryonated, 21-25 by 13-14  $\mu$ m. Vitelline follicles about 10-15, fairly large, sometimes extending along anterior two-thirds of intestinal ceca, slightly entering forebody. Excretory vesicle I-shaped, reaching to midlevel of ovary.

Four immature specimens measured: body 0.69-0.94 by 0.31-0.41; oral sucker 0.10-0.14 by 0.13-0.16; ventral sucker 0.11-0.14 by 0.13-0.15, with sucker width ratio of 1: 0.9-1.0; and seminal vesicle pear-shaped.

5) From lot 15, 10 adults measured (Figs. 9-11). Similar to lot 7. Body 0.69-1.45 by 0.31-0.57; forebody 0.35-0.48 long, 33-50% of total body length. Oral sucker 0.11-0.17 by 0.14-0.17. Pharynx 0.06-0.10 by 0.07-0.10. Esophagus 0.06-0.15 long. Ventral sucker 0.12-0.19 by 0.14-0.19; sucker width ratio 1: 0.9-1.2. Testis smaller than in lot 7, 0.12-0.35 by 0.09-0.22. Sperm ducts two; common sperm duct fairly long. Cirrus pouch 0.15-0.40 by 0.06-0.15. Seminal vesicle usually globular or elliptical, rarely somewhat constricted (Fig. 10). Ovary 0.09-0.25 by 0.10-0.22. Seminal receptacle oval, small, empty or containing a small number of spermatozoa. Uterus rarely extending slightly anterior to ventral sucker. Eggs 21-24 by 13-14 μm. Vitelline follicles slightly entering forebody in most specimens, ending posteriorly with level of intestinal ceca in some specimens.

6) The specimens of lot 16 resembled those of lot 15. The eggs had usually a small knob, or rarely a small hook-like projection, at the anopercular end.

Discussion. Ozaki (1925) described this species from the cloaca [probably the lower part of the rectum] of Mogurnda [= Odontobutis] obscura (type host) taken from a brook in the vicinity of Saijo (type locality) (now Saijo-cho, Higashihiroshima), Hiroshima Prefecture. His original material has not yet been located. The three slides of lot 1 are part of a set of 13 old slides labelled merely "gori" in Japanese by him, which contains the digeneans, A. macrostoma, Coitocoecum plagiorchis Ozaki, 1926, Genarchopsis goppo Ozaki, 1925, and the cestodes, Nippotaenia mogurndae Yamaguti and Miyata, 1940, and Bothriocephalus sp. (all my identifications). Possibly, lot 1 was obtained by him from O. obscura of Saijo; even so it is uncertain whether it constitutes part of the type series of A. macrostoma. The above-mentioned digeneans of three species were described by him from O. obscura of Saijo (Ozaki, 1925, 1929). The cestode N. mogurndae has so far been recorded only from O. obscura in Japan, and it was found in fish of the same species caught in Higashihiroshima (Shimazu and Weekes, 1991, unpublished). Gori is a common Japanese name for gobiid fishes of several species and even for small juveniles of O. obscura in some areas in Japan, although Ozaki (1925) gave the Japanese name "goppo" for this species in his paper.

All lots examined closely resemble one

another. According to Ozaki's (1925) original description for A. macrostoma, the tegumental spines are present only on the anterior half of the body; the oral sucker (0.165 in diameter) is constantly larger than the ventral one (0.138 in diameter); the pharynx (0.12 in diameter) is nearly as large as the ventral sucker; the uterus is coiled in the posterior half of the body; and the eggs are oval and 0.173-0.182 by 0.090-0.112 [sic]. Otherwise, however, all lots correspond with his description in morphology and dimensions. They are all identified as A. macrostoma. In accordance with Yamaguti (1934), Tang (1962) and the present study, the specific diagnosis of this species should be emended as follows: tegumental spines covering whole body, becoming sparser posteriorly; oral and ventral suckers equal or unequal in size, sucker width ratio 1: 0.8-1.3 in mature specimens; pharynx globular, distinctly smaller than ventral sucker; uterus folded in hindbody, sometimes extending a little farther forward than ventral sucker in fully mature specimens; and eggs pear-shaped, operculate at attenuated end, slightly thickened or having a small knob- or hook-like projection at anopercular end, yellow, embryonated, 19-25 by 11-14  $\mu$ m; and vitelline follicles about 10-15 on each side of body, usually extending along anterior two-thirds of intestinal ceca. The oral sucker is certainly larger than the ventral one in the cercarial and metacercarial stages and immature worms obtained from fish (Yamaguti, 1938; Okabe, 1940; Tang, 1962; this paper). It is not clear, however, that the oral sucker tends to change from larger to smaller than the ventral one with further growth of mature worms in fish. Ozaki (1925) said that the vitellaria consist of a number of follicles, and his figure (see Fig. 6) shows more than 20 follicles on each side of the body. It is difficult to count the follicles accurately because larger follicles often have smaller lobes or buds. I observed at most 10-15 of such larger follicles on each side of the body.

The references cited by Ozaki (1925) suggest that he distinguished A. macrostoma from four species which had already been recognized in the genus from cyprinids in Europe: A. perlata (von Nordmann, 1832) Looss, 1899=A. tincae (Modeer, 1790) Lühe, 1909; A. exspinosa

(Hausmann, 1897) Looss, 1899; A. imitans (Mühling, 1898) Looss, 1899; and A. ferruginosa (von Linstow, 1877) Lühe, 1909. He stated that it differs from them in that (1) the oral sucker is larger than the ventral one; (2) the pharynx is nearly as large as the ventral sucker; (3) the vitellaria extend more anteriorly; (4) the anterior half of body has tegumental spines. As mentioned above, the first two differences are unfounded. The third is considered to be the best criterion for distinguishing between A. macrostoma and the four others. In the latter, the vitellaria lie in the posterior half of the body and scarcely reach the posterior border of the ventral sucker (Looss, 1894; Szidat, 1943). Furthermore, this criterion separates A. macrostoma from A. tincae kubanica Isaichikov, 1923 (Bykhovskaya-Pavlovskoya and Kulakova, 1987), from the Kuban River in European Russia, although most presumably Ozaki was unaware of this species at that time.

The species differs from A. japonica in the smaller sucker width ratio (1: 0.8-1.3 versus 1: 1.2-1.6), the tubular (not bipartite) seminal vesicle, the vitellaria distributed largely anterior to (not in) the gonadal zone, and the smaller eggs (19-25 by 11-14  $\mu$ m versus 37-40 by 16-22  $\mu$ m).

The life cycle of this trematode is known in part in Japan. Yamaguti (1934, 1938) found encysted metacercariae of natural infection in fishes, namely, in the peribuccal connective tissue of *Chaenogobius macrognathos* [=C.urotaenia(?)] (Gobiidae) (locality not given) and in the peribuccal connective tissue and gill arches of Gnathopogon elongatus caerulescens (Cyprinidae) from Lake Biwa (Yamaguti, 1938, pl. fig. 6; MPM Coll. No. 22275, 22276, 22282 and 22283) and Cobitis biwae (Cobitididae) from the Katura River, Kyoto Prefecture. He regarded them as A. macrostoma morphologically, not experimentally. In addition, his collection includes one slide of four excysted metacercariae (MPM Coll. No. 22949) obtained from the gills of Acheilognathus lanceolatus (Cyprinidae) of the Katura River on May 2, 1940. Okabe (1940) also reported metacercariae as A. macrostoma from fishes of several species in Fukuoka Prefecture. In China, Tang (1962) found cercariaea developing in rediae in Melania peregrinorum collected in a mountain stream at Yongan, Fujian Province, and fed them to fish of *Puntius* sp., from which he recovered adult worms similar to *A. macrostoma*. He observed that the flame-cell formula in the cercarial stage was 2[(3+3+5)+(3+3+4)]=42.

The species has been recorded from Odontobutis obscura of Kyoto and Hiroshima Prefectures (Ozaki, 1925; Yamaguti, 1934; this paper); a gobiid called "gori" (locality unknown) (this paper); Opsariichthys uncirostris of Shiga Prefecture and the Yodo River (locality not specified); Tribolodon hakonensis of Saitama, Nagano, Toyama, Shiga, Fukui and Hiroshima Prefectures (Yamaguti, 1934; this paper); Hemibarbus barbus of Ibaraki and Shiga Prefectures (Yamaguti, 1934; this paper); Moroco steindachneri of Kyoto and Fukui Prefectures (Yamaguti, 1934; this paper); "ukikamatsuka(?)" of Shiga Prefecture (this paper): a cyprinid called bote of Ibaraki Prefecture (this paper); and Chaenogobius isaza and Tridentiger brevispinis of Shiga Prefecture (this paper). Yamaguti (1934) claimed to have found the species in Pseudorasbora parva (Cyprinidae) of Lake Kobata [=Lake Kohata, Kyoto Prefecture] on May 14, 1928. His collection does include three such specimens on one slide (MPM Coll. No. 22742), but my reexamination has shown that they are Neoplagioporus elongatus (Goto and Ozaki, 1930) Shimazu, 1990 (2 specimens), and Urorchis acheilognathi Yamaguti, 1934 (1 specimen). The species also occurs in a cyprinid of *Puntius* sp. in Yongan, Fujian Province, China (Tang, 1962).

# Genus Anapalaeorchis Fujino and Kifune, 1991

Anapalaeorchis Fujino and Kifune, 1991, p. 35 (type species, Anapalaeorchis hamajimai Fujino and Kifune, 1991).

Generic diagnosis. Lissorchiidae. Intestinal ceca extending half length of hindbody or to midlevel of posterior testis. Testes two, tandem or almost so, slightly overlapping each other. Seminal vesicle bipartite. Genital pore sinistral, marginal or submarginal. Ovary trilobed. Uterus confined to hindbody; metraterm well developed, spined. Eggs not filamented. Vitellaria distributed lateral and ventral to intestinal ceca between ventral sucker and midlevel of anterior testis. Excretory vesicle I-shaped, extending to midlevel of anterior testis. Intestinal parasites of freshwater fishes.

Life cycle unknown.

Discussion. This generic diagnosis is based on Fujino and Kifune (1991) and the present study. Fujino and Kifune distinguished this genus from the genera Asymphylodora, Palaeorchis, Triganodistomum and Neopaleorchis. It seems to me that the genus most resembles the genus Asymphylotrema Dvoryadkin and Besprozvannykh, 1985. Dvoryadkin and Besprozvannykh (1985) described the morphology and life cycle of the type species of the latter, Asymphylodora macracetabulum Belous, 1953, found in Misgurnus anguillicaudatus (Cobitididae) from the Lake Khanka system in Primorskii krai, Russia. The genus differs from the genus Asymphylotrema in that the testes are tandem (not diagonal) and the intestinal ceca are shorter, extending at most to the midlevel (not the posterior border) of the posterior testis. These differences are slight. Future studies may show that they are generically identical. The genus Anapalaeorchis has the following type and only species from Japan.

# Anapalaeorchis hamajimai Fujino and Kifune, 1991 (Figs. 12-16)

Anapalaeorchis hamajimai Fujino and Kifune, 1991, pp. 35-36, figs. 1-8.

Material examined. 1) Lot 1. Seven gravid whole-mounts (NSMT-Pl 3776) (holotype, June 27, 1985; 5 paratypes, May 30, 1985; and 1 paratype, July 20, 1988) of A. hamajimai of Fujino and Kifune (1991) from the intestine of Cobitis biwae (Cobitididae) (type host) from the Tokigawa River (type locality) [Tokigawamura], Saitama Prefecture. They are labelled "adult of C. mono." in Japanese by them.

2) Lot 2. Four immature and 10 gravid whole-mounts (MPM Coll. No. 22034) of Yamaguti (1938) from the intestine of *C. biwae* from the Katura River [Kyoto Prefecture], on May 25, 1936. They are labelled "*Asymphylodora japonica*?" by him.

3) Lot 3. Five gravid whole-mounts (NSMT-Pl 3682) from the intestine of *C. biwae* from the Seki River, Shiraki-cho, Asakita-ku, Hiroshima, Hiroshima Prefecture, on July 25, 1991.

Description. 1) From lot 1, 7 adults measured (Figs. 12-13). Body elliptical, 0.70-0.99 by 0.27-0.34; forebody having numerous gland cells, 0.22-0.37 long, 32-37% of total body length. Tegumental spines covering whole body except for a median ventral field of forebody, becoming thinner and smaller posteriorly, forming a crown of 3 or 4 rows around margin of each sucker. Oral sucker round, subterminal, 0.09-0.13 in diameter. Prepharynx short. Pharvnx oval, 0.05-0.07 in diameter. Esophagus 0.12-0.16 long, bifurcating dorsal to ventral sucker; intestinal ceca short, extending at longest to midlevel of posterior testis. Ventral sucker round, located at about junction of anterior and middle thirds of body, 0.13-0.19 in diameter; sucker width ratio 1: 1.2-1.5.

Testes two, of smooth or irregular outline, tandem or almost so, slightly overlapping each other, in third quarter of hindbody; anterior testis usually spherical, 0.08-0.14 by 0.08-0.11; posterior one usually elongate, 0.12-0.16 by 0.07-0.13. Common sperm duct short. Cirrus pouch clavate, lying diagonally, just behind ventral sucker, 0.14-0.16 by 0.04-0.06; seminal vesicle bipartite; pars prostatica round, small; cirrus fairly long, heavily spinose, protrusible. Genital atrium small. Genital pore on left body margin, a little anterior to posterior border of ventral sucker. Ovary trilobed, to right of median line, anterolateral to anterior testis, 0.11-0.14 by 0.07-0.16. Ootype complex just preovarian. Laurer's canal present. Seminal

Figs. 12-16. Anapalaeorchis hamajimai. 12-13: Holotype (lot 1) from Cobitis biwae from the Tokigawa River, Saitama Prefecture; 12, entire body, ventral view; 13, ovarian complex, dorsal view. 14-15: Adults (lot 2) from C. biwae from the Katura River, Kyoto Prefecture; 14, entire body, ventral view; 15, posterior part of body, slightly dorsal view. 16: Terminal genitalia of an adult (lot 3) from C. biwae from the Seki River, Hiroshima Prefecture, ventral view. (Scale bars: 0.2 mm in Figs. 12 and 14; 0.1 mm in Figs. 13 and 15; 0.05 mm in Figs. 16.)



receptacle globular, small, empty or containing a small number of spermatozoa. Uterus occupying all available space in hindbody, extending anteriorly to ventral sucker, serving as a uterine seminal receptacle; metraterm well developed, shorter than cirrus pouch, thinly spined, surrounded by gland cells. Eggs oval, symmetrical, with a small operculum and small anopercular knob, yellow, embryonated, 27-30 by 16-19  $\mu$ m in balsam. Vitelline follicles 6-7 on each side of body, fairly large, distributed lateral and ventral to intestinal ceca between ventral sucker and midlevel of anterior testis. Excretory vesicle I-shaped, reaching midlevel of anterior testis; excretory pore terminal.

2) Lots 2 (Figs. 14-15) and 3 (Fig. 16) were similar to lot 1 in morphology and measurements. The figured specimen (Fig. 14) of lot 2 measured: body 0.55 by 0.19; forebody 0.21 long, 38% of total body length; oral sucker 0.08 by 0.07; pharynx 0.04-0.05; ventral sucker 0.10 in diameter, with sucker width ratio of 1: 1.3; testes 0.06-0.08 by 0.04-0.05; cirrus pouch 0.12 by 0.04; ovary 0.08 by 0.05; and eggs 25-31 by 16  $\mu$ m. Three specimens of lot 3 measured: body 0.57-0.63 by 0.22-0.28; forebody 0.21-0.25 long, 33-44% of total body length; oral sucker 0.06-0.09 by 0.08-0.10; pharynx 0.05 by 0.04-0.05; ventral sucker 0.12-0.14 by 0.12-0.15, with sucker width ratio of 1: 1.4-1.5; testes 0.07 by 0.05-0.06; cirrus pouch 0.12-0.14 by 0.05; ovary 0.07-0.09 by 0.06-0.07; and eggs 29-32 by 16-17 μm.

Discussion. Fujino and Kifune (1991) described the intestinal ceca as ending at the level of the anterior testis or the posterior end of the ovary, and the vitellaria as arranged in lateral clusters of several follicles at the midbody. They failed to work out either the ovarian complex or the excretory vesicle. The present reexamination of lot 1 has showed that (1) the ceca extend as far as the midlevel of the posterior testis in some specimens; (2) the vitellaria are distributed between the ventral sucker and the midlevel of the anterior testis; (3) the ovarian complex is located in front of the ovary; (4) Laurer's canal and the seminal receptacle are present; and (5) the excretory vesicle extends to the midlevel of the anterior testis.

Lots 2 and 3 are also identified as A. hama-

jimai. Yamaguti (1938) briefly described lot 2 and said that although the specimens of lot 2 were all much smaller than those of his new species, Asymphylodora japonica, yet the nearly complete agreement in essential particulars seemed to warrant their specific identity with A. *japonica*, but he reserved the final identification at that time. According to Fujino and Kifune (1991), who reexamined them but failed to identify them definitely, they have some features identifiable to A. japonica, namely, longer intestinal ceca extending over the posterior end of the testis [sic] and more extensive vitellaria than in A. hamajimai. From the above description and figures for lot 2, it is evident that lot 2 is indistinguishable from A. hamajimai, but distinguishable from A. japonica. A. hamajimai differs from Asymphylotrema macracetabulum. In the latter, the body is larger, 2 by 0.8; the oral sucker is 0.4 in diameter; the ventral sucker is 0.68 in diameter, with a larger sucker width ratio of 1: 1.7 (my calculation); and the eggs are larger, 33-44 by 11-22  $\mu$ m (Dvoryadkin and Besprozvannykh, 1985).

The life cycle of this species is unknown. A helveticum cercaria, Cercaria monostyloides Ito, 1960, could be its cercarial stage. All of the three slides of lot 1 are labelled "adult of C. mono." in Japanese, which however Fujino and Kifune (1991) did not mention at all in their paper. Cercariae of this species are produced in rediae in a freshwater prosobranch snail, Semisulcospira libertina, in Japan (Ito, 1960). The type series of A. hamajimai was collected by Prof. Fusanori Hamajima from Cobitis biwae in the Tokigawa River (Fujino and Kifune, 1991). Hamajima et al. (1982) found C. monostyloides in S. libertina in the same river, and Fukuda et al. (1990) obtained cercariae similar to C. monostyloides when they experimentally exposed S. libertina to eggs of A. hamajimai from the same river. The trematode has been recorded only from Cobitis biwae in Saitama, Kyoto and Hiroshima Prefectures (Fujino and Kifune, 1991; this paper).

#### Genus Palaeorchis Szidat, 1943

Palaeorchis Szidat, 1943, p. 48 (type species, Asymphylodora diplorchis Yamaguti, 1936, by subsequent designation).

Generic diagnosis. Lissorchiidae. Intestinal ceca short, extending only'to midlevel of body or level of ovary. Testes two, symmetrical or diagonal. Seminal vesicle oblong or bipartite. Genital pore sinistral, submarginal. Ovary unlobed, rarely lobed. Uterus restricted to hindbody; metraterm well developed, spined. Eggs not filamented. Vitellaria forming two small bunches in hindbody. Excretory vesicle Ishaped, reaching testes or not.

Life cycle involving two hosts where known. Cercariaea developing in rediae in prosobranch snails; metacercariae encysting in snails of the same species, sometimes attaining sexual maturity in them. Adults parasitic in intestine of freshwater fishes.

Discussion. This generic diagnosis is based on Szidat (1943), Yamaguti (1971), Macy and English (1975), Schell (1985) and the present study. Szidat (1943) erected this genus for trematodes with two testes without designating the type species. Wang (1983) described a new species, P. sinensis, which has the intestinal ceca extending to the midlevel of the post-testicular region, the tandem testes and the irregularly triangular ovary, in Jiangxi Province, China. Zhang (1988) described a new species, P. lobiovaris, the ovary of which is 4-lobed and situated between the two opposite testes, and another new species, P. postovaris, in which the intestinal ceca are very short, located in front of the ventral sucker and the ovary is rounded and almost post-testicular, both in Sichuan Province, China. Thus, the species named in the genus show a considerably wide variation in shape, size and topography of the internal organs, and they need review.

Both the genera *Palaeorchis* and *Anapalaeorchis* have two testes, but the former is different from the latter mainly in that the testes are opposite instead of tandem and the intestinal ceca are shorter, extending barely to the ovarian level instead of reaching to the testicular level. In Japan, only the following species in the genus is known.

# Palaeorchis diplorchis (Yamaguti, 1936) Szidat, 1943 (Figs. 17-22)

Asymphylodora diplorchis Yamaguti, 1936, pp. 4-5, fig. 8.

Steganoderma kamatukae Takeuti, 1936, pp. 581-583, 1 fig.

Palaeorchis diplorchis: Szidat, 1943, p. 48.

Material examined. 1) Lot 1. Twenty-two immature and 18 mature whole-mounts (MPM Coll. No. 22273) of A. diplorchis from the intestine of *Pseudogobio esocinus* (Cyprinidae) (type host) of Lake Suwa (type locality), Nagano Prefecture: holotype and 2 paratypes (gravid) of Yamaguti (1936) on May 18, 1935; and 22 immature and 15 mature whole-mounts of Yamaguti (1936, p. 6, a footnote) on March 30 and 31, 1936.

2) Lot 2. Two immature and 90 mature whole-mounts (NSMT-Pl 3695 and 3979-3983) found in the intestine of P. esocinus of Lake Biwa at Onoe and Omatsu, Shiga Prefecture, on February 4, 1980, and May 1 to 6, 1992. The specimens (NSMT-Pl 3695) were obtained by Nagasawa.

3) Lot 3. Twelve gravid whole-mounts (NSMT-Pl 3696) found in the intestine of *P. esocinus* of the Furukawa River, Yoshikawa, Hachihonmatsu-cho, Higashihiroshima, Hiroshima Prefecture, on July 25, 1991.

Description. 1) From lot 1, 7 adults measured (Figs. 17-19). Body elliptical, 0.82-1.23 by 0.28-0.53; forebody containing numerous gland cells, 0.28-0.35 long, 28-39% of total body length. Tegumental spines like scales, covering whole body, becoming smaller and sparser posteriorly. Oral sucker round, subterminal, 0.08-0.12 by 0.09-0.15. Prepharynx short. Pharynx oval, 0.04-0.05 in diameter. Esophagus short, 0.08-0.17 long, bifurcating anterodorsal to ventral sucker; intestinal ceca short, extending usually to about midlevel of body or midlevel of ovary, rarely to level of anterior part of testicular zone. Ventral sucker round, located at about junction of anterior and middle thirds of body, usually smaller than oral sucker, 0.08-0.13 by 0.09-0.14; sucker width ratio 1: 0.9-1.0.



Figs. 17-22. Palaeorchis diplorchis. 17-18: Holotype (lot 1) from Pseudogobio esocinus from Lake Suwa, Nagano Prefecture; 17, entire body, ventral view; 18, terminal genitalia, ventral view. 19-20: Adult (lot 1) from P. esocinus from Lake Suwa; 19, entire body, ventral view; 20, ovarian complex, dorsal view. 21: Steganoderma kamatukae, an adult [holotype(?)] from P. esocinus from Lake Biwa, Shiga Prefecture, entire body, ventral view, redrawn from Takeuti (1936). 22: Gonads of an adult (lot 2) from P. esocinus from Lake Biwa, ventral view.

(Scale bars: 0.2 mm in Figs. 17, 19, 21 and 22; 0.1 mm in Figs. 18 and 20.)

Testes two, symmetrical or slightly diagonal, in about middle third of hindbody, 0.14-0.24 by 0.12-0.15. Common sperm duct absent. Cirrus pouch clavate, curved, lying obliquely just behind ventral sucker, extending to median line of body, 0.16-0.26 by 0.06-0.09. Seminal vesicle bipartite. Pars prostatica globular, small. Cirrus fairly long, spined. Genital atrium small, shallow. Genital pore submarginal, opening at about level of posterior border of ventral sucker. Ovary triangular or globular, almost median, about equatorial, just pretesticular, 0.11-0.14 by 0.08-0.15. Ootype complex anterior or anterodorsal to ovary. Seminal receptacle elongate oval, small, empty or containing spermatozoa and/or oocytes. Laurer's canal short. Uterus occupying all available space in hindbody, serving as a uterine seminal receptacle; metraterm well developed, one-half to twothirds as long as cirrus pouch, spined, surrounded by gland cells. Eggs oval, symmetrical, operculate, somewhat thickened at anopercular end, dark brown, embryonated, 37-42 by 19-24  $\mu$ m in balsam. Vitelline follicles about 10 on each side of body, large, extending lateral and ventral to intestinal ceca between midlevel of ventral sucker and testes. Excretory vesicle I-shaped, short, not reaching testes in elongated, gravid specimens; excretory pore terminal.

2) After Takeuti's (1936) original English résumé for *S. kamatukae* (Fig. 21). Body 1.05-1.43 by 0.36-0.48, spined in anterior part. Oral sucker subterminal, 0.11-0.14 in diameter. Prepharynx present. Pharynx small, 0.05 in diameter. Esophagus present; intestinal ceca short, terminating at midlevel of body. Ventral sucker one-third of body length from anterior end of body, larger than oral sucker, 0.13-0.18.

Testes more or less elliptical, lying symmetrically at junction of middle and last thirds of body. Cirrus pouch muscular, behind ventral sucker. Seminal vesicle large, bipartite. Pars prostatica undifferentiated. Cirrus protrusible. Genital pore lateral, about at midlevel of ventral sucker. Ovary heart-shaped, median, in front of testes. Uterine coils compact, between genital pore and posterior end of body. Uterine eggs ovoid, yellow, 0.031–0.034 by 0.02–0.021. Vitellaria consisting of an elongated aggregation of globular follicles, lateral, extending from ventral sucker to cecal ends.

3) From lot 2, 10 adults measured (Fig. 22). Similar to lot 1. Body 0.72-1.23 by 0.28-0.44; forebody 0.28-0.44 long, 31-39% of total body length. Oral sucker 0.09-0.12 by 0.11-0.15. Pharynx 0.04-0.05 in diameter. Esophagus 0.08-0.15 long. Ventral sucker 0.09-0.13 by 0.10-0.14; sucker width ratio 1: 0.9-1.1. Testes 0.12-0.22 by 0.11-0.14. Cirrus pouch 0.12-0.22 by 0.06-0.07. Ovary usually globular, rarely 2- or 3-lobed, 0.09-0.12 by 0.08-0.14. Eggs 35-42 by 21-25  $\mu$ m in balsam.

4) In lot 3, the uterus was usually restricted to the hindbody, but in one fully gravid specimen, its loops extended anteriorly on both sides of the ventral sucker to encircle it. Eggs measured 34-38 by  $19-22 \ \mu m$  in balsam.

Discussion, Yamaguti (September 10, 1936) described A. diplorchis on the basis of lot 1 found in the intestine of *P. esocinus* from Lake Suwa. One month later, Takeuti (October 15, 1936) descrided S. kamatukae from the intestine of P. esocinus of Lake Biwa. Yamaguti (1954) listed the latter species as a synonym of the former. Lots 1-3 are closely similar to one another, and accordingly lots 2 and 3 are assigned to A. diplorchis. In lots 1-3, the oral sucker is usually slightly larger than the ventral one, the sucker width ratio being 1: 0.9-1.1. Takeuti described and figured the oral sucker (0.11-0.14 in diameter) as smaller than the ventral one (0.13-0.18 in diameter). One other difference is seen between the two species in egg size (34-42 by  $19-25 \ \mu m$  in this study versus 0.031-0.034 by 0.02-0.021 in Takeuti). The egg size (36-42 by 24-30 µm) given by Yamaguti (1936) for 1 ot 1 is larger in width than that in this study. It seems likely that he measured immature eggs as well, which are usually broader than mature ones in mounted specimens. Takeuti's figure (see Fig. 21) shows a large cirrus pouch which includes a large, bipartite seminal vesicle and presses against the ovary. Such a large cirrus pouch was observed in some of the fully gravid specimens of lot 2. Takeuti (1936) said that in S. kamatukae, the pars prostatica is not definitely formed and the spination of the cirrus is inconspicuous. He mentioned nothing about the metraterm. His original material has not yet been available to me for reexamination to confirm his description. However, the two species closely resemble each other in general morphology, dimensions, host species and locality except for the abovementioned differences. I agree with Yamaguti (1954) that they are specifically identical. Szidat (1943) transferred A. diplorchis to his new genus Palaeorchis. P. diplorchis differs from Anapalaeorchis hamajimai (this paper) chiefly in having an unlobed (not trilobed) ovary and symmetrical (not tandem) testes.

The species has been recorded only from *Pseudogobio esocinus* of Nagano, Shiga and Hiroshima Prefectures, Japan (Yamaguti, 1936; Takeuti, 1936; this paper). Its life cycle is unknown.

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