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Allogenarchopsis gen. nov. (Digenea, Derogenidae, Halipeginae) Parasitic in the Intestine of Freshwater Fishes: a Molecular and Morphological Study of Adult and Cercarial Forms

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Abstract Adults of *Genarchopsis yaritanago* Shimazu *et al.*, 2011 (Digenea, Derogenidae, Halipeginae) were found in the intestine of *Tanakia lanceolata* (Temminck and Schlegel, 1846) (type host), *Tanakia limbata* (Temminck and Schlegel, 1846) (new host record), *Rhodeus ocellatus ocellatus* (Kner, 1866) (new host record) and *Acheilognathus rhombeus* (Temminck and Schlegel, 1846) (new host record) (Cyprinidae, Acheilognathinae) from the Lake Biwa basin in Shiga Prefecture, central Japan. Daughter rediae and cercariae of *Cercaria problematica* Faust, 1924 were found in *Semisulcospira reiniana* (Brot, 1874) (Gastropoda, Sorbeoconcha, Pleuroceridae) from the same basin. The cytochrome *c* oxidase subunit I gene (COI) of the mitochondrial DNA of the adult and cercaria was partially sequenced. Morphological and molecular studies showed that *G. yaritanago* and *C. problematica* are the same species, that the original description by Shimazu *et al.* (2011) includes some errors of taxonomic importance at the generic level, and that *G. yaritanago* should be removed to another genus. *Allogenarchopsis* gen. nov. (Halipeginae) is proposed. The type species is *Allogenarchopsis yaritanago* (Shimazu *et al.*, 2011) comb. nov. The species name of the taxon is changed from *A. yaritanago* to *Allogenarchopsis problematica* (Faust, 1924) comb. nov. (syn. *C. problematica*, *Cercaria manei* Ito, 1960, *G. yaritanago*, *A. yaritanago*). The adult, daughter redia and cercaria are described and figured.

Key words: *Allogenarchopsis problematica* (Faust, 1924) comb. nov., *Cercaria problematica*, *Genarchopsis yaritanago*, COI sequences, morphology.

Introduction

Shimazu *et al.* (2011) described a new species, *Genarchopsis yaritanago* (Digenea, Derogenidae, Halipeginae), on the basis of the type series found in the intestine of *Tanakia lanceolata* (Temminck and Schlegel, 1846) (Cyprinidae, Acheilognathinae) from the Lake Biwa basin in Shiga Prefecture, central Japan. Later, additional adult specimens of *G. yaritanago* and daughter rediae and cercariae of *Cercaria problematica* Faust, 1924 were obtained from the same basin. The cytochrome *c* oxidase subunit I gene (COI) of the mitochondrial DNA of the adult and cercaria was partially sequenced, and the COI sequences were phylogenetically analyzed. Results suggested that *G. yaritanago* and *C. problematica* are the same species; and *G. yaritanago* does not belong to *Genarchopsis* Ozaki, 1925 whose type species is *Genarchopsis goppo* Ozaki, 1925. Subsequent morphological studies of the type and newly collected adults of *G. yaritanago* and cercariae of *C. problematica* proved that the original description of *G. yaritanago* by Shimazu *et al.* (2011) includes some errors of taxonomic importance at the generic level and supported the suggestion made above. In this paper, we present results of the morphological

and molecular studies of the cercaria *C. problematica* and the adult *G. yaritanago*, propose a new halipegine derogenid genus with *G. yaritanago* as the type species, and discuss the species name of the taxon.

Materials and Methods

Morphological study

Material examined. (1) Type series of *Genarchopsis yaritanago*, ex intestine of *Tanakia lanceolata* (type host): holotype (LBM 1340000079), adult, whole-mounted, irrigation canal (type locality) closely connected to Yogo River, Nishiyama, [now in Kinomoto-cho, Nagahama City], Shiga Prefecture, 27 November 2007; and 1 paratype (LBM 1340000080), adult, whole-mounted, irrigation canal, Miyake-cho, Moriyama City, Shiga Prefecture, 27 October 2000 (Shimazu *et al.*, 2011). (2) 1 adult specimen (NSMT-Pl 5854), slightly flattened, whole-mounted, ex intestine of *T. lanceolata*, irrigation canal, Nishiyama, 27 May 2009. (3) 1 adult specimen (NSMT-Pl 5855), slightly flattened, whole-mounted, ex intestine of *Tanakia limbata* (Temminck and Schlegel, 1846) (Acheilognathinae) (new host record), irrigation canal, Nishiyama, 29 July 2012. (4) Many immature and adult specimens (NSMT-Pl 5851 and 5853 and Urabe's personal collection), slightly flattened, whole-mounted, ex intestine of *Rhodeus ocellatus ocellatus* (Kner, 1866) (Acheilognathinae) (new host record), agricultural irrigation canal (new locality record) connected to Hino River, Kominami, Yasu City, Shiga Prefecture, 2 May 2011, 28 October 2011, 11 September 2012. (5) Several immature and adult specimens (NSMT-Pl 5852), hot formalin-fixed, serially sectioned or whole-mounted, ex intestine of *Acheilognathus rhombeus* (Temminck and Schlegel, 1846) (Acheilognathinae) (new host record), irrigation canal, Kominami, 11 September 2012. (6) Daughter rediae (NSMT-Pl 5856–5857), serially sectioned or whole-mounted, ex midgut gland of *Semisulcospira reiniana* (Brot, 1874) (Gastropoda, Sorbeoconcha, Pleuroceridae), irrigation canal, Kominami, 26 April 2010, 28 April 2012. (7) Cercariae (NSMT-Pl 5857), serially sectioned or whole-mounted, ex midgut gland of *S. reiniana*, irrigation canal, Kominami, 26 April 2010, 26 June 2010.

Methods. Newly collected adult specimens were mostly flattened slightly, fixed with AFA, stained with Heidenhain's iron hematoxylin and mounted in Canada balsam. Some were fixed in hot 10% formalin, serially sectioned (sagittal, 5 μm thick) and stained with hematoxylin and eosin. The type series of *G. yaritanago* was borrowed from the Lake Biwa Museum (LBM), Kusatsu, Shiga Prefecture.

Cercariae were observed alive for the excretory system. Some daughter rediae and cercariae were fixed with AFA, stained with Heidenhain's iron hematoxylin and mounted in Canada balsam. Some others were fixed in 10% formalin, serially sectioned (5 μm thick) and stained with hematoxylin and eosin.

Drawings were made with the aid of a camera lucida. Measurements (length by width) are given in millimeters unless otherwise stated. The newly collected specimens have been deposited in the National Museum of Nature and Science (NMNS, collection name code NSMT-Pl), Tsukuba, Ibaraki Prefecture; and in M. Urabe's personal collection.

Molecular study

Samples sequenced. An adult worm found in *Rhodeus ocellatus ocellatus* on 11 December 2010 and two daughter rediae found in the midgut gland of *Semisulcospira reiniana* on 6 April 2010 both from the agricultural irrigation canal at Kominami were sequenced. The whole bodies were rinsed twice with TAE buffer (40 mM Tris, 20 mM acetic acid, 1 mM EDTA; pH 8.0) and preserved at –30°C until the DNA extraction.

Genomic DNA was extracted using a Wizard® SV Genomic DNA Purification System (Promega, Madison, WI, USA).

Sequencing. A partial region (627 bp) of the COI was amplified. The primer set JB3 (5′-TTTTTTGGGCATCCTGAGGTTTAT-3′)

and COI R-Trema (5′-CAACAAATCATGATG-CAAAAGG-3′) (Miura *et al.*, 2005) was used for PCR amplification. PCR amplification was performed in 20 μL of reaction mixture containing 0.5 U *ExTaq* (TaKaRa Bio Inc., Otsu, Shiga, Japan), 10 × ExTaq Buffer (TaKaRa), dNTPs (0.2 mM each), 0.5 μL template, and 0.5 μM forward and reverse primers, using a Mycycler™ thermal cycler (Bio-Rad Laboratories, Hercules, CA, USA). Thermocycling profiles were as follows: 40 cycles of 10 s at 98℃, 30 s at 50℃, and 60 s at 72℃. The PCR products were separated on electrophoresis gels and sequenced directly (DNA sequencing services provided by FASMAC Co., Ltd., Atsugi, Kanagawa, Japan). The analyzed sequences have been deposited in DDBJ (http://ddbj.sakura.ne.jp/).

Phylogenetic analyses. Molecular phylogenetic trees were constructed together with the COI sequences of *Genarchopsis goppo* Ozaki, 1925 (West and Central Japan Groups), *Genarchopsis gigi* Yamaguti, 1939, *Genarchopsis fellicola* Shimazu, 1995 and *Cercaria longicerca* Ito, 1953 in DDBJ (Urabe *et al.*, 2012; accession nos. AB703662–AB703674). The COI sequences of *Fasciola hepatica* Linnaeus, 1758 (Fasciolidae) (Le *et al.*, 2000; accession no. AF216697) and *Fascioloides magna* (Bassi 1875) (Fasciolidae) (Kralova-Hromadova *et al.*, 2008; accession no. EF534996) were used as outgroups.

DNA sequences were aligned using ClustalW with default parameters. It was found that three bases were inserted in the sequences of both *F. hepatica* and *Fa. magna*, and so these positions were excluded from the analyses. Rooted phylogenetic trees were constructed by the maximum likelihood (ML) using the nearest neighbor interchange (NNI) search strategy, and maximum parsimony (MP) methods using the close neighbor interchange (CNI) strategy. All trees were constructed using MEGA5.0 (ver. 1.0) (Tamura *et al.*, 2011). The probabilities were tested using the bootstrap analysis of 1000 replicates. Prior to the ML method analysis, base substitution models were selected according to the values of BIC, and the HKY + G model was selected (Hasegawa *et al.*, 1985). This model was applied for the phylogenetic analysis with a default gamma parameter of 5.

Results

Allogenarchopsis gen. nov.

Diagnosis. Digenea, Derogenidae, Halipeginae. Body spindle-shaped, small; tegument smooth. Oral and ventral suckers well developed; ventral sucker a little posterior to middle of body. Prepharynx absent. Pharynx well developed. Esophagus inverted Y-shaped, small, with ventral pouch. Drüsenmagen present. Intestines forming cyclocoel near posterior extremity of body. Testes two, entire, almost symmetrical, lateral, between ventral sucker and ovary. Seminal vesicle coiled, small. Pars prostatica vesicular; prostatic cells well developed. Ejaculatory duct short, uniting with metraterm to form short hermaphroditic duct running in sinus organ and opening on its apex. Sinus sac membranous, enclosing seminal vesicle, prostatic complex, ejaculatory duct and distal part of metraterm. Sinus organ permanent, conical, small. Genital atrium tubular. Genital pore median, slightly posterior or ventral to esophagus. Ovary entire, lateral, post-testicular. Laurer's canal dilated, including sperm as rudimentary seminal receptacle, leading into Juel's organ. Juel's organ anterior to vitellaria, in dorsal parenchyma. Blind or canalicular seminal receptacle absent. Ootype submedian or rarely median; Mehlis' gland well developed, free in parenchyma. Uterus much coiled in all available field between vitellaria and sinus sac; metraterm long, folded in forebody; uterine seminal receptacle present. Eggs numerous, operculate, fully embryonated, with long, anopercular filament. Vitellaria two, entire, symmetrical or diagonal, close together near posterior extremity of body. Excretory vesicle Y-shaped, bifurcating in hindbody; arms well separated at near intestinal shoulders. Parasitic in intestine of freshwater fishes. Type and only known species: *Allogenarchopsis yaritanago* (Shimazu *et al.*, 2011) comb.

nov. (syn. *Genarchopsis yaritanago* Shimazu *et al.*, 2011).

Etymology. Allo- from the Greek *αλλος*, another, and *genarchopsis* from the generic name *Genarchopsis* of the previously established genus; feminine in gender.

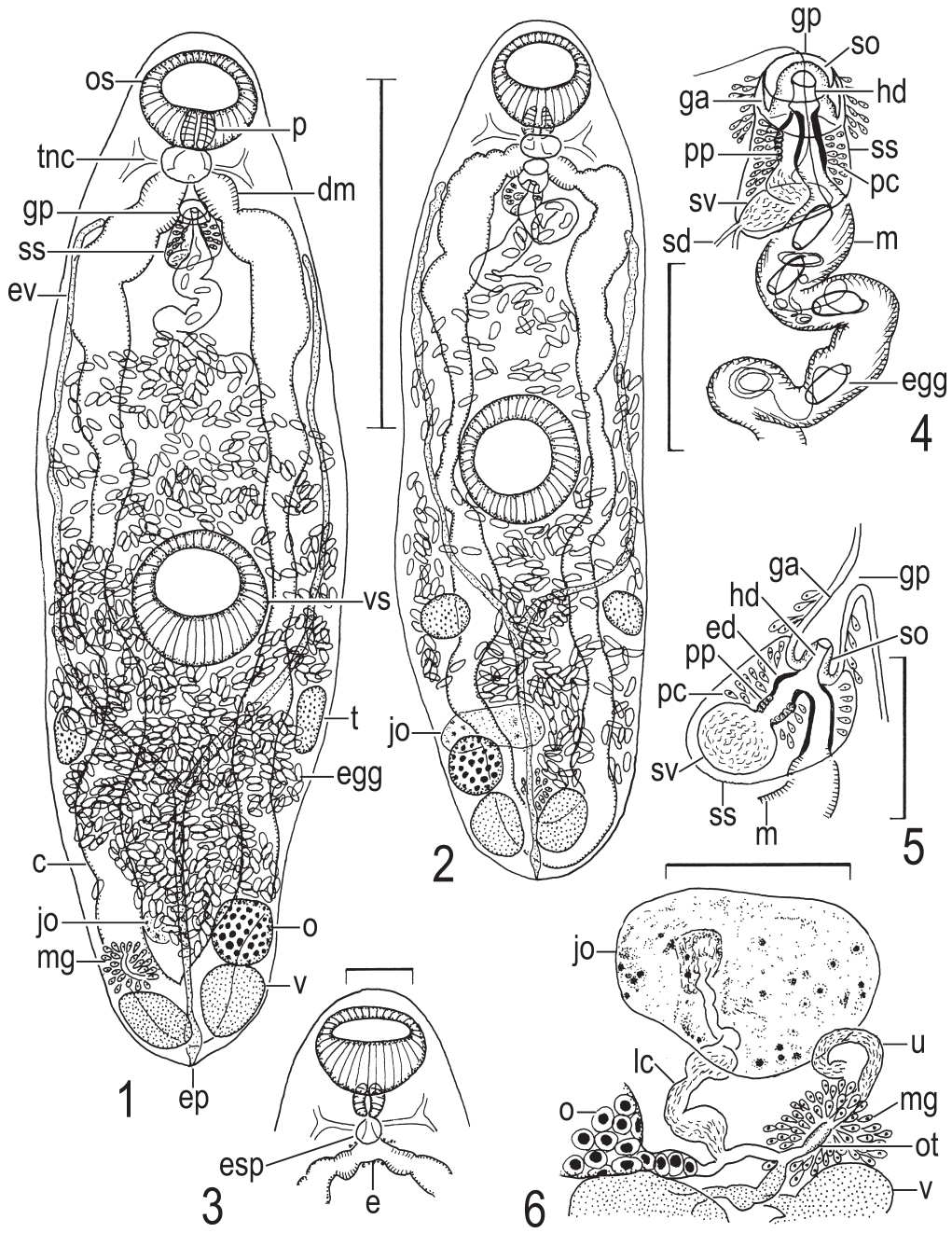
Allogenarchopsis problematica
(Faust, 1924) comb. nov.

[*Cercaria cystophora* cercaria]: Kobayashi, 1915: 52–54, pl. 2, fig. 5.
[*Cercaria cystophora* C]: Kobayashi, 1922: 268.
Cercaria problematica Faust, 1924: 294, table 1.
Cercaria manei Ito, 1960: 70–71, figs. 16–17.
Genarchopsis yaritanago Shimazu, Urabe and Grygier, 2011: 21–23, figs. 25–29.

Morphological study

Adult (Figs. 1–6).

Description. Ten adult specimens including type series measured. Body spindle-shaped, slightly flattened dorsoventrally, with bluntly pointed ends, small, 1.62–3.47 by 0.52–0.97; forebody 0.84–1.84 long, occupying 51–56% of body length; tegument smooth. Preoral lobe present. Transverse nerve commissure dorsal to esophagus. Oral sucker almost globular, 0.20–0.37 by 0.23–0.35, almost ventral, close to anterior extremity of body; sphincter at mouth aperture not developed. Prepharynx absent. Pharynx barrel-shaped, 0.08–0.12 by 0.07–0.11, posterodorsal to oral sucker. Esophagus bifurcating into inverted Y-shape, small, 0.06–0.13 by 0.06–0.10, surrounded by small gland cells; esophageal pouch oval, small, ventral to esophagus. Drüsenmagen small, present between esophageal arm and intestine on either side of body, 0.05–0.09 by 0.06–0.13. Intestines slightly undulating, distally uniting with each other to form cyclocoel overlapping vitelline glands. Ventral sucker almost globular, slightly larger than oral sucker, 0.23–0.39 by 0.26–0.45, a little posterior to middle of body; sucker width ratio 1 : 1.1–1.2; sphincter at aperture of ventral sucker not developed. Testes two, elliptical to globular, slightly irregular in outline, small, 0.11–0.22 by 0.07–0.18, symmetrical or slightly diagonal at about junction of anterior and middle thirds of hindbody, lateral to or slightly overlapping intestines. Sperm ducts long; common sperm duct absent. Seminal vesicle retort-shaped, coiled once, small. Pars prostatica vesicular, large; prostatic cells well developed. Ejaculatory duct very short, joining metraterm dorsally to form short hermaphroditic duct running in sinus organ and opening on apex of it. Sinus sac thin-walled, membranous, small, 0.06–0.11 by 0.109–0.14, median, posterior to esophagus, enclosing seminal vesicle, prostatic complex, ejaculatory duct and distal part of metraterm. Sinus organ permanent, muscular, cone-shaped, small, 0.02–0.05 by 0.03–0.06, projecting into genital atrium. Genital atrium cylindrical, thick-walled, 0.05–0.10 by 0.06–0.10, surrounded by small gland cells. Genital pore wide, median, slightly posterior or ventral to esophagus. Ovary almost globular, larger than testes, 0.16–0.26 by 0.14–0.22, dextrally or sinistrally lateral, post-testicular, usually anterior to vitellaria or slightly overlapping them. Oviduct fairly long; ovovitelline duct short. Laurer's canal dilated, sinuous, including sperm, running forward, leading into Juel's organ, may be connected to inner vesicle of Juel's organ. Juel's organ large, 0.14–0.16 by 0.14–0.31 in 3 specimens (not clearly observed in others), in dorsal parenchyma, anteromedial to ovary and ootype. Ootype usually submedian but rarely median, opposite to ovary, anterior to or overlapping vitelline gland; Mehlis' gland well developed, free in parenchyma. Uterus much coiled in all available space of body between vitellaria and metraterm or sinus sac; metraterm well developed, much longer than sinus sac, folded between middle of forebody and sinus sac, leading into sinus sac, with well-developed sphincter at its anterior end; uterine seminal receptacle well developed in proximal coils of uterus. Eggs numerous, elongate-oblong, somewhat curved, operculate, brown, fully embryonated, 56–64 by 25–32 μm; long anopercular filament present (measuring up to 1.16 long in 1 laid egg). Vitellaria two, elliptical, large, 0.16–0.30 by 0.10–



Figs. 1–6. *Allogenarchopsis problematica*, adults, ex intestine of acheilognathine fishes. — 1, holotype of *Genarchopsis yaritanago* (LBM 1340000079), ex *Tanakia lanceolata*, entire body, ventral view; 2, NSMT-Pl 5851, ex *Rhodeus ocellatus ocellatus*, entire body, ventral view; 3, NSMT-Pl 5851, anterior part of the digestive system, showing inverted Y-shaped esophagus (e) and esophageal pouch (esp), ventral view; 4, LBM 1340000079, terminal genitalia, ventral view; 5, NSMT-Pl 5852, ex *Acheilognathus rhombeus*, terminal genitalia, reconstructed from several sagittal sections, lateral view; 6, NSMT-Pl 5851, ovarian complex and Juel's organ, ventral view. Scale bars: 1 mm in Figs. 1–2; 0.2 mm in Figs. 3–4 and 6; 0.1 mm in Fig. 5.

0.20, symmetrical or diagonal, separate, close to posterior extremity of body. Vitelline ducts very short; common vitelline duct short. Excretory vesicle Y-shaped, ascending in ventral parenchyma along median line of body, bifurcating at about testicular level; arms lateral, running forward, terminating at near intestinal shoulders, well separated there; excretory pore posterotermINal.

Daughter redia (Fig. 7).

Description. Based on 8 slightly flattened specimens. Body cylindrical, rounded at anterior end, tapering posteriorly, 1.4–2.3 by 0.4–0.7. Pharynx globular, small, 0.04–0.06 by 0.04–0.06. Esophagus very short. Intestine long, 0.50–0.96 by 0.09–0.15. Birth pore beside pharynx. Containing 7–18 cercariae and germ balls.

Cercaria (Figs. 8–11).

Description. Based on 12 slightly flattened specimens. Body proper similar to adult in general morphology (Fig. 9), 0.38–0.64 by 0.18–0.28, outside tail in immature cercariae (Figs. 7, 8A, 10) but withdrawn into caudal cavity of tail in fully developed stage (Fig. 8B). Forebody occupying 57–69% of total body length. Oral sucker 0.08–0.10 by 0.09–0.12. Pharynx barrel-shaped, 0.04–0.05 by 0.03–0.04. Esophageal pouch distinct, round, posteroventral of pharynx. Intestines forming cyclocoel. Ventral sucker 0.09–0.11 by 0.09–0.13, located at about junction between second and posterior thirds of body. Two testes, ovary, ootype and terminal genitalia weakly differentiated. Excretory vesicle Y-shaped, bifurcating between cyclocoel and ventral sucker; arms lateral, running forward to near intestinal shoulders, not fused anteriorly (Figs. 9–11); two pairs of flame cells and two collecting canals seen on either side of body proper (Fig. 11).

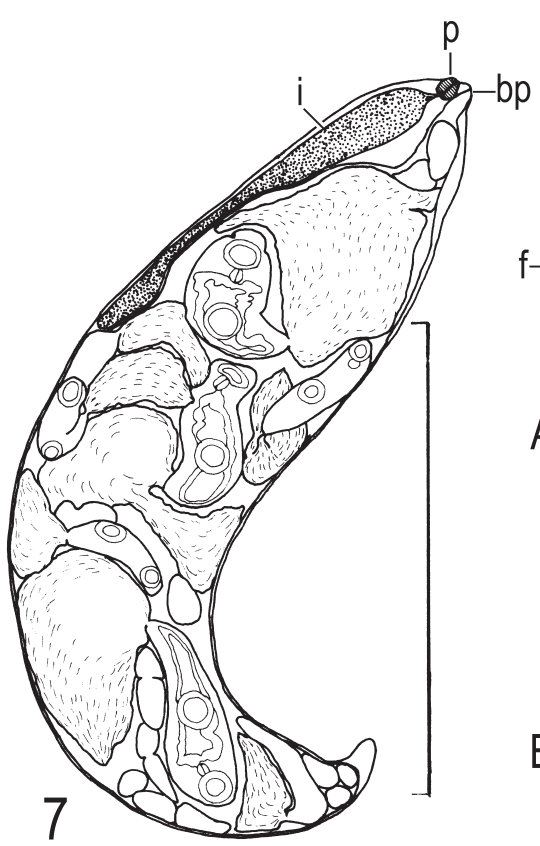
Tail drop-shaped, huge, 0.76–1.10 by 0.41–0.66, with several blunt projections (Fig. 8: pt) in places on lateral sides, sometimes colored black. Delivery tube absent. Caudal cavity globular, present in anterior part of tail, containing retreated cercarial body proper in fully developed cercariae (Fig. 8B). Ten to sixteen tapelike filaments present on tail surface, 0.38–0.64 by 0.02–0.03 (Fig. 8: f). In immature cercariae, primary caudal excretory canal (Fig. 10: pcec, our terminology) running backward, opening as single primary excretory pore on ventral margin of tail near posterior tip of tail (Fig. 10: pep). In fully developed cercariae, secondary caudal excretory canal (Fig. 11: scec, our terminology) arising from proximal part of primary caudal excretory canal, short, anterior to caudal cavity, flowing into it; one pair of flame cells present on either side of tail; two collecting canals may connect to primary caudal excretory canal immediately posterior to secondary caudal excretory canal (Figs. 10–11). Flame cell formula 2[(2) + (2) + (2)] = 12 (Fig. 13).

Molecular study

The 627-bp sequences of the COI (accession no. AB828006) were identical between the adult of *G. yaritanago* and the daughter redia of *C. problematica*, which is shown as only *Genarochopsis yaritanago* in Figs. 12–13.

The topology of MP and ML trees was identical within the clade consisted of *G. goppo*, *G. gigi, G. fellicola* and *C. longicerca*, except for small differences (Figs. 12–13) (see also Urabe *et al.*, 2012). The clade of *G. yaritanago* was situated outside the above-mentioned clade. The DNA sequence divergences between *G. yaritanago* and the other *Genarchopsis* species and *C. longicerca* were 16.9–20.4% (uncollected p-distance).

Figs. 7–11. *Allogenarchopsis problematica*, daughter redia and cercariae, ex midgut gland of *Semisulcospira reiniana*. — 7, daughter redia, lateral view; 8, entire cercariae, body proper outside tail in immature stage (A), withdrawn in caudal cavity of tail in fully developed stage (B); 9, body proper of cercaria, ventral view; 10, excretory system in immature cercaria, lateral view, freehand sketch, scale unknown; 11, excretory system in fully developed cercaria, just before body proper being withdrawn into caudal cavity, ventral view, freehand sketch, scale unknown. Scale bars: 1 mm in Figs. 7–8; 0.2 mm in Fig. 9.



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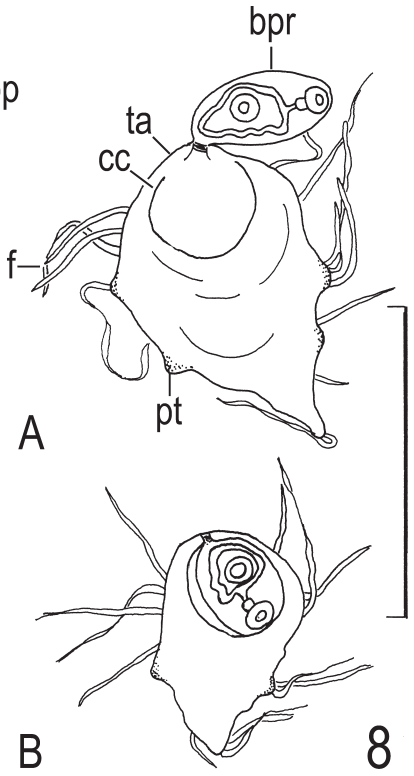

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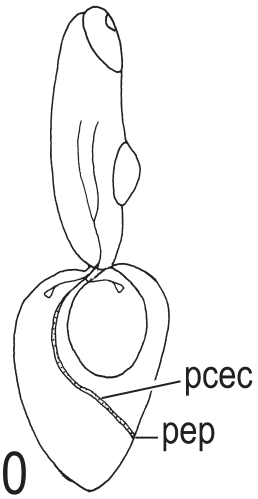

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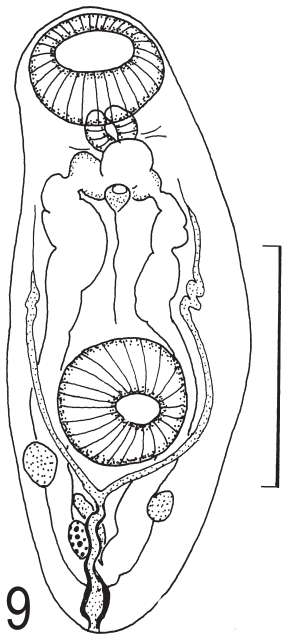

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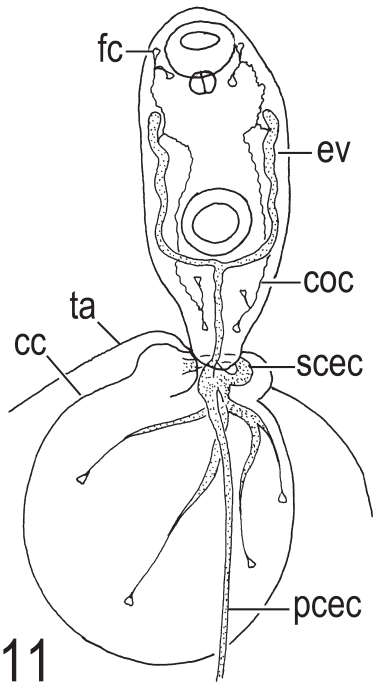

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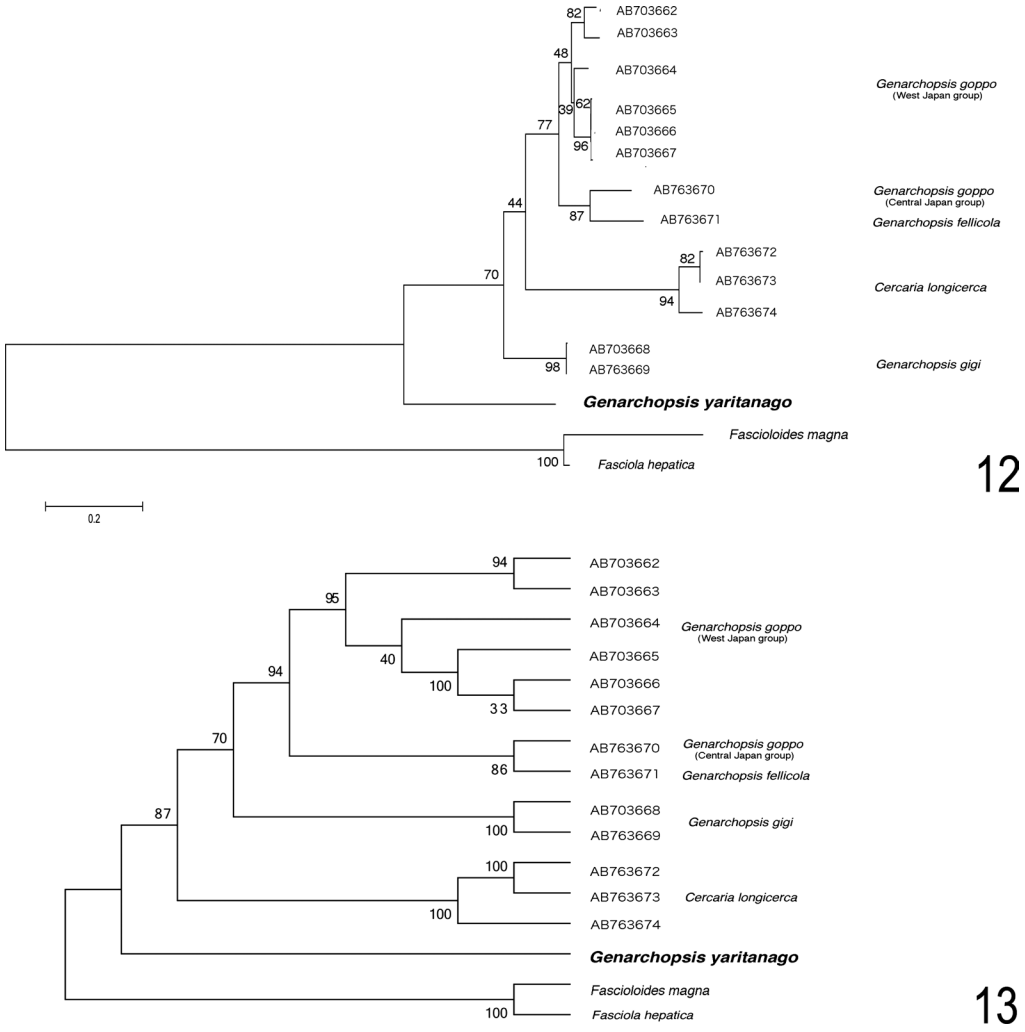
Discussion

The present newly collected adult specimens are identified as *Genarchopsis yaritanago*, because they were very similar to the type series of this species in morphology and measurements.

The present cercaria is identified as *Cercaria problematica* Faust, 1924, because it closely resembled *C. problematica* as described by Kobayashi (1915) in the morphology of the [daughter] redia (Fig. 7) and cercaria (Figs. 8–9, 11). Kobayashi (1915) first briefly described this cercaria as [*Cercaria cystophora* cercaria] with a cyclocoel, which was produced in a [most likely daughter] redia in *Semisulcospira libertina* (Gould, 1859) (syn. *Melania libertina* Gould, 1859) from several places in Japan. He erroneously presumed that this was the cercaria of *Leptolecithum eurytremum* Kobayashi, 1915, or now *Isoparorchis hypselobagri* (Billet, 1898) Ejsmont, 1932 (see Shimazu *et al.*, 2011), with the blindly ending intestines (or ceca), saying that the cyclocoel in the cercarial stage might separate into two ceca in the adult stage. The cercaria appeared as [*Cercaria cystophora* C] in Kobayashi (1922). Faust (1924) gave a new name, *Cercaria problematica*, to this latter cercaria. Later, Ito (1960) described a new cercaria, *Cercaria manei*, produced in a [most likely daughter] redia in *S. libertina* from Iwata City, Shizuoka Prefecture, Japan. We regard *C. manei* as a synonym of *C. problematica*, because these two agree well in the morphology of the [most likely daughter] redia and cercaria and in the host snail. Ishii in Ueno *et al.* (1930) briefly described a similar cystophorous cercaria, Cercaria F, from *S. libertina* collected in Kumamoto Prefecture, Japan. This cercaria is said to have the blind intestines (fig. 21) instead of the cyclocoel in *C. problematica*. This form requires reexamination.

The present morphological study of the adult specimens shows that the original description by Shimazu *et al.* (2011, figs. 25–29) for this species includes some errors. These are: (1) feature 1: the sinus organ is cone-shaped and small (Figs. 1–2, 4–5) instead of cylindrical and large (figs. 25, 27–28); (2) feature 2: Juel's organ is present (Figs. 1–2, 6), but this organ was not mentioned by them; (3) feature 3: Laurer's canal leads into Juel's organ (Fig. 6), but the distal part of Laurer's canal was not clearly observed by them; (4) feature 4: Mehlis' gland is free in the parenchyma (Figs. 1–2, 6) instead of enclosed in the ootype pouch (figs. 25, 29); (5) feature 5: the excretory arms are well separated near the intestinal shoulders (Figs. 1–2) instead of united dorsally to the pharynx or oral sucker (figs. 25–26). Feature 5 was also observed in the present cercaria (Figs. 9–11). We consider that at least features 2, 3 and 5 are of taxonomic importance at the generic level and that a combination of them excludes *G. yaritanago* from *Genarchopsis*. In the type species *G. goppo*, Juel's organ is absent, Laurer's canal opens dorsally to the exterior, and the excretory arms are united dorsally to the pharynx or esophagus (Ozaki, 1924; Shimazu, 1995; Shimazu *et al.*, 2011). Since the type species *G. goppo* does not have Juel's organ, we disagree with Gibson (2002) that *Genarchopsis* is composed of both species with Juel's organ and those without this organ. Furthermore, the present taxon does not fit any of the other known genera in the Derogenidae Nicoll, 1910 sensu Gibson (2002). It is not known at present that features 1 and 4 are also taxonomically important at the generic level. Species of *Genarchopsis* from Japan have a cylindrical large sinus organ and the ootype pouch surrounding Mehlis' gland (Shimazu, 1995; Shimazu *et al.*, 2011). However, neither the terminal genitalia nor the ovarian complex have been studied in detail in species from East, Southeast and South Asia except Japan.

The present molecular study indicates that the adult *G. yaritanago* and the cercaria *C. problematica* belong to the same species (Figs. 12–13). Accordingly, *C. problematica* is the cercaria of *G. yaritanago*. This is morphologically supported in the finding that the excretory arms were separated anteriorly in both adult and cercaria (Figs. 1–2, 9–11). The divergences in the COI sequences between *G. yariatanago* and the other



Figs. 12–13. Molecular phylogenetic trees of *Genarchopsis yaritanago* (or *Cercaria problematica*, or now *Allogenarchopsis problematica*), other *Genarchopsis* species, and *Cercaria longicerca* from Japan, based on 627-bp sequences of the COI, with *Fasciola hepatica* and *Fascioloides magna* as outgroups. — 12, maximum likelihood tree; 13, maximum parsimony tree.

Genarchopsis species and *C. longicerca* were 16.9–20.4% (uncollected p-distance). Congeneric interspecific divergences in the COI sequences have been reported in a few digenean genera: 8.9–26.5% for *Paragonimus* Braun, 1899 (Blair *et al.*, 1997), 9.6–12.8% for *Ichthyocotylurus* Odening, 1969 (Bell *et al.*, 2001) and 9.9–15.1% for *Diplostomum* von Nordmann, 1832 (Locke *et al.*, 2010). The p-distance in the present study is near the maximum, or out of it, of the range of these results. Therefore, the present molecular study (Figs. 12–13) also excludes *G. yaritanago* from *Genarchopsis*. This agrees with the conclusion made above, based on the results of the present morphological study, that *G. yaritanago* is incorrectly assigned to *Genarchopsis*.

We observed the present cercaria in more detail than has previously been reported. The cercaria differs from that of *G. goppo* as described by Urabe (2001) mainly in that the intestines have already formed a cyclocoel instead of ending blindly, the excretory arms are anteriorly sep-

arated instead of united, the tail is drop-shaped instead of elongate and long, the tail possesses ten to sixteen tapelike filaments, and the delivery tube is absent. The primary caudal excretory canal opens to the outside through only the primary excretory pore on the ventral margin near the posterior tip of the tail in immature cercariae (Fig. 12: pcec, pep). In fully developed cercariae, in addition, the primary caudal excretory canal gives off a unique secondary caudal excretory canal, which is short and anterior to the caudal cavity and flows into the caudal cavity (Fig. 11: scec).

As the result of the above discussion, we propose a new monotypic genus, *Allogenarchopsis* gen. nov., to accommodate *G. yaritanago*, or now *Allogenarchopsis yaritanago* (Shimazu *et al.*, 2011) comb. nov., as the type species in the Derogenidae. This new genus is distinctively characterized by that Juel's organ is present (feature 1), Laurer's canal leads into Juel's organ (feature 2), and the excretory arms are well separated near the intestinal shoulders (feature 5) in the family. It is placed in the Halipeginae Poche, 1926, because the sinus sac includes the seminal vesicle, prostatic complex, ejaculatory duct and distal part of the metraterm.

Since *G. yaritanago* Shimazu *et al.*, 2011 and *C. problematica* Faust, 1924 are the same species as discussed above, *G. yaritanago* is a junior synonym of *C. problematica*. The specific name *problematica* has priority over the junior specific name *yaritanago* in accordance with Article 23.3.2.2 of the International Code of Zoological Nomenclature (ICZN) (International Commission on Zoological Nomenclature, 1999). Consequently, the species name of the present taxon is changed from *A. yaritanago* to *Allogenarchopsis problematica* (Faust, 1924) comb. nov. (syn. *C. problematica*, *C. manei*, *G. yaritanago*, *A. yaritanago*).

The presence or absence of Juel's organ is considered to be taxonomically important to separate genera as discussed above. This organ is also present in *Genarchopsis punctati* Agrawal, 1966 found in the stomach of *Ophicephalus punctatus* Bloch, 1793 (Channidae) from India (Agrawal, 1966: figs. 1–4; Anjaneyulu, 1967: figs. 1–2, s; Madhavi and Rao, 1974: fig. 9, PL). We do not consider that such a species with Juel's organ is a member of *Genarchopsis*. However, it is not known at present whether it is also allocated in the present new genus. It is said to have the seminal vesicle free in the parenchyma (Agrawal, 1966), the prostatic complex enclosed in a thin-walled sac (Agrawal, 1966), Mehlis' gland enclosed in a sac (Agrawal, 1966) but free in the parenchyma (Anjaneyulu, 1967; Madhavi and Rao, 1974), the uterus [most likely the metraterm interpreted in this paper] terminally receiving the ejaculatory duct to form a short hermaphroditic duct in a cone-shaped projection [the sinus organ interpreted in this paper] (Anjaneyulu, 1967: fig. 1), and the anteriorly united excretory arms (Agrawal, 1966). If the last feature is correctly described, *G. punctati* does not belong to the present new genus. Careful reexamination of this form is needed.

Abbreviations used in the figures. bp, birth pore; bpr, body proper; c, cyclocoel; cc, caudal cavity; coc, collecting canal; dm, Drüsenmagen; e, esophagus; ed, ejaculatory duct; egg, eggs in uterus; ep, excretory pore; esp, esophageal pouch; ev, excretory vesicle; f, filament of tail; fc, flame cell; ga, genital atrium; gp, genital pore; hd, hermaphroditic duct; i, intestine; jo, Juel's organ; lc, Laurer's canal; m, metraterm; mg, Mehlis' gland; o, ovary; os, oral sucker; ot, ootype; p, pharynx; pc, prostatic cells; pcec, primary caudal excretory canal; pep, primary excretory pore; pp, pars prostatica; pt, projection of tail; scec, secondary caudal excretory canal; sd, sperm duct; so, sinus organ; ss, sinus sac; sv, seminal vesicle; t, testis; ta, tail; tnc, transverse nerve commissure; u, uterus; v, vitellarium; vs, ventral sucker.

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References

Agrawal, V. 1966. Studies on some trematode parasites of fresh water fishes from Lucknow. Annales de Parasitologie (Paris), 41: 217–231.

Anjaneyulu, G. 1967. Observations on the female reproductive system of *Genarchopsis punctati* Agrawal, 1966. Indian Journal of Helminthology, 19: 118–121.

Bell, A. S., C. Sommerville and E. T. Valtonen 2001. A molecular phylogeny of the genus *Ichthyocotylurus* (Digenea, Strigeidae). International Journal for Parasitology, 31: 833–842.

Blair, D., T. Agatsuma, T. Watanobe, M. Okamoto and A. Ito 1997. Geographical genetic structure within the human lung fluke, *Paragonimus wastermani*, detected from DNA sequences. Parasitology, 115: 411–417.

Faust, E. C. 1924. Notes on larval flukes from China. II. Studies on some larval flukes from the central and south coast provinces of China. American Journal of Hygience, 4: 241–301.

Gibson, D. I. 2002. Family Derogenidae Nicoll, 1910. In Gibson, D. I., A. Jones and R. A. Bray (eds.): Keys to the Trematoda, 1, pp. 351–368. CAB International and The Natural History Museum, Wallingford, UK.

Hasegawa, M., H. Kishino and T. Yano 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. Journal of Molecular Evolution, 22: 160–174.

International Commission on Zoological Nomenclature 1999. International Code of Zoological Nomenclature. Fourth edition. xxix + 306 pp. International Trust for Zoological Nomenclature, London.

Ishii, K. 1930. [Nine cercariae and their rediae parasitic in *Semisulcospira libertina*] In Ueno, N., K. Ishii and H. Abe (eds.): [On cercariae parasitic in freshwater snails in the neighborhood of Kumamoto], pp. 970–975, pl. 2, figs. 11–28. Kumamoto Igakkai Zasshi, Kumamoto. (In Japanese.)

Ito, J. 1960. Contributions to the morphology of cercariae obtained from a snail host, *Semisulcospira libertina* in Japan. Japanese Journal of Medical Science & Biology, 13: 59–72.

Kobayashi, H. 1915. [Studies of endoparasitic trematodes from Japan (2).] Dobutsugaku Zasshi, 27: 50–57, pl. 2. (In Japanese.)

Kobayashi, H. 1922. [A review of Japanese cercariae.] Dobutsugaku Zasshi, 34: 252–270. (In Japanese.)

Kralova-Hromadova, I., M. Špakulová, E. Horáčková, L. Turčeková, A. Novobilský, R. Beck, B. Koudela, A. Marinculić, D. Rajský and M. Pybus 2008. Sequence analysis of ribosomal and mitochondrial genes of the giant liver fluke *Fascioloides magna* (Trematoda: Fasciolidae): Intraspecific variation and differentiation from *Fasciola hepatica*. Journal of Parasitology, 94: 58–67.

Le, T. H., D. Blair, T. Agatsuma, P. F. Humair, N. J. Campbell, M. Iwagami, D. T. Littlewood, B. Peacock, D. A. Johnston, J. Bartley, D. Rollinson, E. A. Herniou, D. S. Zarlenga and D. P. McManus 2000. Phylogenies inferred from mitochondrial gene orders—a cautionary tale from the parasitic flatworms. Molecular Biology and Evolution, 17: 1123–1125.

Locke, A. S., J. D. McLaughlin, S. Dayanandan and D. Marcogliese 2010. Diversity and specificity in *Diplostomum* spp. metacercariae in freshwater fishes revealed by cytochrome oxidase I and internal transcribed spacer sequences. International Journal for Parasitology, 40: 333–343.

Madhavi, R. and K. H. Rao 1974. Anatomy of female reproductive system in digenetic trematodes. Part II. Hemiuroidea. Rivista di Parassitologia, 35: 23–36.

Miura, O., A. M. Kuris, M. E. Torchin, R. F. Hechinger, E. J. Dunham and S. Chiba 2005. Molecular-genetic analysis reveal cryptic species of trematodes in the intertidal gastropod, *Batillaria cumingi* (Cross). International Journal for Parasitology, 35: 793–801.

Ozaki, Y. 1925. On a new genus of fish trematodes, *Genarchopsis*, and a new species of *Asymphylodora*. Japanese Journal of Zoology, 1: 101–108.

Shimazu, T. 1995. Trematodes of the genus *Genarchopsis* (Digenea, Derogenidae, Halipeginae) from freshwater fishes of Japan. Proceedings of the Japanese Society of Systematic Zoology, (54): 1–18.

Shimazu, T., M. Urabe and M. J. Grygier 2011. Digeneans (Trematoda) parasitic in freshwater fishes (Osteichthyes) of the Lake Biwa basin in Shiga Prefecture, central Honshu, Japan. National Museum of Nature and Science Monographs, No. 43, 105 pp.

Urabe, M. 2001. Life cycle of *Genarchopsis goppo* (Trematoda: Derogenidae) from Nara, Japan. Journal of Parasitology, 87: 1404–1408.

Urabe, M., T. Nishimura and T. Shimazu 2012. Taxonomic revision of three species of the genus *Genarchopsis* (Digenea: Hemiuroidea: Derogenidae) in Japan by molecular phylogenetic analyses. Parasitology International, 61: 554–560.

Tamura, K., D. Peterson, N. Peterson, G. Steche, M. Nei

and S. Kumar 2011. MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. Molecular Biology and Evolution. doi: 10.1093/molbev/msr121.