

Revision of *Isoparorchis* Southwell, 1913 (Digenea, Hemiuroidea, Isoparorchidae), Parasites of the Air Bladder of Freshwater Catfishes: a Molecular and Morphological Study

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(Received 5 November 2013; accepted 18 December 2013)

Abstract *Isoparorchis* Southwell, 1913 (Digenea, Hemiuroidea, Isoparorchidae), parasites of the air bladder of freshwater catfishes (Osteichthyes, Siluriformes) from East, Southeast and South Asia and Australia, is revised based on a molecular and morphological study. Materials, including syntypes where available, were examined from Russia (Primorskiy Kray), Japan, Vietnam, Cambodia, Bangladesh, India and Australia. The entire second internal transcribed spacer region of the ribosomal DNA (ITS2 rDNA) was sequenced for 18 distinct samples. Four of the five previously described species are considered to be valid: *Isoparorchis trisimilitubis* Southwell, 1913 (type species) from India, *I. hypselobagri* (Billet, 1898) from Vietnam, *I. eurytremum* (Kobayashi, 1915) from Japan and Russia and *I. tandani* Johnston, 1927 from Australia. *Isoparorchis pakistani* Bilquees and Khatoon, 1972 from Pakistan is regarded as a *species inquirenda*. Not only syntypes of *I. trisimilitubis* but also other materials from India and Bangladesh consisted of specimens of two morphologically distinct species. One is identified as *I. trisimilitubis*, and the other remains an undescribed species, *Isoparorchis* sp. 3. Distinctions in the ITS2 sequences obtained for samples from India support the interpretation that there are two species there. One sequence is related to *I. trisimilitubis*, but it remains undetermined whether the other sequence (*Isoparorchis* sp. 1) relates to *Isoparorchis* sp. 3. Some materials from East, Southeast and South Asia require further critical studying for definitive species identification. Each species is described and figured with a summarized life cycle where known. Molecular data are given. A key to the species is presented.

Key words: *Isoparorchis*, Digenea, revision, taxonomy, morphology, life cycle, sequences of ITS2 rDNA.

Introduction

Species of *Isoparorchis* Southwell, 1913 (Digenea, Hemiuroidea, Isoparorchidae) are parasitic in the air bladder of freshwater catfishes

(Osteichthyes, Siluriformes) in Asia and Australia (Gibson, 2002; this paper).

Billet (1898) described a new species, *Distomum hypselobagri*, from the fish “*Hypselobagrus*” (Siluriformes, Bagridae) collected in

Vietnam. Southwell (1913) established a new genus, *Isoparorchis*, with a new species, *Isoparorchis trisimilitubis*, as the type species from *Wallago attu* (Bloch and Schneider, 1801) (Siluriformes, Siluridae) collected in India. Kobayashi (1915a) created a new genus and species, *Leptolecithum eurytremum*, from *Silurus asotus* Linnaeus, 1758 (syn. *Parasilurus asotus* (Linnaeus, 1758)) (Siluridae) collected in Japan. Travassos (1922a, b) recognized *Leptolecithum* as synonymous with *Isoparorchis* and placed *L. eurytremum* in the latter genus as *Isoparorchis eurytremum* (Kobayashi, 1915) (see Shimazu *et al.*, 2011). Bhalerao (1926) considered that *I. trisimilitubis* and *I. eurytremum* are the same species. Johnston (1927) proposed a new species, *Isoparorchis tandani*, from *Tandanus tandanus* (Mitchell, 1838) (Siluriformes, Plotosidae) collected in Australia. Ejsmont (1932) transferred *D. hypselobagri* to *Isoparorchis* as *Isoparorchis hypselobagri* (Billet, 1898) and synonymized *I. trisimilitubis*, *I. eurytremum* and *I. tandani* with *I. hypselobagri* without reexamining any of the type materials. Bilquees and Khatoon (1972) added a new species, *Isoparorchis pakistani*, from *Wallago attu* collected in Pakistan. Bhutta and Khan (1975) and Zaidi and Khan (1977) reduced this species to a synonym of *I. hypselobagri*. It has thus far been believed that *I. hypselobagri* alone is valid in the genus with *I. trisimilitubis*, *I. eurytremum*, *I. tandani* and *I. pakistani* as its synonyms.

Shimazu *et al.* (2011) gave a full account of the morphology of material from Japan under the species name of *I. hypselobagri*. They pointed out that the sinus organ was much smaller in their material than in the specimens of *I. eurytremum* described by Odhner (1927) and of *Isoparorchis* described by Gibson and Bray (1979). The sinus organ is actually much smaller in their material than in those of *I. trisimilitubis* and *I. tandani* as originally described by Southwell (1913) and Johnston (1927), respectively. We thus decided to make a molecular and morphological study of materials of *Isoparorchis* as broad a range of collections as possible to revise the genus. In this

paper, we present the results attained and summarize the life cycle where known.

Materials and Methods

Molecular study

Samples sequenced. Table 1 lists samples for which the entire second internal transcribed spacer region of the ribosomal DNA (ITS2 rDNA) was sequenced and GenBank accession numbers of their ITS2 sequences.

Molecular sample processing. Total genomic DNA was extracted from ethanol preserved worms by the extraction method using a QIAgen DNeasy Kit (QIAGEN INC., Valencia, California) according to the manufacturer's instructions.

The ITS2 rDNA was amplified via Polymerase Chain Reaction (PCR) amplifications (20 μ l) using 1.6 μ l of MgCl₂ (25 mM), 2 μ l of PCR reaction buffer (PROMEGA) (10x), 0.8 μ l of dNTPs (5 mM), 0.75 μ l each of forward primer 3S (5'-GGT ACC GGT TCA CGT GGC TAG TG-3') and reverse primer, ITS2.2 (5'-CCT GGT TAG TTT CTT TTC CTC CGC-3') (10 pmol), 0.25 μ l Taq polymerase (PROMEGA, Madison, USA) (5 units/ μ l), 1–2 μ l of DNA template (5–100 ng), made up to 20 μ l with millipore H₂O and run on a Minicycler (MJ Research, supplied by Bresatec, Watertown, USA). The following thermocycling conditions were used: 4 min denaturation hold at 95°C, 2 min at 45°C, 90 sec at 72°C, 4 cycles of 45 sec at 95°C, 45 sec at 50°C and 90 sec at 72°C; then 25 cycles of 20 sec at 95°C, 20 sec at 52°C and 90 sec at 72°C and 5 min extension hold at 72°C.

Amplified DNA was purified using QIAGEN® QIAquick™ PCR purification kit according to manufacturer's protocol. Cycle sequencing was conducted using the same primers utilized for PCR amplification on the purified DNA products at the Australian Genome Research Facility (AGRF) in Brisbane, Australia. The resulting sequences were edited and contigs constructed using Geneious Pro™ version 5.4 software (Biomatters Ltd.). They have been registered at GenBank (Table 1).

Table 1. Samples of the species of *Isoparorchis* used for sequencing the ITS2 rDNA and GenBank accession numbers of their associated sequences.

Sample, species, developmental stage, site of infection, host, locality and date		GenBank accession no.
1) ISO11	— <i>I. hypselobagri</i> , adult, air bladder, <i>Silurus asotus</i> , Ninh Co River, Vietnam, 10 May 2011	KJ188114
2) ISO22	— <i>I. hypselobagri</i> , adult, air bladder, <i>S. asotus</i> , Hong River, Vietnam, 23–25 November 2011	KJ188115
3) ISO2	— <i>I. eurytremum</i> , juvenile, body cavity, <i>Gnathopogon elongatus elongatus</i> , near Daido River, Lake Biwa basin, Japan, 16 October 2010	KJ188116
4) ISO33	— <i>I. eurytremum</i> , juvenile, body cavity, <i>Sarcocheilichthys variegatus microoculus</i> , Lake Biwa, Japan, 14 December 2011	KJ188117
5) ISO3	— <i>I. eurytremum</i> , adult, air bladder, <i>S. asotus</i> , Lake Biwa, Japan, 30 November 2010	KJ188118
6) ISO4	— <i>I. eurytremum</i> , adult, air bladder, <i>S. asotus</i> , Lake Biwa, Japan, 30 November 2010	KJ188119
7) RUS1262	— <i>I. eurytremum</i> , juvenile, wall of esophagus, <i>Huso dauricus</i> , Amur River, Primorskiy Kray, Russia, 7 September 2010	KJ188120
8) RUS1297	— <i>I. eurytremum</i> , juvenile, wall of esophagus, <i>Lota lota</i> , Amur River, Primorskiy Kray, Russia, 11 September 2010	KJ188121
9) RUS1385	— <i>I. eurytremum</i> , adult, air bladder, <i>Silurus soldatovi</i> , Amur River, Primorskiy Kray, Russia, 25 September 2010	KJ188122
10) RUS1712	— <i>I. eurytremum</i> , adult, air bladder, <i>S. asotus</i> , Lake Khanka, Primorskiy Kray, Russia, 28 July 2011	KJ188123
11) IND375*	— <i>Isoparorchis</i> sp. 1, juvenile, body cavity, <i>Ompok pabo</i> , Mukutmanipur dam lake, West Bengal, India, 18 October 2010	KJ188124
12) ISO1	— <i>Isoparorchis</i> sp. 1, juvenile, body cavity, <i>Mastacembelus favus</i> , India, date unknown	KJ188125
13) 55A†	— <i>I. trisimilitubis</i> , adult, air bladder, <i>Wallago attu</i> , Rishra near Kolkata, West Bengal, India, 15 November 2007	KJ188126
14) 61A†	— <i>I. trisimilitubis</i> , adult, air bladder, <i>W. attu</i> , Balurghat, West Bengal, India, 13 December 2007	KJ188127
15) ISO44†	— <i>I. trisimilitubis</i> , adult, air bladder, <i>W. attu</i> , Balurghat, West Bengal, India, 9 December 2007	KJ188128
16) ISO5†	— <i>I. trisimilitubis</i> , adult, air bladder, <i>W. attu</i> , Balurghat, West Bengal, India, 9 December 2007	KJ188129
17) VNT353#	— <i>Isoparorchis</i> sp. 2, juvenile, body cavity, <i>W. attu</i> , Phnom Penh-market, Cambodia, 18 October 2010	KJ188130
18) ISOAU1	— <i>I. tandani</i> , adult, air bladder, <i>Tandanus tandanus</i> , Moggill Creek, Brisbane, Australia, 20 August 2012	KJ188131

*Collected by T. Scholz and A. Ash; † collected by A. Ash; # collected by T. Scholz and R. Kuchta.

Comparative molecular analysis. The ITS2 data generated were aligned using MUSCLE version 3.7 (Edgar, 2004) with ClustalW sequence weighting and UPGMA clustering for iterations 1 and 2. The resultant alignments were refined by eye using MESQUITE v. 2.75 (Maddison and Maddison, 2009). After alignment of the ITS2 dataset was edited, the ends of each fragment were trimmed to match the shortest sequence in the alignment. Total nucleotide distance matrices were calculated using MEGA v.5 software (Tamura *et al.*, 2011).

Minimum evolution analysis was conducted on the ITS2 dataset using MEGA v.5 software. Nodal support for the minimum evolution analysis

of the ITS2 dataset was inferred by bootstrap analysis using a heuristic search of 10,000 replicates.

Morphological study

Material examined. Specimens examined are listed in *Material examined* of each species. They had been prepared by various methods, either whole-mounted or serially sectioned. Some were borrowed from the National Museum of Nature and Science (NMNS, collection name code NSMT-PI), Tokyo then, Japan; the Meguro Parasitological Museum (MPM), Tokyo, Japan; the Swedish Museum of Natural History (SMNH), Stockholm, Sweden; the National Museum of

Natural History (MNHN), Paris, France; the Department of Life Sciences, The Natural History Museum (NHM, collection name code NHMUK), London, UK; the South Australian Museum (SAM, collection name code AHC), Adelaide, Australia; and the Institute of Parasitology (IPCAS), Biology Centre, Czech Academy of Sciences, Branišovská, České Budějovice, Czech Republic.

Methods. Measurements (length by width) are given in millimeters unless otherwise stated. Drawings were made with the aid of a camera lucida. Most of the present newly collected specimens have been deposited in the NMNS, Tsukuba; the NMNS; the NHM; the IPCAS; and the Queensland Museum (QM), Brisbane, Queensland, Australia.

Abbreviations used in the figures. csd, common sperm duct; dm, Drüsenmagen; e, esophagus; ed, ejaculatory duct; ep, excretory pore; ev, excretory vesicle; ga, genital atrium; gp, genital pore; hd, hermaphroditic duct; ic, intestinal cecum; Lc, Laurer's canal; m, metraterm; md, male duct; Mg, Mehlis' gland; o, ovary; os, oral sucker; ot, ootype; p, pharynx; pc, prostatic cells; pac, parturient canal; pp, pars prostatica; rsr, rudimentary seminal receptacle; sd, sperm duct; so, sinus organ; ss, sinus sac; sv, seminal vesicle; t, testis; tnc, transverse nerve commissure; u, uterus; v, vitellarium; vd, vitelline duct; vs, ventral sucker.

Results

Genus *Isoparorchis* Southwell, 1913

Isoparorchis Southwell, 1913: 91–92.

Leptolecithum Kobayashi, 1915a: 50–52; Kobayashi, 1921: 396–397.

Diagnosis. Digenea, Hemiuroidea, Isoparorchidae *sensu* Gibson (2002). Body broad-ovate, large, dorsoventrally flat. Ecsoma absent. Preoral lobe present. Oral sucker subterminal. Prepharynx absent. Pharynx posterodorsal to oral sucker. Esophagus inverted T- or Y-shaped, short. Drüsenmagen present at commencement of intestinal cecum, with several internal longitudinal

grooves. Intestinal ceca undulating, ending blindly near posterior extremity of body. Ventral sucker in anterior half of body. Testes two, entire, almost symmetrical, posterolateral to ventral sucker. Sperm ducts long; common sperm duct short, anterior to ventral sucker. Seminal vesicle tubular, sinuous, anterior to ventral sucker, free in parenchyma. Male duct lacking prostatic cells, long, connecting seminal vesicle and pars prostatica. Pars prostatica tubular, long, surrounded by prostatic cells, posterodorsal to sinus sac, free in parenchyma. Sinus sac snifter-shaped, globular or elliptical, large, composed of thick outer layer of diffuse musculature and thin inner layer of dense musculature, median, between ventral sucker and esophagus; its base protractile with sinus organ on it; small thin-walled tubular portion present between sinus sac and genital pore. Sinus organ large or small, complex, with diffuse musculature, arising from base of sinus sac. Ejaculatory duct long, convoluted in wall of sinus sac, joining to middle of hermaphroditic duct dorsally. Genital atrium consisting of anterior lumen of thin-walled tubular portion and posterior lumen of thick-walled sinus sac. Genital pore large, median, immediately posterior to esophagus. Ovary tubular, dextral or sinistral, lying transversely between ootype and relevant intestinal cecum, well posterior to testes in hind-body. Oviduct and ovovitelline duct very short. Laurer's canal opening dorsally, forming small rudimentary seminal receptacle proximally. Ootype small, median; Mehlis' gland well developed. Uterus long, preovarian, embracing ovary on ovarian side of body, folding transversely in intercecal field between vitellaria and sinus sac, in dorsal parenchyma; uterine seminal receptacle present; metraterm thin, long, convoluted in wall of sinus sac, terminally forming hermaphroditic duct in sinus organ. Hermaphroditic duct not eversible, clavate, thickened, short, opening on apex of sinus organ. Eggs numerous, ovate, operculate, not filamented, fully embryonated. Vitellaria two, dendritic (may be compound follicular glands with many small follicles), large, median, diagonal; anterior vitellarium located on anti-

ovarian side of body, in dorsal parenchyma; vitelline area mainly between ovary and cecal ends, extending a little anteriorly to ovary on antiovarian side of body, overlapping ceca. Excretory vesicle Y-shaped, in ventral parenchyma; stem swollen in median longitudinal posterior portion, folding transversely in intercercal field, bifurcating in hindbody; arms running forward, turning backward laterally to pharynx, separate there; excretory pore postero-ventral, -terminal or -dorsal. Parasitic in air bladder of freshwater catfishes (East, Southeast and South Asia and Australia). Type species: *Isoparorchis trisimilitubis* Southwell, 1913.

Discussion. The above diagnosis is based on the present study. Some other details will be added in the description for *I. trisimilitubis*.

The short esophagus bifurcates into an inverted Y- or T-shape with a short stem (Kobayashi, 1915a, c, 1921; Shimazu *et al.*, 2011; this paper). The Drüsenmagen is well differentiated at the commencement of the intestinal cecum on either side of the body (Kobayashi, 1915a, c, 1921; Shimazu *et al.*, 2011; this paper). The anatomy of the terminal genitalia is complex (Kobayashi, 1915a, b, 1921; Bovien, 1927; Johnston, 1927; Odhner, 1927; Gibson and Bray, 1979; Shimazu *et al.*, 2011; this paper). The anatomy reported in this paper is essentially the same as that described by Bovien (1927, fig. 3) and Shimazu *et al.* (2011). Bovien (1927) referred to the male duct connecting the seminal vesicle and pars prostatica as the first section of the pars prostatica lacking the prostatic cells. The male duct and pars prostatica are lined with a single layer of epithelial cells. The terminal thickening of the metraterm dorsally receives the ejaculatory duct at about the middle and then becomes a true uneversible hermaphroditic duct (Fig. 17). Kobayashi (1915a) erroneously stated that the metraterm joined to the ejaculatory duct laterally. The hermaphroditic duct should be interpreted as a continuation of the terminal thickening of the metraterm, because the posterior terminal thickening and anterior hermaphroditic duct are substantially the same in the thickness and histology.

The small, thin-walled tubular portion following the sinus sac may be a true genital atrium (see Figs. 13–15). In this paper, for convenience sake, the hermaphroditic duct means a combination of the terminal thickening of the metraterm and the true hermaphroditic duct; and the genital atrium means a combination of the anterior lumen of the thin-walled tubular portion and posterior lumen of the thick-walled sinus sac (Figs. 13–15).

Molecular study

ITS2 Analysis. Alignment of the ITS2 dataset yielded 468 characters (including 54 nucleotides of the 5' flanking 5.8S region and 56 nucleotides of the 3' flanking 28S region) for analysis. The total pairwise nucleotide differences observed between the species of *Isoparorchis* examined are shown in Table 2. No intraspecific variation was observed in any of the species sequenced. Interspecific variation observed among these taxa ranged from 3–18 nucleotides over the ITS2 region. Minimum evolution analysis of the ITS2 dataset resulted in a phylogram with the species *I. eurytremum*, *I. hypselobagri* and *I. tandani* forming a well-supported clade sister to *Isoparorchis* sp. 1, *I. trisimilitubis* and *Isoparorchis* sp. 2 (Fig. 1). Each of the species clades resolved in the analysis was resolved with strong bootstrap support.

Morphological study

Isoparorchis trisimilitubis Southwell, 1913

(Figs. 2–7)

Isoparorchis trisimilitubis Southwell, 1913, in part (?): 92–94 (?), pl. 8, figs. 9 (?) and 11 (?), pl. 9, fig. 12 (?).

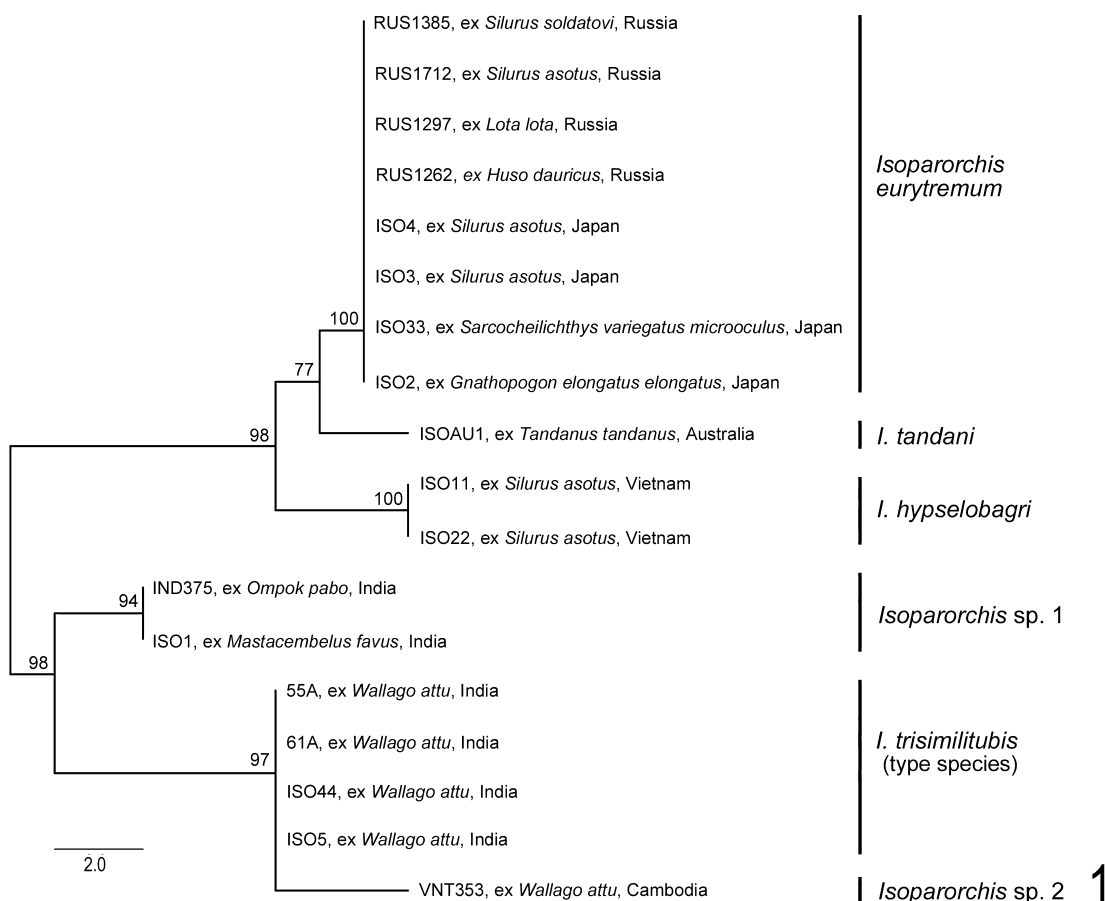
Isoparorchis hypselobagri: Ejsmont, 1932: 456.

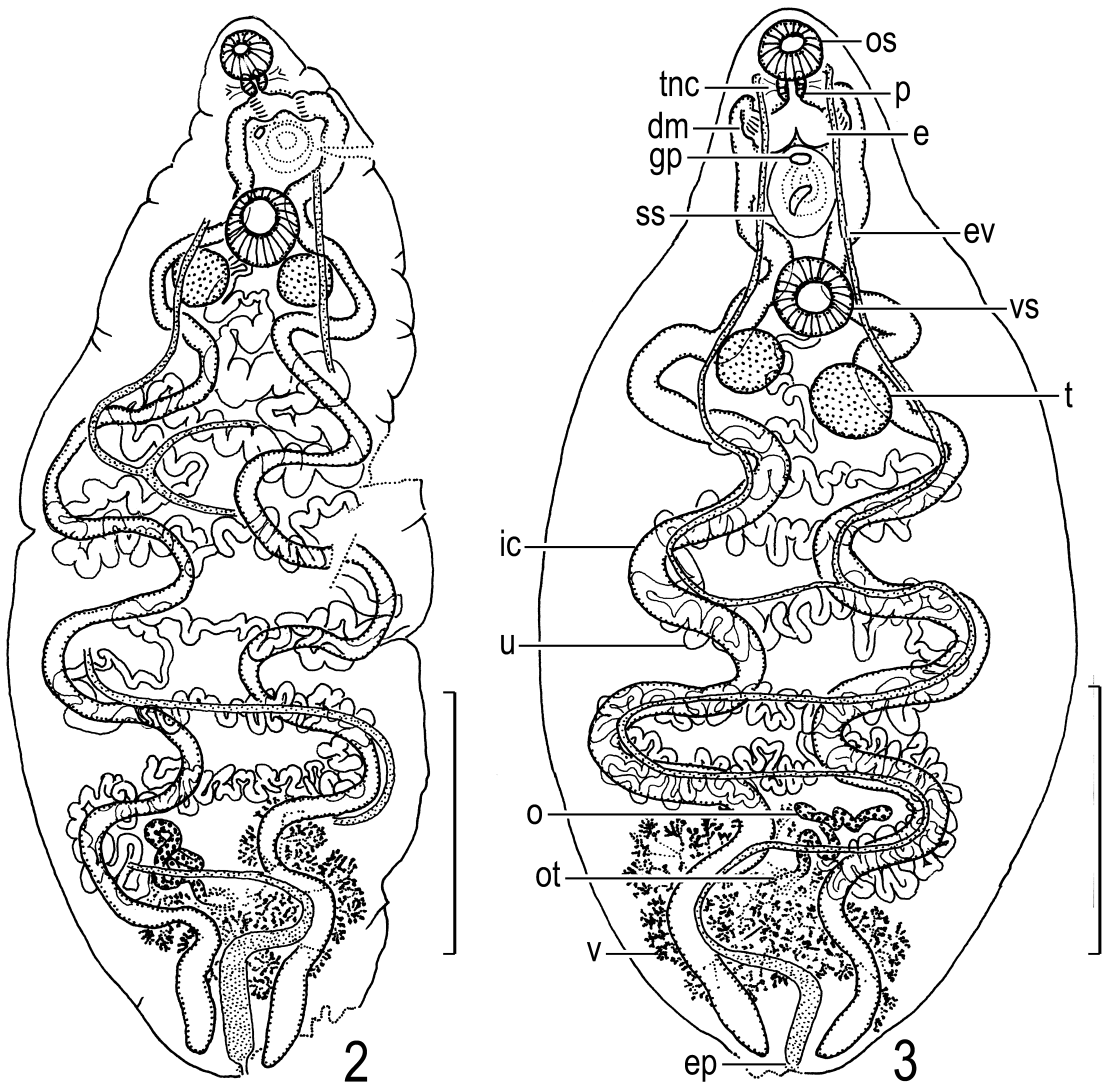
Host and localities. *Wallago attu* (Bloch and Schneider, 1801) (Siluriformes, Siluridae) (type host) from a freshwater tank (type locality) at Bankipur [in Patna, Bihar State (?)], India (Southwell, 1913); and Balurghat and Rishra near Kolkata, West Bengal, India (this paper).

Site of infection. Air bladder (Southwell, 1913; this paper).

Table 2. Number of nucleotide differences observed in the ITS2 rDNA dataset between the species of *Isoparorchis* examined.

Sample, species, and locality	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1) ISO11 — <i>I. hypselobagri</i> , Vietnam																		
2) ISO22 — <i>I. hypselobagri</i> , Vietnam	0																	
3) ISO2 — <i>I. eurytremum</i> , Japan	5	5																
4) ISO33 — <i>I. eurytremum</i> , Japan	5	5	0															
5) ISO3 — <i>I. eurytremum</i> , Japan	5	5	0	0														
6) ISO4 — <i>I. eurytremum</i> , Japan	5	5	0	0	0													
7) RUS1262 — <i>I. eurytremum</i> , Russia	5	5	0	0	0	0												
8) RUS1297 — <i>I. eurytremum</i> , Russia	5	5	0	0	0	0	0											
9) RUS1385 — <i>I. eurytremum</i> , Russia	5	5	0	0	0	0	0	0										
10) RUS1712 — <i>I. eurytremum</i> , Russia	5	5	0	0	0	0	0	0	0									
11) IND375 — <i>Isoparorchis</i> sp. 1, India	12	12	11	11	11	11	11	11	11	11								
12) ISO1 — <i>Isoparorchis</i> sp. 1, India	12	12	11	11	11	11	11	11	11	11	0							
13) 55A — <i>I. trisimilitubis</i> , India	15	15	14	14	14	14	14	14	14	14	7	7						
14) 61A — <i>I. trisimilitubis</i> , India	15	15	14	14	14	14	14	14	14	14	7	7	0					
15) ISO44 — <i>I. trisimilitubis</i> , India	15	15	14	14	14	14	14	14	14	14	7	7	0	0				
16) ISO5 — <i>I. trisimilitubis</i> , India	15	15	14	14	14	14	14	14	14	14	7	7	0	0	0			
17) VNT353 — <i>Isoparorchis</i> sp. 2, Cambodia	18	18	17	17	17	17	17	17	17	17	10	10	3	3	3	3		
18) ISOAU1 — <i>I. tandani</i> , Australia	6	6	3	3	3	3	3	3	3	3	12	12	15	15	15	15	18	

Fig. 1. Relationships between the species of *Isoparorchis* examined here based on minimum evolution analysis of the ITS2 rDNA dataset. Bootstrap values based on 10,000 replicates are indicated at the nodes.

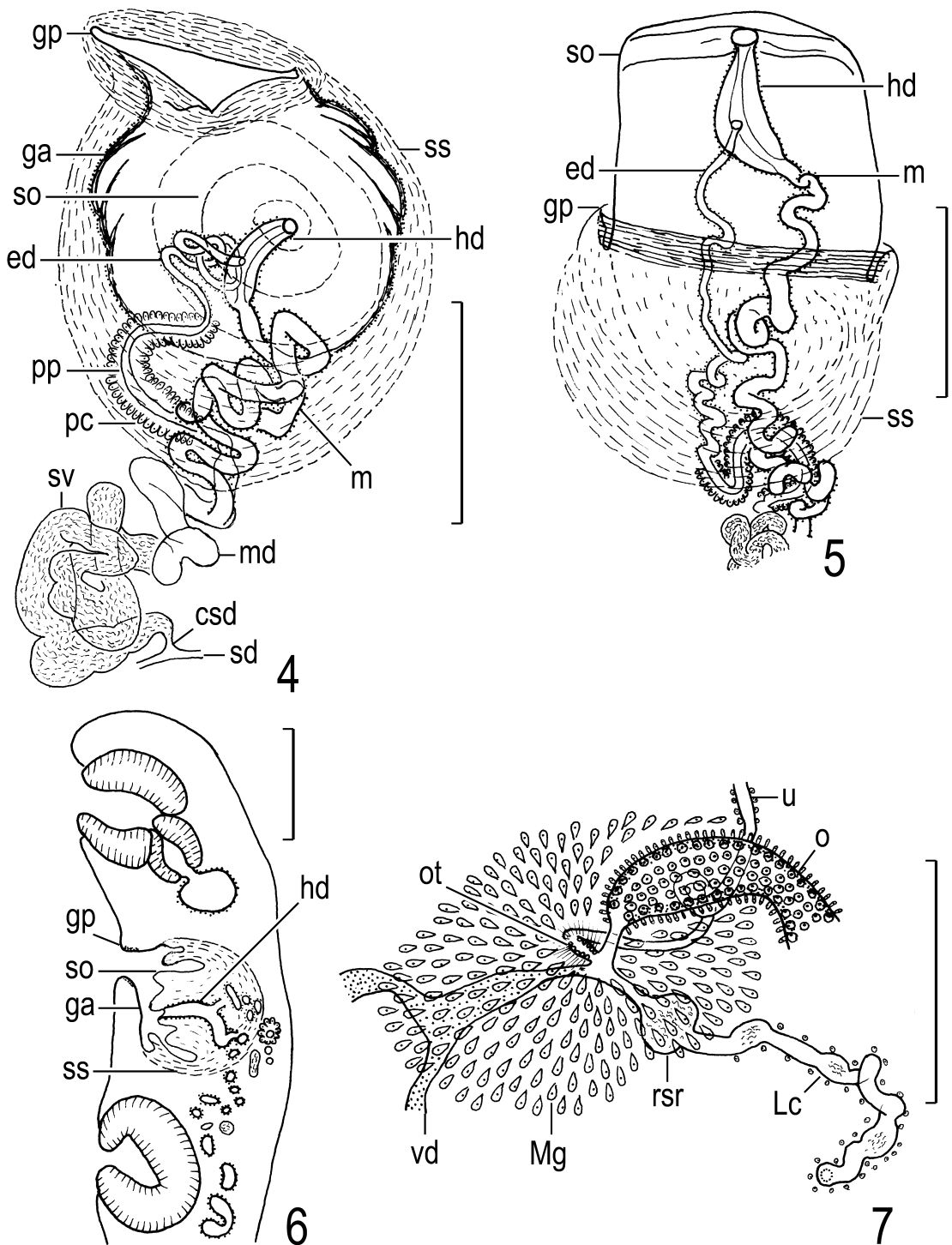


Figs. 2–3. *Isoparorchis trisimilitubis*, adult specimens ex air bladder of *Wallago attu* from India. — 2, syntype 3 (NHMUK 2000.4.10.78–80), entire body, ventral view; 3, adult (NSMT-PI 5887) from Balurghat, entire body, ventral view. Scale bars: 5 mm in Figs. 2–3.

Material examined. (1) 2 specimens (NHMUK 2000.4.10.78–80, syntypes 3 and 4 of *I. trisimilitubis*), adult, whole-mounted, ex air bladder of *W. attu*, freshwater tank at Bankipur, March 1912 (Southwell, 1913). (2) 11 (NSMT-PI 5887–5888, 1 whole-mounted immature, 2 whole-mounted adult, 3 serially sectioned adult; IPCAS D-694, 2 whole-mounted adult; NHMUK 2013.7.16.1, 1 whole-mounted adult; QM G 234252, 1 whole-

mounted adult; Nguyen Van Ha's personal collection, 1 whole-mounted adult), hot formalin-fixed, ex air bladder of *W. attu*, Balurghat, 9 and 13 December 2007 (another 2 ethanol-fixed samples 61A and ISO44 sequenced) (supplied by T. Scholz). The ceca and uterus were seriously damaged in the hot formalin-fixed specimens.

Description. 1) Based on 2 adult syntypes 3 and 4 (Fig. 2). Body torn in parts, wrinkled,



Figs. 4–7. *Isoparorchis trisimilitubis* (continued), adult specimens ex air bladder of *Wallago attu* from Balurghat, India. — 4, adult (NSMT-PI 5888), terminal genitalia, ventral view; 5, adult (IPCAS D-694), terminal genitalia, showing sinus organ protruding through genital pore, ventral view; 6, adult (NSMT-PI 5888), sagittal section, showing sinus sac and sinus organ, lateral view; 7, adult (IPCAS D-694), ovarian complex, ventral view. Scale bars: 1 mm in Figs. 4–6; 0.5 mm in Fig. 7.

scratched, ovate, large, 20–29 by 8–13; forebody 4–7 long, occupying 20–24% of body length. Oral sucker 1.00–1.50 by 1.06–1.67. Pharynx 0.56–0.76 by 0.44–0.67. Esophagus 0.16–0.19 by 0.63–0.95. Drüsenmagen 0.32–0.47 by 0.59–0.68. Ventral sucker 1.47–1.90 by 1.51–1.90; sucker width ratio 1:1.1–1.4 (sucker length ratio 1:1.3–1.5). Testes slightly smaller than ventral sucker, 1.03–2.06 by 0.95–1.59. Seminal vesicle anterior to ventral sucker. Male duct thick. Pars prostatica posterodorsal to sinus sac. Sinus sac globular, 0.79–1.74 by 0.84–1.58, occupying 20–25% of forebody length. Genital atrium globular, 0.44–0.95 by 0.48–1.03, occupying 55–56% of sinus sac length. Sinus organ not clearly observed, large, filling genital atrium. Hermaphroditic duct 0.44 by 0.09 in syntype 3. Ovary 5.86–6.75 by 0.18–0.28, dextral or sinistral. Laurer's canal not clearly traced; rudimentary seminal receptacle 0.06–0.12 by 0.06–0.08. Uterine seminal receptacle seen. Eggs 51–59 by 24–27 μm in syntype 3 and 44–48 by 22 μm in syntype 4. Vitelline area 4.12–4.76 by 4.60–5.08. Excretory vesicle bifurcating greatly anteriorly to ovary or slightly anteriorly to middle of hindbody in syntype 3.

2) Based on newly collected specimens, 7 adult whole-mounts measured (Figs. 3–7). *Isoparorchis*. Body large, 19–27 by 10–15; forebody rapidly tapering anteriorly, with squaring shoulders at about middle, 5–6 long, occupying 20–26% of body length; hindbody round to elliptical. Oral sucker almost globular, 1.11–1.35 by 1.19–1.43. Pharynx barrel-shaped, 0.56–0.74 by 0.56–0.71. Esophagus 0.08–0.40 by 0.79–1.59, surrounded by small gland cells. Drüsenmagen globular, small, 0.49–0.68 by 0.24–0.48. Intestinal ceca undulating 5–9 times in hindbody. Ventral sucker almost globular, 1.43–1.90 in diameter, located at about junction of anterior and second fourths of body; sucker width ratio 1:1.2–1.4. Testes globular to elliptical, smaller than ventral sucker, 1.27–2.30 by 1.11–2.14, symmetrical, directly medial to or overlapping ceca. Sperm ducts long; common sperm duct short, anterior to ventral sucker. Seminal vesicle

anterior to or overlapping ventral sucker. Male duct thick, lined with large epithelial cells. Pars prostatica thick, curved, posterior or posterodorsal to sinus sac. Ejaculatory duct surrounded by small gland cells. Sinus sac almost globular, 1.50–2.06 by 1.23–1.67, occupying 25–34% of forebody length. Genital atrium globular, 0.95–1.27 by 0.89–1.27, occupying 58–74% of sinus sac length; inner layer grooved spirally, surrounded by small gland cells located in outer layer. Sinus organ fairly large, contracted like double volcano (Figs. 4, 6), or extended on protracted base of sinus sac, protruding to outside through genital pore (Fig. 5), filling genital atrium. Hermaphroditic duct 0.48–0.66 by 0.22–0.28, surrounded by tall gland cells. Genital pore with sphincter. Ovary straight or folded once or twice, 5.32–8.39 by 0.21–0.32, surrounded by tall gland cells, dextral or sinistral, at about junction of middle and posterior thirds of hindbody. Laurer's canal fairly long; rudimentary seminal receptacle almost globular, 0.08–0.12 by 0.07–0.15 in 3 specimens, but not clearly observed in other specimens. Uterus surrounded by small gland cells, forming some 7 large transverse turns across ceca between ootype and testes and then some 5 small median transverse turns; metraterm surrounded by small gland cells; uterine seminal receptacle seen. Eggs light brown, 41–51 by 22–24 μm . Vitelline area 1.49–4.28 by 4.44–5.71. Excretory vesicle bifurcating greatly anteriorly to ovary or a little anteriorly to middle of hindbody; excretory pore postero-terminal to -ventral.

Remarks. Southwell (1913) established *Isoparorchis* Southwell, 1913 and described the type species *Isoparorchis trisimilitubis* Southwell, 1913 on the basis of adult [and possibly juvenile] specimens found in the air bladder of *Wallago attu* collected in a freshwater tank at Bankipur. Odhner (1927) reexamined some of Southwell's original specimens received from the Indian Museum in Calcutta [now Kolkata] and gave a specific diagnosis of *I. trisimilitubis*. Ejsmont (1932) regarded *I. trisimilitubis* as a junior synonym of *Isoparorchis hypselobagri* (Billet, 1898)

Ejsmont, 1932 in spite of the fact that *I. trisimilitubis* is the type species of the genus.

Some wet specimens (NHMUK 2000.4.10.78–80) of Southwell's original material have been deposited in the Department of Life Sciences, NHM (Eileen Harris, Senior Curator, personal communication). Odhner deposited none of the specimens reexamined but a serially sectioned juvenile specimen (SMNH Register Nos. 121143 and 121145). Neither a holotype nor a lectotype has previously been fixed for *I. trisimilitubis*. Accordingly, the wet and Odhner's specimens are syntypes of *I. trisimilitubis* in accordance with Articles 72.4 and 73.2 of the International Code of Zoological Nomenclature (ICZN) (International Commission on Zoological Nomenclature, 1999). As far as we know, nobody else has previously reexamined or redescribed the type material.

We borrowed four wet syntypes 1–4 from the NHM and made one (syntype 2) of them into serial sections and the others (syntypes 1, 3 and 4) into stained whole-mounts in Canada balsam. The four syntypes reexamined were adults but poor preparations. However, the two suckers were clearly observed in them. The syntypes 3 and 4 had a low sucker width ratio of 1:1.1–1.4 (sucker length ratio 1:1.3–1.5). The syntype 1 had a high sucker width ratio of 1:1.9, and the syntype 2 had a high sucker length ratio of 1:2.0. Furthermore, another difference was detected in the position of bifurcation of the Y-shaped excretory vesicle in the hindbody: either greatly or slightly anterior to the ovary. The excretory vesicle could be traced in places in the four syntypes. It appeared that the excretory vesicle bifurcated greatly anteriorly to the ovary in the syntype 3 (Fig. 2) with the low sucker ratio and slightly anteriorly to the ovary in the syntype 1 (Fig. 30) with the high sucker ratio. These differences in the sucker ratio and position of bifurcation of the excretory vesicle were also clearly observed in the new specimens from India and Bangladesh.

The two above-mentioned morphological differences are correlated and considered to be of taxonomic importance at the species level. The

present specimens from India and Bangladesh are classified into two different species: (1) species 1 (syntypes 3 and 4), in which the sucker ratio is low, and the excretory vesicle bifurcates greatly anteriorly to the ovary; and (2) species 2 (syntypes 1 and 2), in which the sucker ratio is high, and the excretory vesicle bifurcates slightly anteriorly to the ovary. Since *I. trisimilitubis* is the type species of the genus, this species must be retained. Southwell (1913) gave the body of the 10 specimens as 10–35 mm long by 4.5–20 mm wide, and the oral sucker as 0.7 mm in diameter and the ventral sucker as 1 mm in diameter in the largest adult specimen 35 mm long by 18 mm wide. Since the sucker width ratio is 1:1.4 (our calculation), this specimen probably belongs to species 1. On the other hand, he described the excretory vesicle as running forward a little distance from the more or less globular or cylindrical contractile vesicle [the posterior longitudinal swollen part of the excretory vesicle in this paper]. He figured an adult specimen (pl. 8, fig. 10) that had the sucker width ratio of 1:2.3 (our calculation) and the excretory vesicle bifurcating slightly anteriorly to the ovary. This specimen probably belongs to species 2. It seems likely that his original description for *I. trisimilitubis* was based on specimens of two different species, or our species 1 and 2. We here apply species 1 to *I. trisimilitubis*, because we have collected the 11 new better adult specimens of this species (described above). We abstain at present from designating a lectotype for *I. trisimilitubis* from the two syntypes 3 and 4, because they are poor whole-mounts. Species 2 will be described as *Isoparorchis* sp. 3 below.

Odhner (1927) described the oral sucker as 1.3–1.4 mm in diameter and the ventral sucker as 2.5–2.8 mm in diameter, with the sucker ratio of 1:2, in the specimens (19–35 mm long by 9–20 mm wide) reexamined. In the sagittal sections of the juvenile syntype of *I. trisimilitubis*, the sucker length ratio was 1:2.0 (see *Isoparorchis* sp. 3). These specimens evidently belong to species 2. Odhner stated that Southwell's measurements of the two suckers were completely

incorrect. However, it seems that both Southwell and Odhner were correct. Odhner may have dealt, by chance, with only the adult and juvenile specimens of species 2.

In the present molecular study, the ITS2 sequences obtained for the samples from India also formed two different clades (Fig. 1). The samples 55A, 61A, ISO44 and ISO5 all from *Wallago attu* (*I. trisimilitubis* in Table 1 and Fig. 1) had been morphologically identified as *I. trisimilitubis* prior to DNA sequencing. The ITS2 sequences for these four samples were identical and they formed a well-supported clade with the specimen VNT353 (*Isoparorchis* sp. 2 in Table 1 and Fig. 1) from the same host (but different locality, Cambodia). The clade was sister to the clade formed from the samples IND375 (juvenile) from *Ompok pabo* (Hamilton, 1822) (Siluridae) and the sample ISO1 (juvenile) from *Mastacembelus favus* Hora, 1924 (Mastacembelidae) (*Isoparorchis* sp. 1 in Table 1 and Fig. 1). Therefore, the clade associated with the four samples from the type host (*Wallago attu*) collected in India is assigned to *I. trisimilitubis*. The samples IND375 and ISO1 had been sequenced before morphological observations for precise identification could be undertaken, because we supposed that they also belonged to *I. trisimilitubis*. However, soon after that, we concluded that the syntypes of *I. trisimilitubis* consisted of two species as mentioned above. Since nothing is known at present about their morphology, the samples IND375 and ISO1 remain as an unidentified species, *Isoparorchis* sp. 1. Morphological observation of adult specimens is necessary for species identification of *Isoparorchis* sp. 1.

Neither Southwell (1913) nor Odhner (1927) described the seminal vesicle, or the pars prostatica, or the anatomy of the terminal genitalia. The present study shows that the seminal vesicle and pars prostatica are present, posterior and posterodorsal to the sinus sac, respectively; a long thick male duct connects the seminal vesicle and pars prostatica; the sinus sac is globular and small (occupying 20–25% of the forebody length in the syntypes 3 and 4); the sinus organ is like a

double volcano and large, filling the genital atrium; the genital atrium is globular and small (occupying 55–56% of the sinus sac in the syntypes 3 and 4); the ejaculatory duct and metratrum are long and convoluted in the wall of the sinus sac; and the metratrum is differentiated into an unversible clavate terminal thickening (the hermaphroditic duct interpreted in this paper), which receives the ejaculatory duct dorsally at about the middle and then becomes a true hermaphroditic duct (Figs. 4–6) (see also Figs. 16–17). Southwell (1913) did not say anything about eggs. The egg size differs in the two syntypes: 51–59 by 24–27 μm in the syntype 3 and 44–48 by 22 μm in the syntype 4. The syntype 3 has the largest eggs among the present specimens of *I. trisimilitubis*.

Gibson and Bray (1979) illustrated the anatomy of the sinus sac in *Isoparorchis*. Their specimens (NHMUK 1954.9.14.360–389) were part of the material collected by W. N. F. Woodland from the air bladder of *W. attu* collected in the Ganges River at Allahabad, India, in 1921 (D. I. Gibson, personal communication; see also Bhalerao, 1926). In the sagittally sectioned specimen 29 mm long, the oral sucker was 0.94 mm long, and the ventral sucker was 1.46 mm long (D. I. Gibson, personal communication). The sucker length ratio is 1:1.5 (our calculation). Accordingly, this specimen is likely to belong to *I. trisimilitubis*.

Since Southwell (1913), many papers have been published on adult materials under the species name of either *I. trisimilitubis* or *I. hypselobagri* from India (e.g. Bhalerao, 1926; Simha and Rao, 1977; Srivastava, 1977), Bangladesh (e.g. Bashirullah, 1972; Chandra and Banerjee, 1993), and Pakistan (e.g. Bilqees and Khatoon, 1972; Bhutta and Khan, 1975; Zaidi and Khan, 1977). Most of these materials lack details of the adult morphology, so that it is not possible at present for us to identify them definitely. In Bhutta and Khan's (1975) material, the sucker ratio was 1:1.5–3.0. We consider that this range is too wide to be consistent with a single species. Zaidi and Khan (1977, fig. 25) figured an adult, in which the sucker ratio is about 1:1.3, but the

excretory vesicle bifurcates posteriorly to the ovary. These materials need reexamination.

Life cycle. The final host is apparently confined to *W. attu* (this paper). According to Das and Manna (1993) and Manna and Das (2003), a first intermediate host of *I. hypselobagri* in India is the planorbid snail *Indoplanorbis exustus* (Deshayes, 1834). Chattopadhyay and Manna (1987) studied the chromosomes (karyotype) of adults of *I. hypselobagri* from India. Immature worms have been recorded from the body cavity and other organs of freshwater fishes, a turtle and a crocodile (e.g. Bhalerao, 1936; Simha, 1958; Dollfus, 1959; Srivastava, 1977). Accidental human infection has also been reported (Chandler, 1926; Bhalerao, 1936). The morphology of the cercaria, juvenile and adult is quite unknown, because none of them has been described. It is not possible for us to identify them.

Isoparorchis hypselobagri (Billet, 1898)

(Figs. 8–11)

Distomum hypselobagri Billet, 1898: 288–290, pl. 13, fig. 8.
Isoparorchis hypselobagri: Ejsmont, 1932: 456.

Hosts and localities. “*Hypselobagrus*” [most likely *Hemibagrus* sp.] (Siluriformes, Bagridae) (type host) from Bang Giang River (type locality) in Cao Bang Province (Billet, 1898; this paper); and *Silurus asotus* Linnaeus, 1758 (Siluriformes, Siluridae) from Ha Bac water at Tu Son District, Bac Ninh Province (Kha Ki [Ha Ky], 1968; this paper); Thanh Tri District, Hanoi, Day River at Kim Son District, Ninh Binh Province, and Nhue River at Kim Bang District, Ha Nam Province (Nguyen Van Ha, 2003; this paper); and Ninh Co River at Hai Hau District and Hong River at Balat District, both in Nam Dinh Province (this paper), all in northern Vietnam.

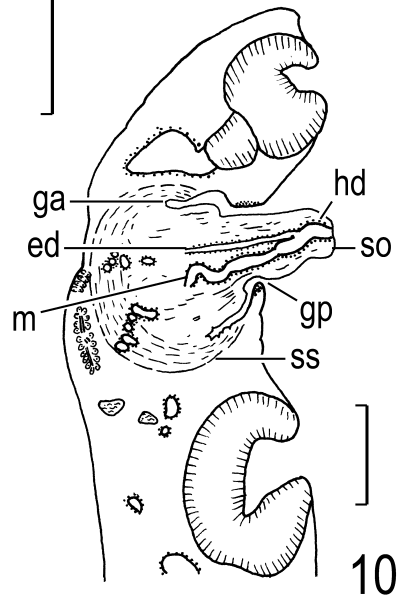
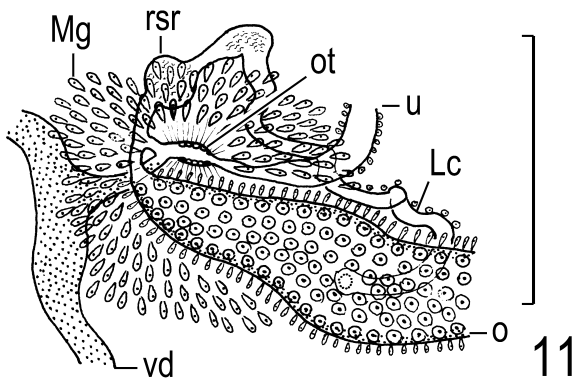
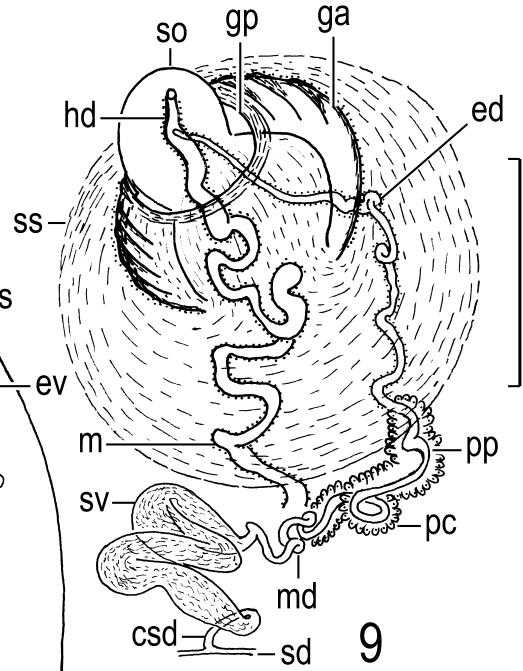
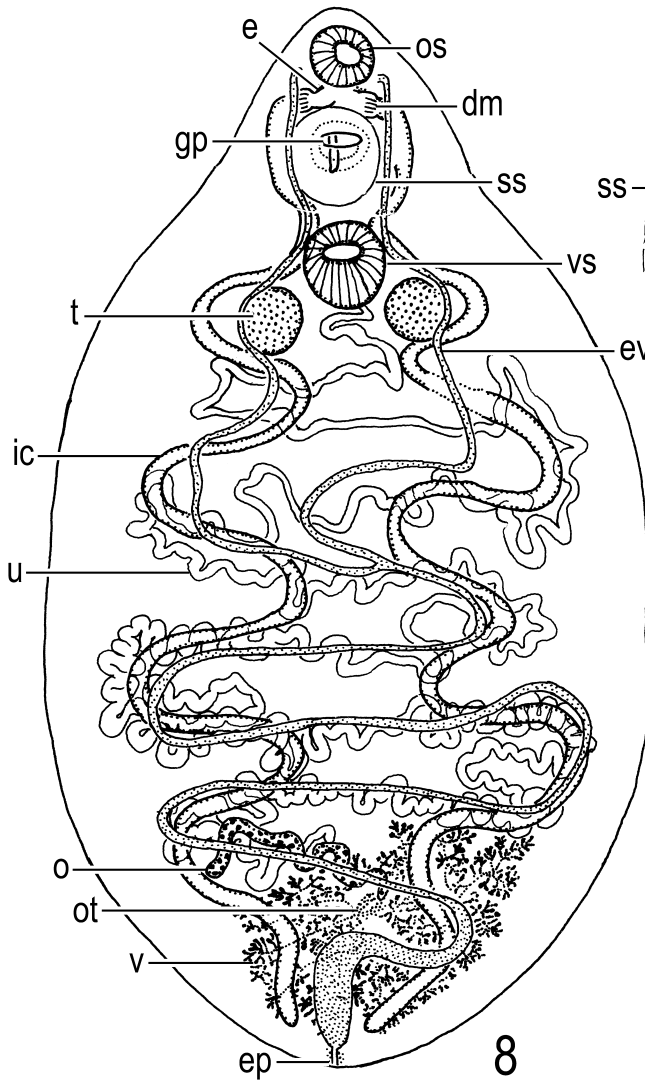
Site of infection. Air bladder (Kha Ki [Ha

Ky], 1968; Nguyen Van Ha, 2003; this paper).

Material examined. (1) 2 specimens of *I. hypselobagri*, adult, whole-mounted, ex air bladder of *S. asotus*, Day River, 11 September 2001 (Nguyen Van Ha, 2003; his personal collection). (2) 1 (NSMT-PI 5884), juvenile, whole-mounted, ex air bladder of *S. asotus*, Ninh Co River, 10 May 2011 (another 1 sample ISO11 sequenced). (3) 5 (NSMT-PI 5885, 2 whole-mounted, 1 serially sectioned; NHMUK 2013.7.16.2, 1 whole-mounted; IPCAS D-213, 1 whole-mounted), adult, killed in hot water, ex air bladder of *S. asotus*, Hong River, 23–25 November 2011 (another 1 ethanol-fixed sample ISO22 sequenced). The ceca and uterus were found seriously damaged in them. (4) 7 (NSMT-PI 5886, 5 whole-mounted, 1 serially sectioned; QM G 234253, 1 whole-mounted), adult, hot formalin-fixed, ex air bladder of *S. asotus*, Day River, 7 May 2012. The ceca and uterus were seriously damaged.

Description. Based on adult specimens (Figs. 8–11); juvenile specimen in parentheses. Body large, 12–23 by 6–16 (2.45 by 0.95); forebody with squaring shoulders, especially in large specimens, 3.25–5.95 (1.03) long, occupying 21–29% of body length; hindbody broad-elliptical. Oral sucker 0.95–1.42 by 1.06–1.42 (0.27 by 0.29). Pharynx 0.52–0.79 by 0.51–0.73 (0.18 in diameter). Esophagus 0.22–0.27 by 0.63–1.11 (0.05 by 0.19). Drüsenmagen 0.32–0.63 by 0.35–0.60 (0.08–0.09 by 0.06–0.12). Intestinal ceca undulating about 8 times in hindbody. Ventral sucker 1.30–2.06 by 1.31–2.11 (0.32 by 0.35); sucker width ratio 1:1.2–1.6 (1:1.2). Testes smaller than ventral sucker, 0.71–1.90 by 0.71–1.59. Seminal vesicle anterodorsal to ventral sucker. Male duct thin, lined with small epithelial cells. Pars prostatica posterolateral to sinus sac. Sinus sac almost globular, small, 1.11–2.06 by 1.34–2.02 (0.09 by 0.05), occupying 28–40 (8)% of forebody length; genital atrium globular, small,

Figs. 8–11. *Isoparorchis hypselobagri*, adult specimens (NSMT-PI 5886) ex air bladder of *Silurus asotus* from northern Vietnam. — 8, entire body, ventral view; 9, terminal genitalia, ventral view; 10, forebody, sagittal section, reconstructed from several sections; 11, ovarian complex, ventral view. Scale bars: 5 mm in Fig. 8; 1 mm in Figs. 9–10; 0.5 mm in Fig. 11.



0.71–1.35 by 0.79–1.42, occupying 45–69% of sinus sac length. Sinus organ large (small), contracted like double volcano or extended on protracted base of sinus sac to protrude to outside through genital pore, filling genital atrium. Hermaphroditic duct 0.40–0.73 by 0.06–0.13 (in 9 specimens, but not clearly observed in 2 others). Ovary dextral or sinistral, 3.18–7.12 by 0.21–0.36. Rudimentary seminal receptacle 0.09–0.15 by 0.05–0.12. Eggs 38–51 by 21–25 μm . Vitelline area 2.22–4.76 by 2.36–6.66. Excretory vesicle bifurcating greatly anteriorly to ovary or a little anteriorly to middle of hindbody; excretory pore postero-ventral to -terminal.

Remarks. Billet (1898) briefly described *Distomum hypselobagri* Billet, 1898 on the basis of adult specimens found in the air bladder of the fish “*Hypselobagrus*” collected in “Song-Bang-Giang” [the Bang Giang River] in “Haut-Tonkin” [Cao Bang Province, northern Vietnam]. The current scientific name of the fish “*Hypselobagrus*” is unknown. *Hypselobagrus* Bleeker, 1862 is a junior synonym of *Mystus* Scopoli, 1777 (Siluriformes, Bagridae) (Eschmeyer (ed.), 2013). Arthur and Bui Quang Te (2006) thus regarded the fish “*Hypselobagrus*” as a species of *Mystus*. However, this genus does not occur in Cao Bang Province. Instead, four species of *Hemibagrus* (Bagridae) are known there (Ministry of Fisheries, Vietnam, 1996). The original host may have been one of them. Odhner (1927) stated that *D. hypselobagri* should be temporarily treated as a *species inquirenda* for lack of almost all measurements. Billet obtained many adult specimens but actually gave only the measurement of the body (about 25 by 7 mm) of one of them. Ejsmont (1932) first placed *D. hypselobagri* in *Iso-parorchis* as *I. hypselobagri*.

Judging from our present knowledge of the genus, there are some problems to solve in Billet’s description and figure (pl. 13, fig. 8) of the entire body. (1) The intestinal cecum has a small anterior diverticulum (*d*, *d'*) on either side of the body. This diverticulum is interpreted as a longitudinal small fold of the intestine (this paper, Fig. 3; see also Dollfus, 1959, fig. 3). (2) The testes (*t*, *t'*)

are anterolateral to the ventral sucker. We doubt if this is correct, because the testes are always posterolateral in all the present specimens. (3) The ovary (*ger*) is globular and median. It seems that the tubular ovary is misinterpreted as the seminal receptacle (*rs*); and Mehlis’ gland, as the ovary (*ger*). (4) A globular organ (*glc*) of unknown nature is present anterior to Mehlis’ gland. It is possible that this organ is the rudimentary seminal receptacle of Laurer’s canal. (5) The excretory system is dorsal to the digestive system. The excretory system should be ventral. Billet’s original specimens do need reexamination, but none of them were made available to Odhner (1927) or to us. It is believed that all of them were lost. Therefore, the morphology of *D. hypselobagri*, or now *I. hypselobagri*, is vaguely understood at present. We attempted to obtain new specimens from *Hemibagrus* spp. and *S. asotus* in Cao Bang City, Cao Bang Province, in early August 2012 without success.

Kha Ki [Ha Ky] (1968) described adult specimens, under the species name of *I. pseudobagri* [sic, should be *hypselobagri*], found in the air bladder of *S. asotus* (syn. *Parasilurus asotus*) from the Kha Bak [Ha Bac] water [at several places near the Cau River at Tu Son District, Bac Ninh Province], northern Vietnam. Neither the sinus organ nor eggs nor the excretory vesicle was mentioned. None of the specimens were made available to us for reexamination.

Nguyen Van Ha (2003, fig. 3.14 b) also reported five adult specimens, under the species name of *I. hypselobagri*, from the air bladder of *S. asotus* collected in Thanh Tri District, Hanoi; the Day River at Kim Son District, Ninh Binh Province; and the Nhue River at Kim Bang District, Ha Nam Province, all in northern Vietnam. Neither the sinus organ nor the excretory vesicle was mentioned. We reexamined two of the five specimens in the present study.

In the specimen of *I. hypselobagri* figured by Billet (1898, fig. 10), the body is elongate; the sucker width ratio is 1 : 1.6 (our calculation); the testes are anterolateral to the ventral sucker; and the excretory vesicle bifurcates a little anteriorly

to the ovary, or posteriorly to midway between the ventral sucker and ovary. We could not obtain any new specimens from *Hemibagrus* spp. collected in northern Vietnam. In the present specimens (Fig. 1), the body was broad-elliptical; the sucker width ratio was 1:1.2–1.6; the testes were posterolateral to the ventral sucker; and the excretory vesicle bifurcated greatly anteriorly to the ovary or a little anteriorly to the midlevel of the hindbody. We here tentatively identify Kha Ki's [Ha Ky's] (1968), Nguyen Van Ha's (2003) and the present specimens from *S. asotus* in northern Vietnam as *I. hypselobagri*.

Morphologically, *I. hypselobagri* resembles *I. trisimilitubis* (this paper) but differs from it mainly in having a broader body, a higher sucker ratio (1:1.2–1.6 instead of 1:1.1–1.4), a larger sinus sac (occupying 28–40% instead of 20–34% of the forebody length), a smaller genital atrium (occupying 45–69% instead of 55–74% of the sinus sac length) and a thinner male duct. These differences are slight. However, *I. hypselobagri* is phylogenetically distinct from *I. trisimilitubis* (Fig. 1).

Life cycle. The entire life cycle is not known. The final hosts are "*Hypselobagrus*" [most likely *Hemibagrus* sp.] (Billet, 1898) and *Silurus asotus* (Kha Ki [Ha Ky], 1968; Nguyen Van Ha, 2003; this paper). Juveniles [not larvae or metacercariae] were recorded in northern Vietnam: from the intestine of *Channa maculata* (Lacépède, 1801) (Perciformes, Channidae) (Moravec and Sey, 1989; IPCAS D-213/1; 5.39 by 1.63, forebody 2.22 long, sinus sac 0.26 by 0.23, sinus organ large, domelike, 0.11 by 0.12 in our reexamination); the musculature of *Ophicephalus maculatus* (Kha Ki [Ha Ky], 1968); the intestine of *Channa maculata* and *Hypophthalmichthys molitrix* (Valenciennes, 1844) (Cypriniformes, Cyprinidae); and the body cavity of *Anabas testudineus* (Bloch, 1792) (Perciformes, Anabantidae) (Nguyen Van Ha, 2003). Dollfus (1959) also recorded juveniles from the subcutaneous tissue, musculature, body cavity, etc. of fishes: *Tylosurus annulatus* (Valenciennes, 1846) (Beloniformes, Belonidae), *Ophicephalus striatus*, "Cà trèn" and

"Cà trê" from "Région de Càntho (Cochinchine)" [Can Tho Province, southern Vietnam]; and *Ophicephalus striatus* from the La Nga River, central Vietnam, and Saigon City, southern Vietnam. The juveniles at least from northern Vietnam are likely to belong to *I. hypselobagri*.

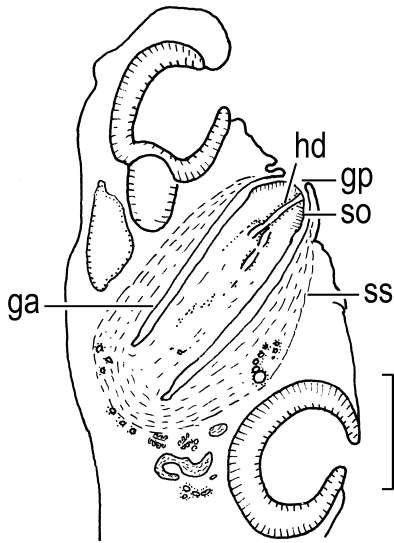
Isoparorchis eurytremum (Kobayashi, 1915)

(Figs. 12–24)

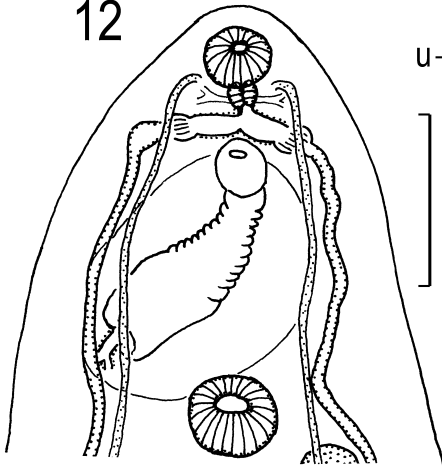
Leptolecithum eurytremum Kobayashi, 1915a: 50–52, pl. 2, figs. 1–3; Kobayashi, 1921: 397–399, pl. 26, fig. 1. Cercaria [U]: Ando, 1918: 619, fig. 14 b. **Syn. nov.** *Isoparorchis euritremen* [sic, should be *eurytremum*]: Travassos, 1922a: 20. *Isoparorchis eurytrema* [sic, should be *eurytremum*]: Travassos, 1922b: 230. *Cercaria introverta* Faust, 1924: 294, table 1; Ito, 1953: 487–488, fig. 1; Ito, 1964: 478, fig. 97; Makita *et al.*, 1996: 313, fig. 8. **Syn. nov.** *Isoparorchis eurytremum*: Odhner, 1927: 2, fig. 1. *Isoparorchis hypselobagri*: Ejsmont, 1932: 456; Shimazu *et al.*, 2011: 24–26, figs. 30–33. *Isoparorchis trisimilitubis*: Bhalerao, 1926: 248; Yamaguti, 1934: 502, fig. 129.

Hosts and localities. 1) Japan: *Silurus asotus* Linnaeus, 1758 (Siluridae) (type host) from Lake Kasumigaura (type locality) in Ibaraki Prefecture; Sawara (type locality), now in Katori City, Chiba Prefecture; Lake Biwa (type locality) in Shiga Prefecture; various places (not specified) (type localities) in Okayama Prefecture; Lake Kizaki in Oomachi City and Lake Suwa at Suwa City, Nagano Prefecture; Lake Biwa basin in Shiga Prefecture; Uji River at Uji City and Kizu River at Kasagi Town, Kyoto Prefectures; and Chikugo River at Hita City, Oita Prefecture (Kobayashi, 1915a, 1921; Yamaguti, 1934; Sawada and Osako, 1969; Shimazu, 2003, 2007; Shimazu *et al.*, 2011; this paper); *Silurus biwaensis* (Tomoda, 1961) from Lake Biwa (Shimazu *et al.*, 2011); and "*Pseudobagrus aurantiacus*" ((?), vague) (Siluriformes, Bagridae) (type host) (locality unknown) (Kobayashi, 1915a, 1921; Shimazu *et al.*, 2011).

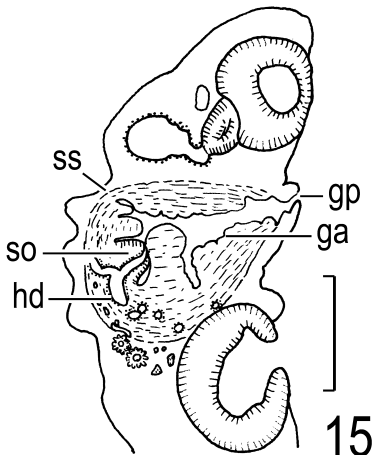
2) Primorskiy Kray, Russia: *S. asotus* (Layman, 1930; Zmeev, 1936; Belous, 1952; Akhmerov,



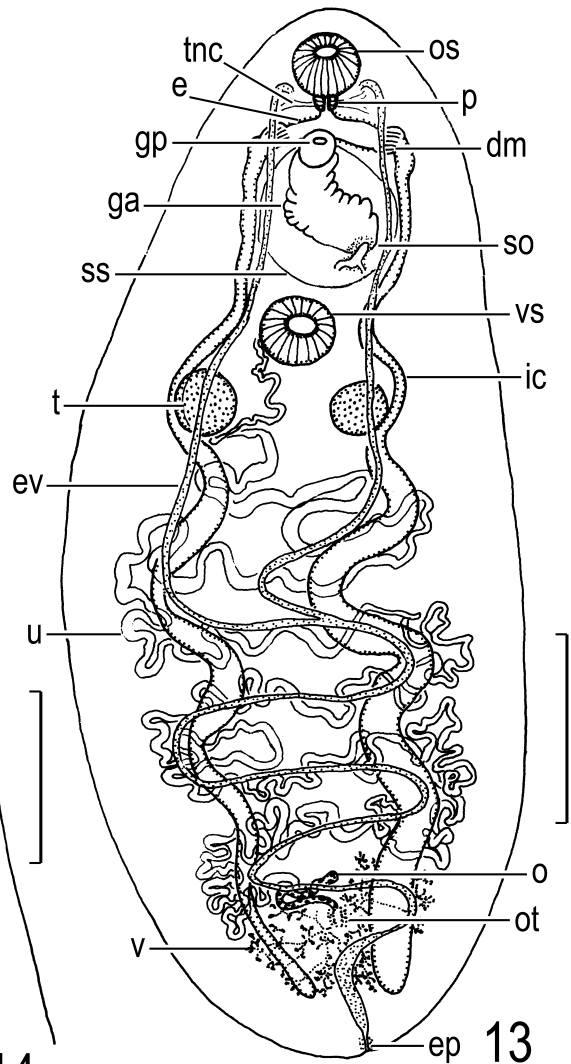
12



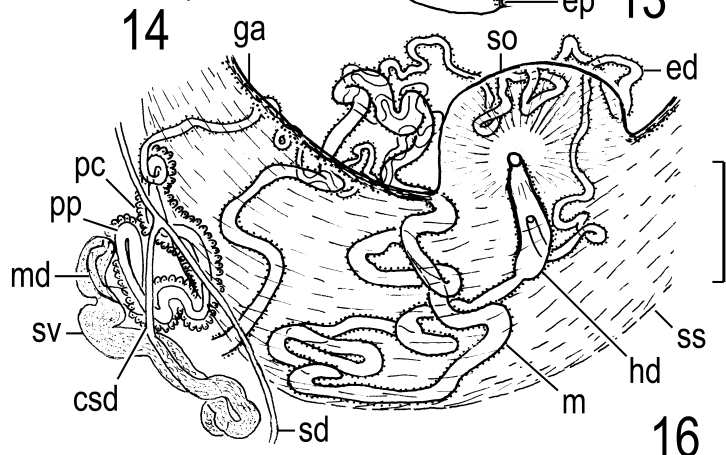
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13



16

[1961]; Bykhovskaya-Pavlovskaya, 1962; Strelkov, 1971; Bykhovskaya-Pavlovskaya and Kulakova, 1987; Ermolenko and Besprozvannykh, 1987a, b; Ermolenko, 1992; Butorina and Ermolenko, 1998; this paper); and *Silurus soldatovi* Nikolskii and Soin, 1948 (Akhmerov, [1961]; Strelkov, 1971; this paper).

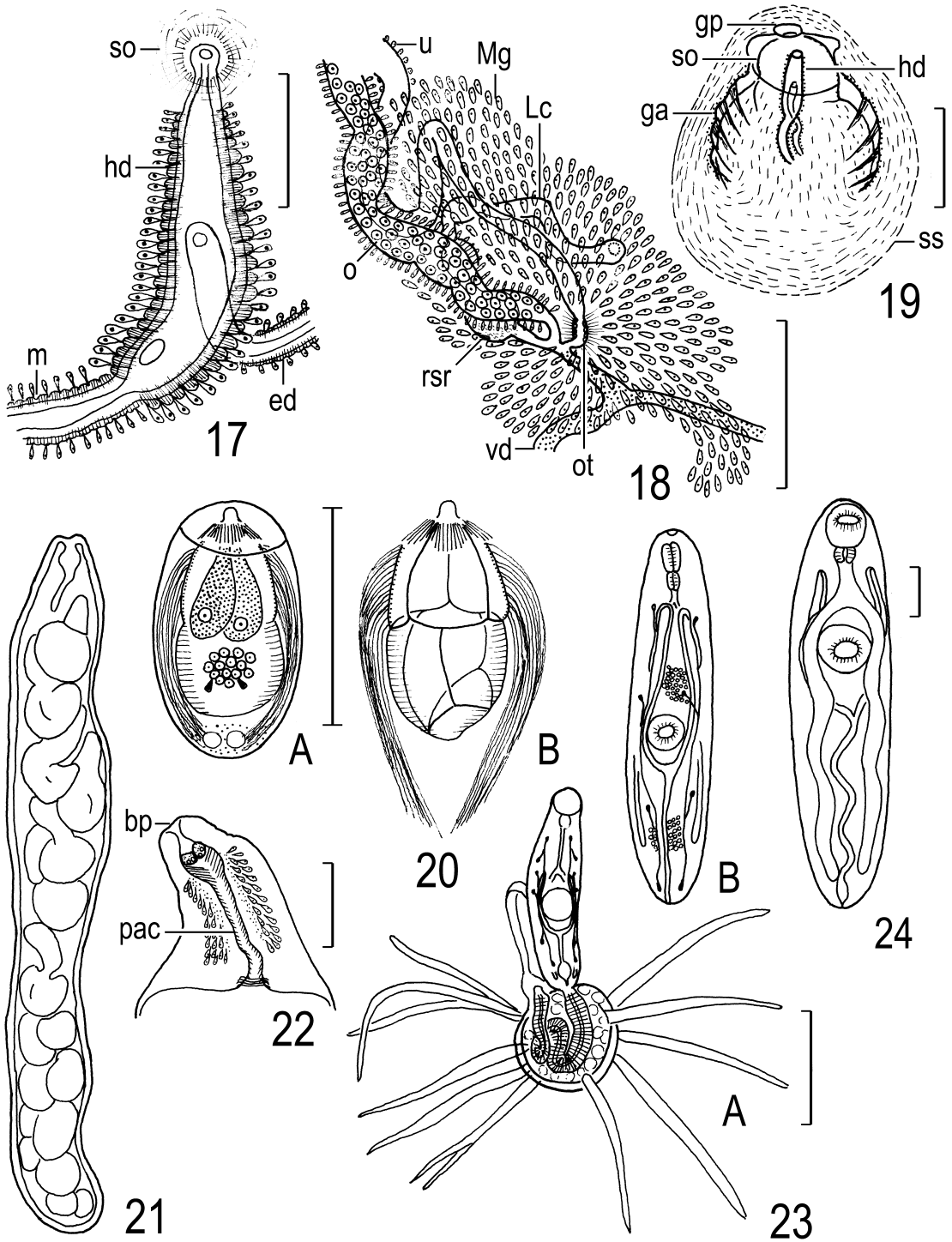
Site of infection. Air bladder (Kobayashi, 1915a; Shimazu *et al.*, 2011; Bykhovskaya-Pavlovskaya and Kulakova, 1987; this paper).

Material examined. (1) 1 specimen (SMNH Register Nos. 121146–121147, a syntype of *Leptolecithum eurytremum*) of *I. eurytremum*, adult, serially sectioned (other data not given) (Odhner, 1927). (2) Specimens of *I. hypselobagri*, ex air bladder of *Silurus asotus*, Nagano Prefecture, Japan: 3 (NSMT-PI 5407), adult, whole-mounted, Lake Kizaki in Oomachi City, 14 October 1980; and 10 (NSMT-PI 5408–5409), 1 juvenile, 9 adult, Lake Suwa at Suwa City, 19 May 1992 and 29 May 1993 (Shimazu, 2007). (3) 4 (Urabe's personal collection), adult, whole-mounted, ex air bladder of *S. asotus*, Chikugo River at Kobuchi Bridge, Miyoshikobuchi-machi, Hita City, Oita Prefecture, Japan, 25 August 2003. (4) Specimens, ex air bladder of *S. asotus*, Lake Biwa basin in Shiga Prefecture, Japan: 2 (NSMT-PI 5861), adult, whole-mounted, Lake Biwa off Isakifudo, Shirao-cho, Omihachiman City, 30 November 2010; 10 (NSMT-PI 5862–5864), adult, whole-mounted, Lake Biwa off Imazuhamu, Takashima City, 2 and 5 December 2010; 7 (NSMT-PI 5865, 6; NHMUK 2013.7.16.3, 1), adult, whole-mounted, Lake Biwa off mouth of Echi River, Higashiomi City, 17 December 2010; 11 (NSMT-PI 5866, 6; IPCAS D-695, 2; QM G 234254, 1; Nguyen Van Ha's personal collection, 2), adult, whole-mounted, Lake Biwa off Notogawa-cho, Higashiomi City, 18 December

2010; and 2 (NSMT-PI 5867, 1; NHMUK 2013.7.16.4, 1), adult, whole-mounted, Iba-naiko (a lake connected to Lake Biwa by Daido River), Higashiomi City, 20 December 2010. (3) 20 (NSMT-PI 5868, 17 whole-mounted, 2 serially sectioned; QM G 234255, 1 whole-mounted), adult, ex air bladder of *S. asotus*, Uji River at Uji City, Kyoto Prefecture, Japan, 20 May 2012. (5) 2 (NSMT-PI 5869), juvenile, serially sectioned, ex body cavity of *Gnathopogon elongatus elongatus* (Temminck and Schlegel, 1846) (Cyprinidae), irrigation canal closely connected to Yogo River at Nishiyama, Kinomoto-cho, Nagahama City, Shiga Prefecture, 27 May 2009. (6) 1 (NSMT-PI 5870), adult, hot formalin-fixed, whole-mounted, ex body cavity of *Sarcocheilichthys variegatus microoculus* Mori, 1927 (Cyprinidae), Lake Biwa off Momose-gyoko Fishing Port, Chinai, Makino-cho, Takashima City, 14 December 2011 (another 1 ethanol-fixed sample ISO33 sequenced). (7) Specimens from Primorskiy Kray, Russia: 5 (NSMT-PI 5871), adult, flattened, whole-mounted, ex air bladder of *S. asotus*, Razdol'naya River, 5 December 2004; 2 (NSMT-PI 5872), adult, flattened, whole-mounted, ex air bladder of *S. asotus*, Lake Khanka, June, 2001; 6 (NSMT-PI 5873), 1 juvenile, 5 adult, flattened, whole-mounted, ex air bladder of *S. asotus*, Amur River near Khabarovsk, 8 July 2011; 2 (NSMT-PI 5874), adult, flattened, whole-mounted, ex air bladder of *S. soldatovi*, Amur River near Khabarovsk, 8 July 2011; and 1 (IPCAS D-695), adult, flattened, whole-mounted, ex air bladder of *S. asotus*, Ilistaya River of Lake Khanka basin near Chernigovka, 22 June 2011.

Description. 1) Based on Odhner's serial sagittal sections of anterior half of body of 1 adult (Fig. 12; see also Odhner, 1927, fig. 1): oral sucker 1.00 long; ventral sucker 1.43 long;

Figs. 12–16. *Isoparorchis eurytremum*, adult specimens from Japan. — 12, syntype of *Leptolecithum eurytremum* (SMNH register nos. 121146–121147), forebody, sagittal section, reconstructed from several sections, neither host nor locality known; 13, small adult (NSMT-PI 5866) ex air bladder of *Silurus asotus*, entire body, ventral view; 14, large adult (NSMT-PI 5865) ex air bladder of *S. asotus*, forebody, ventral view; 15, large adult (NSMT-PI 5868) ex air bladder of *S. asotus*, forebody, sagittal section, reconstructed from several sections; 16, adult (NSMT-PI 5866) ex air bladder of *S. asotus*, part of terminal genitalia, ventral view. Scale bars: 5 mm in Figs. 13–14; 1 mm in Figs. 12 and 15; 0.5 mm in Fig. 16.



sucker length ratio 1:1.4; sinus sac 2.30 long, occupying 63% of forebody length, its base protracted like long thick column with small dome-like sinus organ 0.36 by 0.51 on it, genital atrium elongate, 2.11 long, occupying 89% of length of sinus sac; and hermaphroditic duct 0.49 long.

2) Based on other Japanese specimens (Figs. 13–18); 10 of adult specimens from Lake Biwa basin and Uji River measured. Body very large, 20–47 by 11–18; forebody without squaring shoulders, 7–15 long, occupying 27–37% of body length; hindbody elliptical. Oral sucker 1.26–2.46 by 1.39–2.22. Pharynx 0.63–1.27 in diameter. Esophagus 0.08–0.24 by 1.05–3.65. Drüsenmagen 0.32–1.19 by 0.51–1.03. Intestinal ceca undulating about 7 times in hindbody. Ventral sucker 1.11–3.49 by 1.66–3.65; sucker width ratio 1:1.2–1.4. Testes smaller than ventral sucker, 1.26–4.44 by 1.19–3.96. Seminal vesicle posterior to sinus sac. Male duct thin, lined with small epithelial cells. Pars prostatica posterodorsal to sinus sac. Sinus sac elliptical, large, 3.09–11.11 by 2.06–7.93, occupying 43–74% of forebody length, anterior to or overlapping ventral sucker (Figs. 13–14); genital atrium elliptical, elongated, 2.30–6.66 by 1.34–2.85, occupying 72–95% of sinus sac length. Sinus organ dome-like, small, 0.40–0.79 by 1.59 (Figs. 13–16). Ejaculatory duct long. Genital pore with sphincter. Ovary 3.28–10.92 by 0.14–0.21, dextral or sinistral. Rudimentary seminal receptacle 0.06–0.14 by 0.09–0.14. Uterus transversely folding about 7 times in intercecal field in hindbody; metraterm long; hermaphroditic duct 0.51–0.92 by 0.10–0.22 (Figs. 16–17); uterine seminal receptacle

present. Eggs light brown, 41–54 by 24–32 μ m. Vitelline area 2.06–6.82 by 2.78–12.22. Excretory vesicle bifurcating greatly anteriorly to ovary or a little anterior to middle of hindbody; excretory pore postero-terminal or -dorsal.

In adult specimen 13.25 by 7.30 from *S. variegatus microoculus*, base of sinus sac protracted like column with small domelike sinus organ on it (Fig. 19) as in Odhner's specimen (Fig. 12).

Remarks. Kobayashi (1915a) established *Leptolecithum eurytremum* as a new genus and species in Japanese. Later, Kobayashi (1921) gave a generic diagnosis of the new genus and a description of the type species in English. He must have found several specimens in the air bladder of *S. asotus* (syn. *Parasilurus asotus*) and "*Pseudobagrus aurantiacus*" (Japanese name: "Gigi" of Kobayashi) from Japan, but he seems to have presented measurements of only a single adult specimen. He listed the localities (type localities) including Lake Kasumigaura in Ibaraki Prefecture; Sawara, now in Katori City, Chiba Prefecture; Lake Biwa in Shiga Prefecture; and various places (not specified) in Okayama Prefecture. There is no doubt that *S. asotus* lived in all of these localities at that time as well as today. The question is where "*Pseudobagrus aurantiacus*" was really found infected, but he did not indicate it at all. The current species names of three related bagrids in Japan are: *Pseudobagrus tokiensis* Döderlein, 1869 (Japanese name: Gibachi) in Ibaraki and Chiba prefectures, *Tachysurus nudiceps* (Sauvage, 1883) (syn. *Pelteobagrus nudiceps* (Sauvage, 1883)) (Japanese name: Gigi) in Shiga and Okayama prefec-

Figs. 17–24. *Isoparorchis eurytremum* (continued), adult specimens from Japan and life cycle. — 17, adult (NSMT-PI 5866) ex air bladder of *Silurus asotus*, terminal thickening (hermaphroditic duct) of metraterm and ejaculatory duct, ventral view; 18, adult (NSMT-PI 5866) ex air bladder of *S. asotus*, ovarian complex, ventral view; 19, adult (NSMT-PI 5870) ex body cavity of *Sarcocheilichthys variegatus microoculus*, sinus sac and sinus organ, ventral view; 20, fully embryonated egg, egg (A), miracidium (B); 21, [most likely daughter] sporocyst ex *Juga* sp. from Primorskiy Kray, Russia, scale not given; 22, daughter sporocyst (NSMT-PI 5875) ex *Semisulcospira libertina* from Japan, anterior part of body; 23, *Cercaria introverta*, entire body ex *Se. libertina* from Japan (A), cercarial body proper (B), scale not given; 24, metacercaria ex an experimental second intermediate host, 21 days after infection, excretory vesicle should be ventral to intestinal ceca. Fig. 23A redrawn from Ito (1953); Figs. 21, 23B and 24 redrawn from Besprozvannykh (2000). Scale bars: 0.5 mm in Figs. 18, 19 and 22; 0.2 mm in Fig. 17; 0.1 mm in Figs. 23A and 24; 0.05 mm in Fig. 20.

tures, and *Pseudobagrus aurantiacus* (Temminck and Schlegel, 1846) (Japanese name: Ariakegibachi) in western Kyushu (Eschmeyer (ed.), 2013; Nakabo (ed.), 2013). No records of adult worms of *I. eurytremum* have appeared from bagrids in Japan since Kobayashi (1915a, 1921) (see *Hosts and localities*). Kobayashi did not designate a holotype nor a type host nor a type locality (see also Shimazu *et al.*, 2011).

Travassos (1922a, b) recognized *Leptolecithum* as a junior synonym of *Isoparorchis* and transferred *L. eurytremum* to *Isoparorchis* as *I. eurytremum* (see Shimazu *et al.*, 2011). Travassos (1922a) spelled the specific name *euritremen* [not *euritrema* in Shimazu *et al.*, 2011]. Ejsmont (1932) synonymized *I. eurytremum* with *I. hypselobagri*.

Odhner (1927, fig. 1) examined two adult ones of Kobayashi's syntypes and provided a specific diagnosis of *I. eurytremum* and a photomicrograph of a sagittal section through the genital pore of one of them. We reexamined this serially sectioned specimen (SMNH Register Nos. 121146–121147). Neither the host fish nor the locality of the specimen was indicated. Because it is believed that all the other syntypes were lost, Odhner's specimen is considered to be the only existent syntype of *L. eurytremum*.

The present molecular and morphological study shows that the present specimens from Japan and Primorskiy Kray, Russia, belong to the same species. The present phylogenetic tree (Fig. 1) indicates that it forms, together with the most similar *I. tandani*, a clade sister to *I. hypselobagri*, being dissimilar to the clade formed by *I. trisimilitubis* and *Isoparorchis* spp. 1 and 2. Kobayashi (1915a, pl. 2, fig. 1; 1921, pl. 26, fig. 1) did not clearly describe the anatomy of the terminal genitalia. In Odhner's syntype of *I. eurytremum* (Fig. 12), the sinus sac was large and elliptical, and the sinus organ was small and domed as discussed above. The present adult specimens had a large elliptical sinus sac, a large elongated genital atrium and a small domelike sinus organ (Figs. 13–16). Kobayashi (1915a, 1921) described the excretory vesicle as bifurcating greatly anteriorly

to the ovary or a little anterior to the middle of the hindbody. This feature was also observed in the present specimens. Consequently, we identify the species in Japan and Primorskiy Kray as *I. eurytremum*. Morphologically, *I. eurytremum* is distinct from *I. trisimilitubis* and *I. hypselobagri* (this paper) in the large elliptical sinus sac, large elongated genital atrium and small domelike sinus organ.

In Odhner's specimen, the base of the sinus sac was protracted like a long column, on which a small domelike sinus organ lay, into the large elongate lumen of the sinus sac (Odhner, 1927, fig. 1, gp; this paper, Fig. 12). Odhner (1927) referred to this long column as "einige gewaltige Genitalpapille." The terminal thickening of the metraterm stretched over the sinus organ and the anterior part of the column, and the ejaculatory duct and metraterm were longitudinally extended in the column. Obviously, the base of the sinus sac was protracted, but the sinus organ was not extended at all. The base of the sinus sac underneath the sinus organ is protractile by an unknown mechanism. Such a protraction should be apparently very rare in *I. eurytremum*, because, among the present specimens, the protraction was observed only in the adult specimen (Fig. 19) from the body cavity of *S. variegatus microoculus* (see also Shimazu *et al.*, 2011). On the other hand, it appears that the sinus organ itself is capable of contracting or extending in the other species (this paper; see also Gibson and Bray, 1979, fig. 2A, B).

In addition to Kobayashi's (1915a, 1921) records, *I. eurytremum* has previously been reported as *I. trisimilitubis* by Yamaguti (1934) (see Shimazu *et al.*, 2011) and as *I. hypselobagri* by Sawada and Osako (1969), Kifune (1978), Shimazu (2007) and Shimazu *et al.* (2011) from *Silurus asotus* and *S. biwaensis* in Japan. The description and figures by Shimazu *et al.* (2011) for the terminal genitalia include some misinterpretations: the metraterm in the wall of the sinus sac as the continuation of the uterus (figs. 31–32, u); the clavate terminal thickening of the metraterm as the hermaphroditic duct (figs. 31–32,

hd); and the sinus sac as the posterior portion of the genital atrium (figs. 30–31, ga). Shimazu *et al.* (2011) stated that the uterine seminal receptacle was not seen, but the present study shows that it is present as in the other species.

In Primorskiy Kray, Russia, adult specimens have previously been recorded as *L. eurytremum*, *I. trisimilitubis* or *I. pseudobagri* [sic, should be *hypselobagri*] from *S. asotus* (syn. *Parasilurus asotus*) (Layman, 1930; Zmeev, 1936; Belous, 1952; Akhmerov, [1961]; Strelkov, 1971; Ermolenko and Besprozvannykh, 1987a, b; Ermolenko, 1992; Butorina and Ermolenko, 1998) and from *S. soldatovi* (Akhmerov, [1961]; Strelkov, 1971). The site of infection was the air bladder in all of these records except Layman's (1930), in which the worm was obtained from the stomach of *S. asotus* (locality not given). None of these records were accompanied with a morphological description except Layman's (1930), which merely presented a few measurements of his material. In addition, Bykhovskaya-Pavlovskaya (1962) and Bykhovskaya-Pavlovskaya and Kulakova (1987) listed their hosts for *I. pseudobagri* or *I. hypselobagri*: *S. asotus*; *Pseudobagrus fulvidraco*, *Leiocassis ussuriensis* and *Leiocassis brashnikowi* (Bagridae); and *Esox reichertii* (Esociformes, Esocidae). Little was mentioned of the site of infection, developmental stage, etc. of these records. Belous (1952) found juveniles of *I. trisimilitubis* in the air bladder of *Ps. fulvidraco*. Bykhovskaya-Pavlovskaya (1962, fig. 985) and Bykhovskaya-Pavlovskaya and Kulakova (1987, fig. 94-1) gave the same diagnosis and figure [original (?)] of an adult specimen. Ermolenko and Besprozvannykh (1987a) figured an adult specimen found in the body cavity of *Leuciscus waleckii tumensis* (Cyprinidae). An elliptical large sinus sac and a long genital atrium suggest that these specimens are referred to *I. eurytremum*, though the position of bifurcation of the excretory vesicle is not shown. Bykhovskaya-Pavlovskaya (1962, fig. 986) and Bykhovskaya-Pavlovskaya and Kulakova (1987, fig. 94-2) presented the same figure of the terminal genitalia. However, this figure is a copy of the figure given by

Bovien (1927) for a barely mature specimen of *I. eurytremum* found in the body cavity of *Wallago attu* from Java. The specimen is certainly not *I. eurytremum* as will be discussed below in *Isoparorchis* sp. 4.

In China, Wu (1938, plate fig.) described adult specimens of *I. hypselobagri* from the air bladder of *Silurus asotus* (syn. *Parasilurus asotus*), *Pelteobagrus fulvidraco* and *Odontobutis obscura* (Odontobutidae) from Hangchow [Hangzhou]. A large elongate organ that he referred to as the sinus sac is possibly the sinus sac lumen (or the genital atrium). The excretory vesicle bifurcates a little anterior to the middle of the hindbody. Both the Institute of Hydrobiology, Hubei Province, China (chief ed.) (1973, pl. 53, fig. 8) and Song (1991, fig. 326) present the same figure of an adult that has a large globular sinus sac with a large elongated lumen, though the sinus organ was not mentioned. No adult specimens from China were made available to us for the present study. We reexamined the two juveniles (IPCAS D-213/2) of *I. hypselobagri* found in the body cavity of *Leiocassis longirostris* taken in the Yangtze River at Wuhan, Hubei Province (Moravec *et al.*, 2003). One of them had a large elliptical sinus sac, a large elongated lumen and a small domelike sinus organ. The other was a hindbody part only. The anatomy of the sinus sac suggests that these Chinese adults and juveniles are not *I. hypselobagri* but *I. eurytremum*.

Life cycle. Live eggs (54–62 by 30–33 μm) were fully embryonated in the uterus of adults (Fig. 20A, B). They did not hatch in water. The epidermal plates appeared to be arranged in 3:3:1 in fully formed miracidia. The plates in the first row bore many apical spines (3–13 μm) in the anterior third and many cilia slightly longer than the miracidial body in the posterior two-thirds. The other plates were naked. This naked part of the body was elastic. Two large cephalic gland cells, two flame cells, and posterior germ cells were observed.

Besprozvannykh and Ermolenko (1989) and Besprozvannykh (2000) studied the life cycle of *I. eurytremum*, under the species name *I. hypselo-*

bagri, in Primorskiy Kray, Russia. Natural first intermediate hosts were identified by them as species of *Juga* or *Parajuga* (Gastropoda, Pachychilidae): *Parajuga tegulata* and *Parajuga extersa* in the Razdol'naya River and *Parajuga heukelomiana* and *Parajuga nodosa* in the Mel'gunovka River of the Lake Khanka basin. Cystophorous cercariae (*Cercaria introverta*) were produced in [most likely daughter] sporocysts (Fig. 21). The mother sporocyst is not known. Metacercariae (Fig. 24) developed unencysted in the body cavity of cyclops (*Mesocyclops* sp. and *Acanthocyclops* sp.), a mayfly larva (*Ecdyonurus aurarius*) and an amphipod (*Gammarus* cf. *lacustris*) (experimental second intermediate hosts). The metacercariae were infective to larvae of fish (third intermediate hosts).

Adults live in the air bladder of the final hosts, *Silurus asotus*, *S. biwaensis* and *S. soldatovi*. Not only juvenile but also adult specimens have been found in the body cavity, musculature and internal organs of fishes of many species (e.g. Belous, 1952; Strelkov, 1971; Ermolenko and Besprozvannykh, 1987a, b; Shimazu, 2003; Shimazu *et al.*, 2011; this paper). We also obtained the following specimens in Primorskiy Kray, Russia: 2 adult (NSMT-PI 5875), ex body cavity, *Leuciscus waleckii* (Dybowski, 1869) (Cyprinidae), Razdol'naya River, June 2001; 1 adult (NSMT-PI 5876), ex musculature, *Hemibarbus maculatus* Bleeker, 1871 (Cyprinidae), Amur River near Khabarovsk, 2 July 2011: 14 juvenile and 1 adult (NSMT-PI 5877–5880), wall of esophagus and body cavity, *Huso dauricus* (Georgi, 1775) (Acipenseridae), Amur River near Khabarovsk, 3, 5 and 6 September 2011 (another 1 sample RUS1262 sequenced); 2 juvenile and 3 adult (NSMT-PI 5881), body cavity, *Pelteobagrus ussuriensis*, 4 September 2011; 1 adult (NSMT-PI 5882), wall of esophagus, *Siniperca chuatsi* (Basilevsky, 1855) (Percichthyidae), Lake Khanka, 22 June 2012; and the sample RUS1297 sequenced, juvenile, wall of esophagus, *Lota lota* (Linnaeus, 1758) (Lotidae), Amur River, near Nikolaevsk-na-Amure, 11 September 2010. Since the catfishes of *Silurus* are unlikely to eat the second

intermediate host, the intervention of the third intermediate host (fish paratenic hosts) between the second and final hosts is probably essential for completion of the life cycle. It remains unknown why worms are sometimes found fully gravid even in the organs other than the air bladder of fish paratenic hosts (Shimazu *et al.*, 2011). Yamaguti (1934) experimentally showed that juveniles in the intestine penetrated the intestinal wall into the body cavity and, eventually, entered the air bladder to mature sexually there, without taking a route through the pneumatic duct, in *Silurus asotus*.

The cystophorous cercaria reported by Besprozvannykh and Ermolenko (1989) and Besprozvannykh (2000) closely resembles *Cercaria introverta* Faust, 1924 (Besprozvannykh and Ermolenko, 1989; Besprozvannykh, 2000; Shimazu, 2003). The cercaria was first briefly described by Ando (1918) as *Cercaria* [U] from *Semisulcospira libertina* (Gould, 1859) (syn. *Melania libertina* Gould, 1859) (Gastropoda, Pleuroceridae) collected in Gifu Prefecture, Japan. Faust (1924) gave a new name, *Cercaria introverta*, to the cercaria, which he erroneously referred to as *Cercaria* sp. XIV of Ando, 1918. The cercaria (Fig. 23A) has been reported from *Semisulcospira* spp. at various places in Japan (Kobayashi, 1922; Ito, 1953; Makita *et al.*, 1996; Urabe, 2003). It has filaments on the cystophore (Ito, 1953, 1964; Besprozvannykh and Ermolenko, 1989; Makita *et al.*, 1996; Besprozvannykh, 2000), though neither Ando (1918) nor Kobayashi (1922) described the filaments. The cytochrome *c* oxidase I gene of the mitochondrial DNA (COI mtDNA) was sequenced from two samples: (1) adults of *I. eurytremum* found in *Silurus asotus* from Notogawa-cho, Higashiomi City, Shiga Prefecture; and (2) cercariae of *C. introverta* found in *Semisulcospira reiniana* (Brot, 1877) from Yoshida, Akitakada City, Hiroshima Prefecture. The partial (630 bp) sequences of the COI obtained proved to be identical between the two samples (DDBJ accession no. AB872958) (sequenced by M. Urabe), and we thus conclude that *C. introverta* is the cercaria of

I. eurytremum.

Kobayashi (1915a) erroneously presumed that *Cercaria problematica* Faust, 1924 from *Se. libertina* was the cercaria of *Leptolecithum eurytremum*. Urabe and Shimazu (2013) have recently confirmed that *C. problematica* is the cercaria of *Genarchopsis yaritanago* Shimazu, Urabe and Grygier, 2011, or now *Allogenarchopsis problematica* (Faust, 1924) (Derogenidae, Halipeginae).

Ando (1918) and Kobayashi (1922) referred to the [most likely daughter] germinal sac as a redia; but Ito (1953), Besprozvannykh and Ermolenko (1989) and Besprozvannykh (2000), as a sporocyst. We reexamined some of the formalin-preserved germinal sacs and cercariae (NSMT-PI 5883) obtained by Makita *et al.* (1996) from *Se. libertina* collected in [the Taki River at Nagasaka, Akame-cho, Nabari City, Mie Prefecture, on 31 August 1995]. The germinal sac was apparently a sporocyst, lacking a pharynx (Fig. 22). A long parturient canal had a sphincterlike structure in the anterior part and a sphincter in the posterior part. It was ciliated and surrounded by gland cells posteriorly to this structure. Ito (1953) gave the flame cell formula in the cercaria as $2[(2) + (2 + 2)] = 12$ (Fig. 23A); but Besprozvannykh and Ermolenko (1989) and Besprozvannykh (2000), as $2[(1 + 1) + (1 + 1)] = 8$ or $2[(2) + (2)] = 8$ (Fig. 23B). The flame cell formula needs determining definitively. The excretory arms in the cercaria (Fig. 23A, B) and metacercaria (Fig. 24) should turn backward laterally to the pharynx and then run backward ventrally to the ceca as in the stenostomate type.

Isoparorchis tandani Johnston, 1927

(Figs. 25–29)

A species of *Isoparorchis*: Johnston, 1914: 69.

Isoparorchis sp.: Johnston, 1916: 61.

Isoparorchis tandani Johnston, 1927: 129, 131–132, figs. 1–4.

Isoparorchis hypselobagri: Ejsmont, 1932: 456.

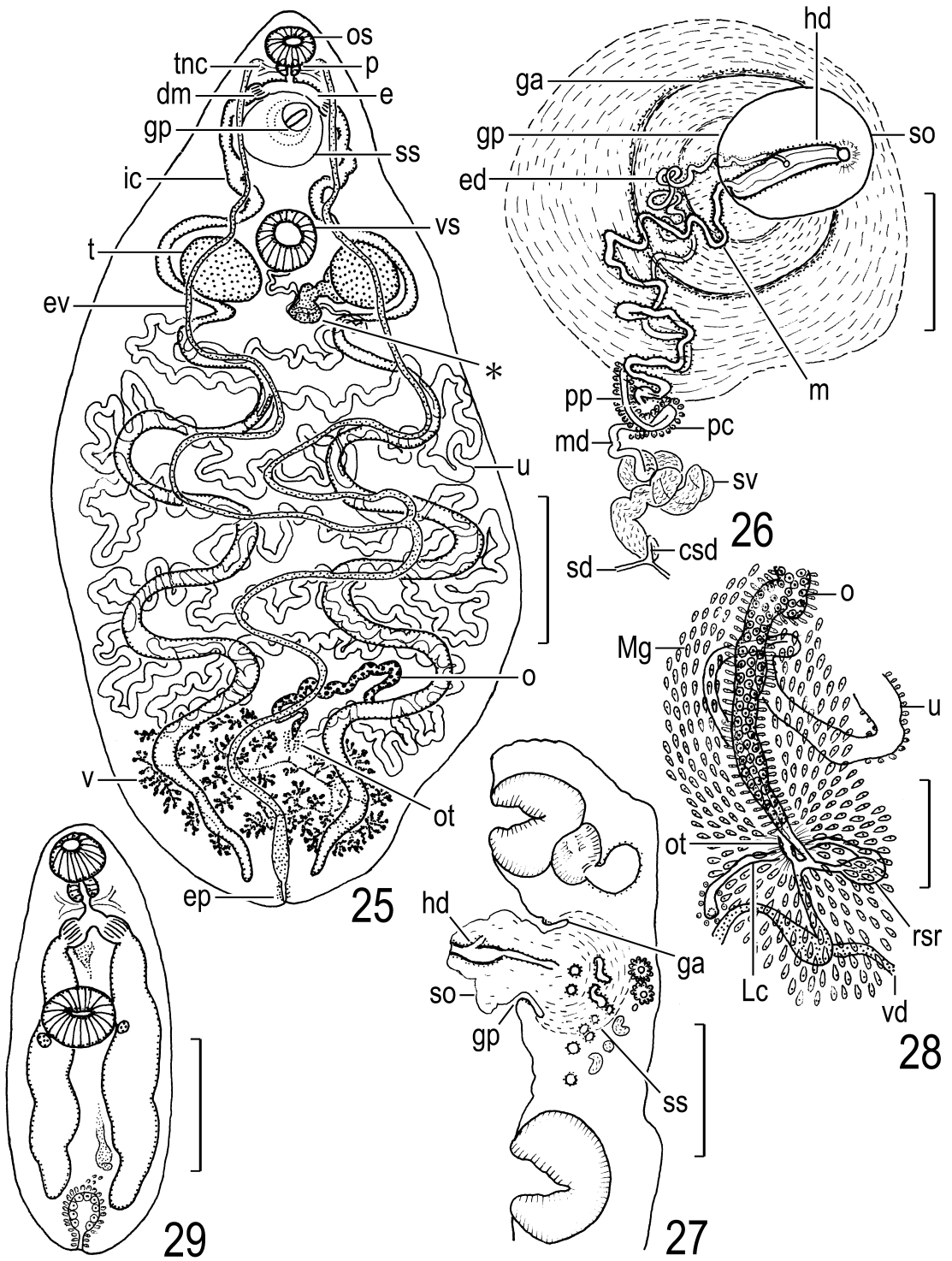
Host and localities. *Tandanus tandanus* (Mitchell, 1838) (Siluriformes, Plotosidae) (type

host) from Condamine River (type locality) near Warwick, Burnett River (type locality) at Eidsvold, and Dee River, all in Queensland (Johnston, 1914, 1916, 1927; this paper); Moggill Creek at Kenmore, Brisbane, Queensland (Cribb, 1988; this paper); and Gwydir River in New South Wales (NHMUK 1988.6.28.7; D. I. Gibson, personal communication), all in eastern Australia.

Site of infection. Air bladder (Johnston, 1914, 1916, 1927; Cribb, 1988; this paper).

Material examined. (1) 2 syntypes of *I. tandani*, adult, whole-mounted (remounted by us), ex air bladder of *Tandanus tandanus*, Queensland, Australia (Johnston, 1927): AHC 43089 (somewhat compressed), Burnett River (date not given); and AHC 43090, Condamine River, February 1911. (2) 2 specimens: one in Yamaguti's Collection (MPM Coll. No. 22013), adult, strongly flattened, whole-mounted, labeled "*Isoparorchis hypselobagri* (Billet, 1898) Host: *Tandanus tandanus*" (other data not given) (Shimazu, 2007); and the other formerly in J. Pearson's personal collection and now QM G 234256, adult, strongly flattened, whole-mounted, labeled "Hemiuroidea *Isoparorchis* Prof. Manter" (other data not given). The register of the former says that the specimen was collected and determined by H. W. Manter. It may be that H. W. Manter prepared these two specimens and donated one each to S. Yamaguti and J. Pearson. (3) Specimens (QM G 234257–65, 9 whole-mounted, February–July 1996; QM G 234266, 1 serially sectioned, February 1983; T. H. Cribb's personal collection, 1 whole-mounted, several sets of serially sectioned), adult, unflattened, ex air bladder of *T. tandanus*, Moggill Creek, 23 February 1983 (Cribb, 1988) and February, May and June 1996.

Description. 1) Based on 2 adult syntypes and Cribb's sagittal sections (Figs. 25–28). Body large, 25–30 by 12–15; forebody gradually tapering anteriorly, without squaring shoulders, 6–8 long, occupying 24–27% of body length; hindbody elliptical. Oral sucker 1.28–1.47 by 1.46–1.66. Pharynx 0.71 by 0.68–0.71. Esophagus 0.19–0.20 long. Drüsenmagen 0.55–0.79 by 0.40–0.52. Intestinal ceca undulating about 9 times in hind-



body. Ventral sucker 1.90–2.06 by 2.06–2.14; sucker width ratio 1:1.2–1.5, located at about junction of anterior and second fourths of body. Testes larger than ventral sucker, 2.22–2.85 by 1.98–2.22. Sperm ducts long; common sperm duct short, anterior to ventral sucker. Seminal vesicle long, thick, convoluted, anterior to or overlapping ventral sucker; male duct thin, lined with large epithelial cells. Pars prostatica long, curved, posterodorsal or posterior to sinus sac. Sinus sac small, globular, 1.98–2.54 by 2.30–2.85, occupying 32–33% of forebody length; genital atrium globular, small, 1.00–1.16 by 0.71–1.11, occupying 46–50% of sinus sac length. Sinus organ large, contracted like double volcano and occupying most of genital atrium, or extending and protruding to outside through genital pore. Hermaphroditic duct 0.49–0.90 by 0.14–0.20. Ovary sinistral, located at about junction of third and posterior fourths of body, 8.14–9.53 by 0.28–0.32. Laurer's canal fairly long, opening to dorsal outside; rudimentary seminal receptacle 0.12–0.14 by 0.09–0.12. Uterine seminal receptacle present. Eggs 45–52 by 24–25 μm . Vitelline area 4.92–6.03 by 6.03–8.80. Excretory vesicle bifurcating greatly anteriorly to ovary or at about middle of hindbody; excretory pore posteroterminal.

2) Based on 2 strongly flattened specimens; 8 of 10 unflattened specimens in parentheses. Body very large, 36–37 by 14–15 (13–23 by 7–12); forebody 7–9 long (4–8 long), occupying 19–25% (24–31%) of body length. Oral sucker 1.27–1.66 by 1.58–1.90 (1.00–1.63 by 1.00–1.95). Pharynx 0.71–0.84 by 0.79 (0.40–0.87 by 0.47–0.82). Esophagus 0.17–0.19 long (0.16–0.17 by 0.71–1.08); Drüsenmagen 0.35–0.43 by 0.46–0.79 (not clearly observed). Ventral sucker 2.14–2.38 by 2.06–2.38 (1.28–2.14 by 1.24–2.38);

sucker width ratio 1:1.2–1.3 (1:1.2–1.4). Testes larger than ventral sucker, 1.90–2.54 by 1.90–2.54 (1.59–2.78 by 1.35–2.46). Sinus sac small, globular, 2.78–2.85 by 2.38 (1.42–2.22 by 1.59–2.06), occupying 32–40% (28–42%) of forebody length. Genital atrium small, globular, 1.19–1.42 by 1.06–1.26 (0.87–1.10 by 0.87–1.03), occupying 43–50% (43–68%) of sinus sac length. Hermaphroditic duct 0.63–0.71 by 0.19–0.28 (not clearly observed). Ovary sinistral, 8.75–9.39 by 0.32 (not clearly observed). Vitelline area 7.45–8.73 by 7.93–9.20 (not clearly observed). Eggs 43–51 by 22–29 μm (44–59 by 24–31 μm). Excretory vesicle bifurcating a little anterior to middle of hindbody (not clearly observed).

Remarks. Johnston (1914) recorded a species of *Isoparorchis* from the air bladder of *Copidoglanis* (now *Tandanus*) *tandanus* from the Condamine River (Murray-Darling system) near Warwick, Queensland; and, later (Johnston, 1916), *Isoparorchis* sp. from the air bladder of *Copidoglanis tandanus* from the Burnett River at Eidsvold and Dee River (Dawson-Fitzroy system) in Queensland (see also Johnston, 1927). Johnston (1927) described this species as *Isoparorchis tandani* Johnston, 1927 from four adult specimens (syntypes) found in the air bladder of *Tandanus tandanus* (Mitchell, 1838) from the Condamine, Dee and Burnett rivers. He mentioned the body size in four specimens, but the position of the ovary in six. His original material including the four syntypes has been deposited in the SAM. We reexamined two syntypes. The two others are: AHC 21700, Burnett River, June 1916; and AHC 21701, Burnett River (date not given) (Leslie Chisholm, Curator, personal communication). As far as we know, nobody else has previously examined or described any specimens of *I. tandani* including the four syntypes.

Figs. 25–29. *Isoparorchis tandani*, adult specimens ex air bladder of *Tandanus tandanus* from Australia and life cycle. — 25, syntype (AHC 43089), entire body, contents (*) pressed out of left testis, ventral view; 26, syntype (AHC 43089), terminal genitalia, ventral view; 27, adult (QM G 234266), forebody, sagittal section, reconstructed from several sections; 28, syntype (AHC 43089), ovarian complex, ventral view; 29, metacercaria, ex cephalothorax of either *Caridina indistincta* or *Paratya australiensis*. Scale bars: 5 mm in Fig. 25; 1 mm in Figs. 26–27; 0.5 mm in Fig. 28; 0.2 mm in Fig. 29.

The present reexamination of the two syntypes shows that Johnston (1927) overlooked the common sperm duct and pars prostatica, and he misinterpreted the hermaphroditic duct (fig. 3, *d.h.*) as eversible and Laurer's canal as terminating blindly in a rounded or pyriform seminal receptacle (fig. 2, *r.s.*). This seminal receptacle is actually a rudimentary seminal receptacle (Fig. 28, *rsr*), or a distinct dilatation of Laurer's canal with a dorsal opening (Fig. 28, *Lc*) (see also Cribb, 1988).

Johnston (1927) stated that *I. tandani* differed markedly from *I. trisimilitubis* and *I. eurytremum* in the shape of the body, sucker ratio, relative size of the forebody length to the body length, size of the testes and position of bifurcation of the excretory vesicle. In spite of that, Ejsmont (1932) reduced *I. tandani* to a junior synonym of *I. hypselobagri*.

Morphologically, *I. tandani* differs from *I. trisimilitubis*, *I. hypselobagri* and *I. eurytremum* (this paper) chiefly in that the testes are larger than the ventral sucker. It is further distinguished from *I. trisimilitubis* by the shape of the forebody (without instead of with shoulders) and a smaller genital atrium (occupying 46–50% instead of 55–74% of the sinus sac length). *Isoparorchis tandani* is also phylogenetically distinct from the other species sequenced here (Fig. 1). Moreover, *I. tandani* is geographically endemic in the eastern part of Australia. Consequently, we retain *I. tandani* as a valid species. Shimazu (2007) was mistaken in saying that the strongly flattened specimen (MPM Coll. No. 22013) was morphologically similar to the Japanese specimens of *I. hypselobagri*, or now *I. eurytremum*.

Life cycle. A natural final host is confined to *T. tandanus* in eastern Australia (Johnston, 1927; Cribb, 1988; this paper). Cribb (1988) studied the life cycle in the field and laboratory. Two unidentified cystophorous cercariae were obtained from the prosobranch snail *Posticobia brazieri* (Smith, 1882) (Hydrobiidae) collected in Moggill Creek. When eggs were experimentally exposed to experimental first intermediate hosts, *P. brazieri* and *Melanoides tuberculata* (Müller,

1774) (Thiaridae), tubular mother sporocysts were recovered from both.

Natural second intermediate hosts are atyid shrimps, *Caridina indistincta* Calman, 1926 and *Paratya australiensis* Kemp, 1917, in the cephalothorax of which metacercariae were found unencysted in Moggill Creek in 1991 and 1996. In metacercariae (0.33–2.54 by 0.12–0.95) (QM G 234267–76, 10 metacercariae, October 1991; T. H. Cribb's personal collection), the stem of the Y-shaped excretory vesicle formed a dilatation lined with large epithelial cells in the posterior part (Fig. 29).

Small juveniles (QM G 234277–86, 10 worms, ex liver or body cavity, 28 February 1983, 15 February 1984, 11 March 1984, 12 May 1984; T. H. Cribb's personal collection) were found in the liver and body cavity of *T. tandanus* from Moggill Creek. Neither juveniles nor adults have been recorded from any fishes other than *T. tandanus*. Since the final host *T. tandanus* feeds on insect larvae, prawns, crayfish, mollusks and small fishes, it is probable that it becomes infected with metacercarial worms by eating the second intermediate hosts *Caridina indistincta* and *Paratya australiensis*.

***Isoparorchis pakistani* Bilquees and Khatoon, 1972, species inquirenda**

Isoparorchis pakistani Bilquees and Khatoon, 1972: 49, figs. 1–3.

Isoparorchis hypselobagri: Bhutta and Khan, 1975: 16; Zaidi and Khan, 1977: 29.

Host and locality. *Ophiocephalus striatus* [now *Channa striata* (Bloch, 1793)] (Perciformes, Channidae) (type host) from Pakistan (type locality) (Bilquees and Khatoon, 1972).

Remarks. Bilquees and Khatoon (1972) described *Isoparorchis pakistani* Bilquees and Khatoon, 1972 on the basis of two adult specimens found in the air bladder of *Ophiocephalus striatus* from West Pakistan. They also made a histological study of adults of this species. Their description says: a pair of the "oral glands" and three "testes glands" are present; the sucker width

ratio is low, 1:0.9–1.0 (our calculation); the male and female openings, each encircled by a prominent sucker, are present in the hermaphroditic sac [the sinus sac interpreted in this paper]; Laurer's canal is absent; the stem of the excretory vesicle is I-shaped, extending forward [to posterior to the ventral sucker (?)]; and eggs are 39–55 by 25–29 μm . If this description is correct, *I. pakistani* is quite different from *I. trisimilitubis* (this paper) and might represent a new genus and species mainly because a hermaphroditic duct is absent and Laurer's canal is absent. On the contrary, Bhutta and Khan (1975) said that *I. pakistani* resembles *I. hypselobagri* in all essential features. Bhutta and Khan (1975) and Zaidi and Khan (1977) synonymized *I. pakistani* with *I. hypselobagri* [*I. trisimilitubis* in this paper]. Our request to the senior author for the loan of the type material was not answered. We here regard *I. pakistani* as a *species inquirenda*.

In addition, Bilqees and Khatoon (1972) reported two [adult (?)] specimens (fig. 5) of *I. trisimilitubis* with the sucker width ratio of 1:1.4 (our calculation from fig. 5) from *Ophiocephalus striatus* and an *Isoparorchis* metacercaria [juvenile] (fig. 6) with the sucker width ratio of 1:2.4 (our calculation) from the body cavity of *Entropiichthys vacha* Hamilton, 1822 (Siluriformes, Schilbeidae). This suggests that both *I. trisimilitubis* and *Isoparorchis* sp. 3 (this paper) occur in Pakistan as well as in India.

Life cycle. Not known.

Isoparorchis sp. 3

(Figs. 30–33)

Isoparorchis trisimilitubis Southwell, 1913, in part (?): 92–94 (?), pl. 8, fig. 10 (?).

Isoparorchis trisimilitubis: Odhner, 1927: 1.

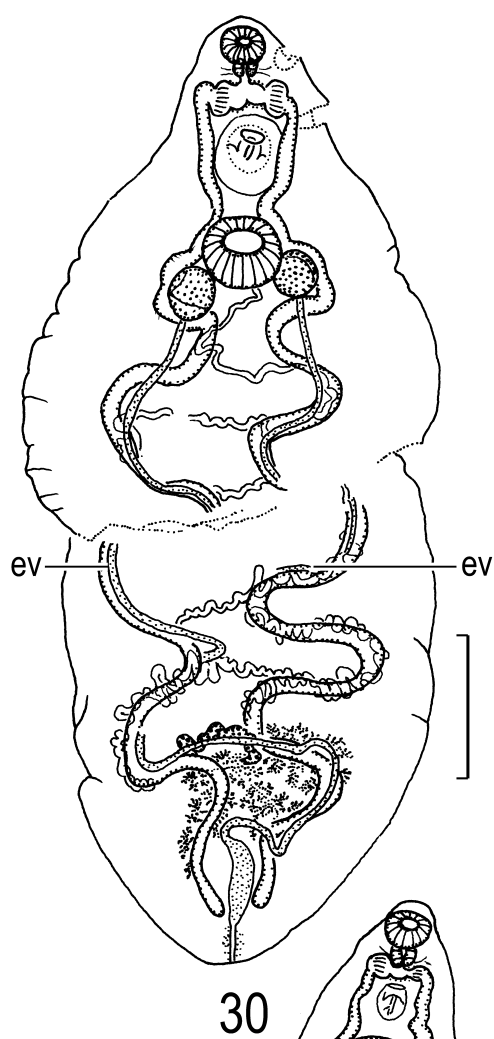
Host and localities. *Wallago attu* from a freshwater tank at Bankipur [in Patna, Bihar State (?)], India (Southwell, 1913); Lucknow, India (this paper); and Bangladesh (this paper).

Site of infection. Air bladder.

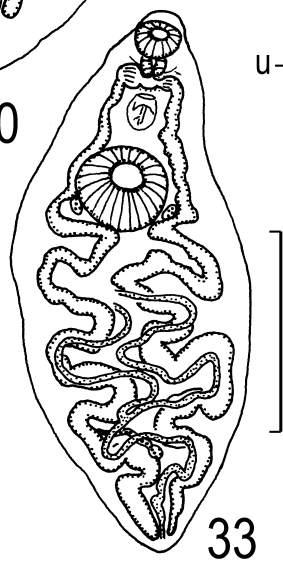
Material examined. (1) 2 syntypes 1 and 2

(NHMUK 2000.4.10.78–80) of *I. trisimilitubis* (species 2), adult, 1—whole-mounted, 2—serially sectioned, ex air bladder of *Wallago attu*, freshwater tank at Bankipur, March 1912 (Southwell, 1913; see *I. trisimilitubis* in this paper); and 1 syntype (SMNH Register Nos. 121143 and 121145) of *I. trisimilitubis*, juvenile, anterior half of body, serial sagittal sections (Odhner, 1927). (2) 4 specimens of *I. hypselobagri*: NHMUK 1953.7.20.46–48, 1 adult, whole-mounted, ex *W. attu*, Bangladesh (other data not given); NHMUK 1976.1.5.101–107, 2 juvenile, whole-mounted, ex *W. attu*, Lucknow, India (other data not given); and NHMUK 1990.10.23.41–43, 1 juvenile, whole-mounted, ex *Ompok pabda* (Hamilton, 1822) (Siluridae), Bangladesh (other data not given).

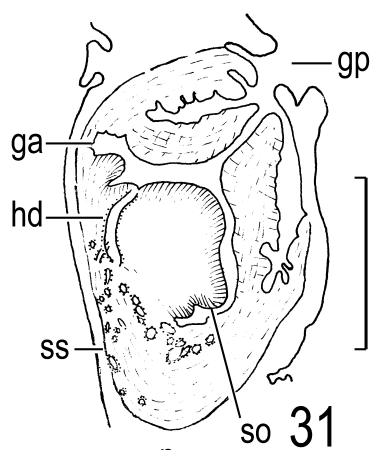
Description. 1) Based on 2 adult syntypes 1 and 2 of *I. trisimilitubis* (Figs. 30–31). Body torn in parts, wrinkled, scratched, large, 32 by 15; forebody rapidly tapering anteriorly, with squaring shoulders at about middle, 8 long, occupying 25% of body length; hindbody almost elliptical. Oral sucker 1.30 by 1.47 (1.27 long in sagittal section). Pharynx 0.95 by 0.94. Esophagus 0.16 by 1.19. Drüsenmagen 1.29–1.43 by 0.95–1.27. Intestinal ceca undulating about 8 times in hindbody. Ventral sucker 2.62 by 2.73 (2.54 long in sagittal section); sucker width ratio 1:1.9 (sucker length ratio 1:2.0) (sucker length ratio 1:2.0 in sagittal section). Testes smaller than ventral sucker, 1.32–1.66 by 1.35–1.59. Seminal vesicle anterodorsal to ventral sucker. Male duct fairly long, thick, lined with large epithelial cells. Pars prostatica posterodorsal to sinus sac. Sinus sac almost globular, 3.01 by 2.12, occupying 38% of forebody length; genital atrium globular, 1.62 by 1.38, occupying 54% of sinus sac length. Sinus organ large, contracted like double volcano, filling genital atrium. Ovary dextral, 8.86 by 0.25. Rudimentary seminal receptacle 0.13 by 0.09 in sagittal section. Hermaphroditic duct 0.41 by 0.16 in sagittal section; uterine seminal receptacle seen. Eggs 43–49 by 21–24 μm . Vitelline area 5.87 by 6.03. Excretory vesicle not clearly traced, but fragments of two arms seen slightly



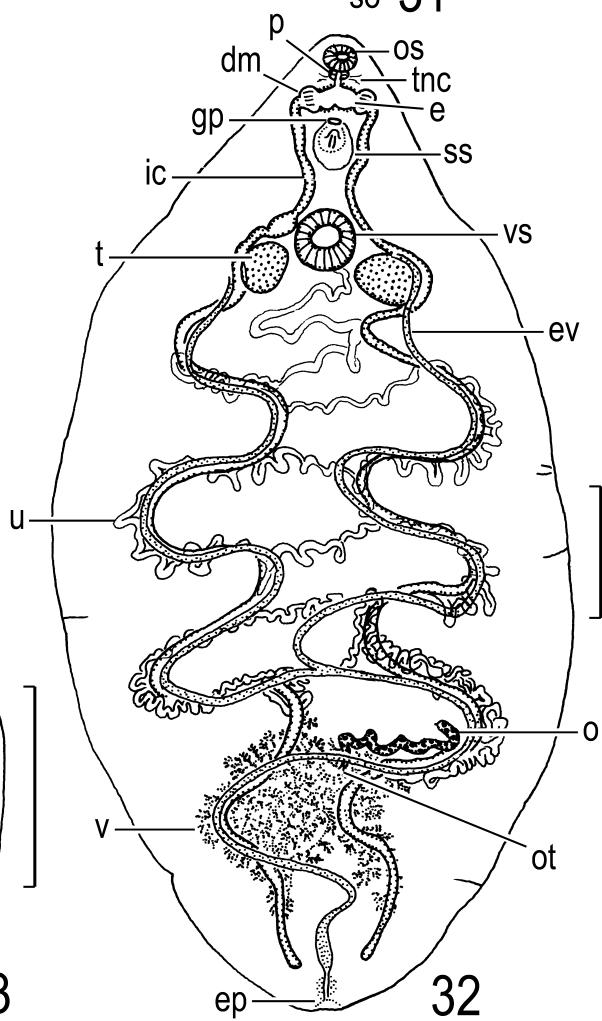
30



33



31



32

anterior to ovary; excretory pore posterodorsal.

2) Based on 1 adult (Fig. 32); 3 juveniles in parentheses (Fig. 33). Body very large, 37 by 19 (7.06–13.65 by 3.33–6.34); forebody squaring shoulders, 7 long, (with or without squaring shoulders, 2.70–4.44 long), occupying 19 (32–38)% of body length; hindbody broad-elliptical. Oral sucker 1.05 by 1.27 (0.71–1.00 by 0.84–1.08). Pharynx 0.87 by 0.85 (0.46–0.63 by 0.46–0.63). Esophagus 1.10 by 1.60 (0.43–0.46 by 0.39–0.53). Drüsenmagen 0.94–0.95 by 0.59–0.71 (0.50–0.71 by 0.39–0.50). Ventral sucker 2.35 by 2.22 (1.59–2.22 by 1.67–2.38); sucker width ratio 1:1.7 (1:1.9–2.2). Testes smaller than ventral sucker, 2.30–2.38 by 1.67 (small). Seminal vesicle anterior to ventral sucker. Male duct fairly long, thick, lined with large epithelial cells. Pars prostatica curved. Ejaculatory duct long, much convoluted. Hermaphroditic duct not clearly observed. Sinus sac elliptical, small, 2.22 by 1.35 (elliptical, 0.51–0.95 by 0.36–0.79), occupying 32 (18–21)% of forebody length; genital atrium elliptical, small, 0.90 by 0.79 (elliptical, 0.16–0.55 by 0.17–0.52), occupying 40 (31–58)% of sinus sac length. Sinus organ large, not clearly observed. Ovary sinistral, 7.57 by 0.36 (dextral or sinistral, 0.21–1.43 long). Rudimentary seminal receptacle 0.14 by 0.08. Uterine seminal receptacle seen. Metraterm long, much convoluted. Eggs 40–48 by 24–29 μ m. Vitelline area 8.73 by 8.25 (vitellaria undeveloped). Excretory arms bifurcating slightly anteriorly to ovary (bifurcating slightly anteriorly to ovary); excretory pore posterodorsal.

3) Based on 1 juvenile syntype. Oral sucker 0.71 long; ventral sucker 1.43 long; sucker length ratio 1:2.0; sinus sac 0.47 by 0.27, occupying 16% of forebody length; lumen of sinus sac 0.33 long, occupying 71% of sinus sac; and

sinus organ small, 0.11 long.

Remarks. The two adult syntypes 1 and 2 and juvenile syntype of *I. trisimilitubis* (species 2) and four other specimens had a high sucker ratio of 1:1.7–2.2. The two excretory arms were seen, though incompletely, slightly anterior to the ovary (Fig. 30) in the syntype 1. This suggests that the excretory vesicle bifurcated slightly anteriorly to the ovary. The excretory vesicle could not be traced in the sectioned syntype 2. However, it bifurcated slightly anteriorly to the ovary in the adult from Bangladesh (Fig. 32) and the three juveniles (Fig. 33). These specimens are considered to belong to the same species, which has the two correlated morphological features: a high sucker ratio and the excretory vesicle bifurcating slightly anteriorly to the ovary. This species is readily separated by these two characteristics from *I. trisimilitubis*, *I. hypselobagri*, *I. eurytremum* and *I. tandani* (this paper).

The syntypes 1 and 2 are fully gravid but poor whole-mounts. The juvenile syntype is only the sectioned anterior part of the body. The specimen (NHMUK 1953.7.20.46–48) is fully gravid and a better whole-mount but from Bangladesh. The three others are immature. Although the species is obviously an undescribed species, it remains as *Isoparorchis* sp. 3. As mentioned above under *I. trisimilitubis*, it is unknown at present whether *Isoparorchis* sp. 1 and 3 (Table 1, Fig. 1) are the same species. We need some well-preserved additional adult specimens of *Isoparorchis* spp. 1 and 3 for sequencing, morphological observation and description of a new species, but no additional adult specimens were made available to us.

The present materials had the sucker ratio of 1:1.7–2.2, which was almost the same as that (1:2) of Odhner's (1927); and the excretory vesicle bifurcating slightly anteriorly to the ovary as

Figs. 30–33. *Isoparorchis* sp. 3, adults and juvenile. — 30, adult, syntype 1 (NHMUK 2000.4.10.78–80) of *I. trisimilitubis*, ex air bladder of *Wallago attu* from India, entire body, ventral view; 31, syntype 2 (NHMUK 2000.4.10.78–80), forebody, sagittal section, showing terminal genitalia; 32, adult (NHMUK 1953.7.20.46–48) of *I. hypselobagri*, ex *W. attu* from Bangladesh, entire body, ventral view; juvenile (NHMUK 1976.1.5.101–107) of *I. hypselobagri*, ex *W. attu* from Lucknow, India, entire body, ventral view. Scale bars: 5 mm in Figs. 30, 32 and 33; 1 mm in Fig. 31.

figured by Southwell (1913, fig. 10). Eggs were slightly larger than described by Odhner (1927), 40–49 by 21–24 μm instead of 0.04–0.043 by 0.2 [sic, most likely 0.02] mm.

Achan (1956) reported specimens as *I. hypselobagri* found in the air bladder of *W. attu* from Madras State, India. Although the ovary is omitted in the figure (fig. 2), this material is likely to belong to *Isoparorchis* sp. 3 because of a high sucker width ratio, 1:2.6 (our calculation from fig. 2); a small globular cirrus sac [the sinus sac in this paper] occupying 28% (our calculation from fig. 2) of the forebody length; and the excretory vesicle bifurcating just anterior to the ootype. Devaraj (1972) obtained immature and adult worms from the air bladder of *W. attu* taken in the Bhavanisagar Reservoir, India. In one (fig. 1) of the immature specimens, the sucker width ratio was 1:2.4 (our calculation). In this respect, it is probably identified as *Isoparorchis* sp. 3. As mentioned above, Bilqees and Khatoon (1972) reported a juvenile with the sucker width ratio of 1:2.4 from Pakistan.

Life cycle. Not known. Devaraj (1972) observed that ciliated epidermal cells were arranged in six tiers in the miracidium.

Isoparorchis sp. 4

(Figs. 34–36)

Isoparorchis: Dollfus, 1959: fig. 2, table 2.

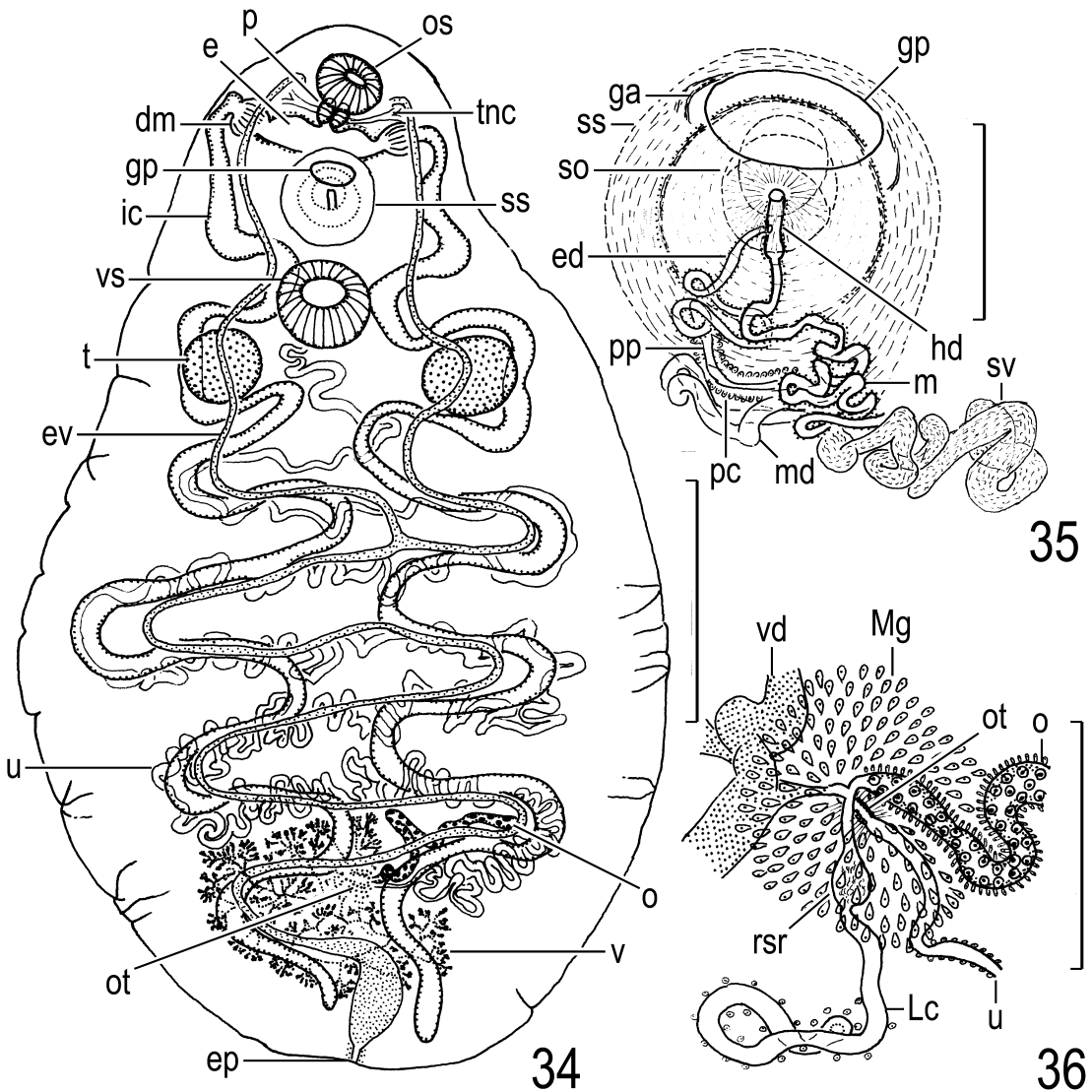
Host and locality. “Cà léo” (*Wallago attu*) from “Région de Cà-Tho (Cochinchine)” [Can Tho Province, southern Vietnam] (Dollfus, 1959; Arthur and Bui Quang Te, 2006; this paper).

Site of infection. Air bladder (Dollfus, 1959).

Material examined. 2 specimens of Dollfus (1959) (MNHN, Collection Dollfus: BD 60-17 and BD 60-20, adult, whole-mounted) of “*Isoparorchis hypselobagri* (A. Billet 1898),” ex air bladder of “Cà léo” (*Wallago attu*, determined by Arthur and Bui Quang Te, 2006), “Région de Cà-Tho (Cochinchine)” [Can Tho Province, southern Vietnam], 28 January 1928 [labeled 1929].

Description. Body fairly large, 13–19 by 8–12; forebody 4–5 long, occupying 26–28% of body length, with squaring shoulders. Oral sucker 0.95–1.16 by 1.11–1.32. Pharynx 0.63–0.65 by 0.50–0.68. Esophagus 0.32–0.60 by 0.95–1.98. Drüsenmagen 0.47–0.63 by 0.35–0.82. Intestinal ceca undulating about 8 times in hindbody. Ventral sucker 1.43–1.59 by 1.47–1.75; sucker width ratio 1:1.3. Testes slightly smaller than ventral sucker, 0.82–1.47 by 0.73–1.74. Seminal vesicle convoluted. Male duct thick, long, convoluted. Pars prostatica curved. Ejaculatory duct fairly long, convoluted. Hermaphroditic duct 0.38 by 0.05 (in BD 60-17). Sinus sac globular, 1.27–1.71 by 1.11–1.74, occupying 28–35% of forebody length. Genital atrium globular, 1.17 by 1.21 (in BD 60-17), occupying 68% (in BD 60-17) of sinus sac length. Sinus organ like double volcano, large, filling up genital atrium. Ovary sinistral, 2.51–4.86 by 0.17–0.22. Rudimentary seminal receptacle 0.10 by 0.07. Uterine seminal receptacle not seen. Eggs 40–48 by 21–25 μm . Vitelline area 2.22–3.41 by 2.85–4.60. Excretory vesicle bifurcating greatly anterior to ovary or about halfway between ovary and testes.

Remarks. Dollfus (1959, fig. 2) seems to have briefly described and figured the forebody of one (BD 60-17, Fig. 34) of these two adult specimens, but he erroneously repeated the measurements of the oral and ventral suckers and pharynx of an immature specimen (fig. 1) for this adult specimen. Our measurements of BD 60-17 were: oral sucker 0.95 by 1.11, ventral sucker 1.43 by 1.47, and pharynx 0.63 by 0.50. Although the two specimens are similar in morphology to *I. trisimilitubis* (this paper), we here treat them as *Isoparorchis* sp. 4. Since it is possible that both *Isoparorchis* sp. 4 from southern Vietnam and *Isoparorchis* sp. 2 from Cambodia (Table 1, Fig. 1) were from *W. attu* collected in the Mekong River basin, these two may be the same species. Because *Isoparorchis* sp. 2 (sample VNT353) had been sequenced before morphological observations for precise identification could be undertaken, nothing is known at present about its morphology. However, it is phylogenetically similar



Figs. 34–36. *Isoparorchis* sp. 4, Dollfus' (1959) adult specimen (MNHN BD 60-17) of *I. hypselobagri*, ex air bladder of *Wallago attu* from southern Vietnam. — 34, entire body, ventral view; 35, terminal genitalia, ventral view; 36, ovarian complex, ventral view. Scale bars: 5 mm in Fig. 34; 1 mm in Fig. 35; 0.5 mm in Fig. 36.

to *I. trisimilitubis* (Fig. 1). Further molecular and morphological studies are needed of new materials from southern Vietnam and Cambodia.

In addition, Bovien (1927, fig. 3) described adult specimens as *I. eurytremum* found in the body cavity of *W. attu* from Java. We do not accept this identification. They are different from *I. eurytremum* (this paper) but similar to *I. trisimilitubis* (this paper) in that the sucker length

ratio is low (1:1.2, our calculation); the sinus sac is smaller, occupying only 32% (our calculation) of the forebody length; and the genital papilla [the sinus organ in this paper] is large and like a double volcano in the contracted state (fig. 5), filling the genital atrium. Ejsmont (1932) reported adult specimens as *I. hypselobagri* found in a fish (not specified) from the Chao Phraya River near Bangkok, Thailand. In the text figure (p. 455),

the sucker width ratio is about 1:1.1, the sinus sac occupies about 27% of the forebody length, and the sinus organ occupies about 73% of the length of the sinus sac (our calculation). These

two materials resemble *I. trisimilitubis* (this paper), but further molecular and morphological studies are needed of new materials from these localities to identify them definitively.

Key to the species of *Isoparorchis* in this paper

- 1.1. Sucker (width) ratio high, 1:1.7–2.0; excretory vesicle bifurcating slightly anteriorly to ovary; India and Bangladesh..... *Isoparorchis* sp. 3
- 1.2. Sucker (width) ratio low, 1:1.1–1.6; excretory vesicle bifurcating greatly anteriorly to ovary 2
- 2.1. Sinus sac elliptical, large; genital atrium elongated, large; sinus organ domelike, small; Japan and Primorskiy Kray (Russia)..... *I. eurytremum*
- 2.2. Sinus sac globular, small; genital atrium globular, small; sinus organ like double-volcano, large..... 3
- 3.1. Testes larger than ventral sucker; Australia *I. tandani*
- 3.2. Testes smaller than ventral sucker 4
- 4.1. Sucker (width) ratio 1:1.1–1.4; sinus sac occupying 20–34% of forebody length; genital atrium occupying 55–74% of sinus sac length; male duct thick; India..... *I. trisimilitubis*
- 4.2. Sucker (width) ratio 1:1.2–1.6; sinus sac occupying 28–40% of forebody length; genital atrium occupying 45–69% of sinus sac length; male duct thin; northern Vietnam..... *I. hypselobagri*

Taxonomic conclusion

On the basis of combined molecular and morphological analysis, we hypothesize that four (*I. trisimilitubis*, *I. tandani*, *I. eurytremum* and *I. hypselobagri*) of the existing nominal species of *Isoparorchis* should be recognized as valid along with an undescribed species, *Isoparorchis* sp. 3. We consider *I. pakistani* to be a *species inquirenda*. There is considerable complexity and some uncertainty associated with this hypothesis relating to the size and difficulty of the specimens, the fact that the material is very poor or untraceable for several reports (especially original species descriptions, type specimens), the fact that reported host identifications are sometimes apparently unreliable, and the fact that our analyses suggest that some fish species harbor two species of the genus, in one case apparently in sympatry.

The type species, *I. trisimilitubis*, was described from India on the basis of specimens which we conclude represent two co-occurring

species, *I. trisimilitubis* and *Isoparorchis* sp. 3, which differ especially in the sucker ratio and in the position of bifurcation of the excretory vesicle. Specimens consistent with *I. trisimilitubis* are known only from India whereas *Isoparorchis* sp. 3 is known from India and Bangladesh and possibly from Pakistan. Reports of species of *Isoparorchis* from India are sometimes ascribable to one of these two species, but often there is no basis on which to make a decision because of a lack of detailed description or available specimens. Both species are known convincingly as adults only in the bagrid *Wallago attu*.

Isoparorchis hypselobagri is perhaps the least well-understood species. Its circumscription suffers from difficulties with type material, original description and figure and original identification of its type host. However, this species is presently known only in northern Vietnam where it is most frequently a parasite of the silurid *Silurus asotus*.

Isoparorchis eurytremum occurs in Japan where it is evidently the only species of the

genus present and infects *Silurus asotus* and *S. biwaensis* at least. This species is also found in Primorskiy Kray, Russia, again principally in *S. asotus* and *S. soldatovi*.

Isoparorchis tandani is restricted to the Australian plotosid catfish *Tandanus tandanus* and is the only species known in the eastern part of the continent.

Sequences of the ITS2 rDNA support the distinction between the five species recognized above with at least 3 and up to 18 base pair differences between each pair of putative species. Sequence replication ranges from only one sequence (*I. tandani*) to eight (*I. eurytremum*), but no intraspecific variation was detected. The most phylogenetically similar pair of species, *I. eurytremum* and *I. tandani*, which differ by just three bases, are distinct morphologically and geographically. In addition to the five named species discussed here, a single sequence from a specimen from *Wallago attu* from Cambodia differs from the sequences of *I. trimilitubis* by three base pairs. There is insufficient satisfactory material to explore the morphology of this form, but it remains possible that it corresponds to the sixth distinct species, possibly *Isoparorchis* sp. 4. The same problem relates to the identity of specimens reported from Java and Thailand for which there are also no molecular data.

General Discussion

As shown in the Introduction, the history of *Isoparorchis* is one of the relatively rapid proposal of four species (between 1898 and 1927) followed by apparently somewhat uncritical revision not based on the examination of type specimens which led to their synonymy by 1932 (Ejsmont, 1932). Subsequently, there were many published reports of the genus but none that critically reconsidered taxonomy of the genus, though considerable progress was made with aspects of biology including life history. Here, careful revision of as much morphological material as could be gathered together with new molecular data together clearly suggest that there

are at least five distinct species in the genus and the possibility of more. With the benefit of hindsight, this history is unsurprising. Probably, a key contributor to the problem has been the sheer size of the worms and the corresponding difficulty with which their morphology is studied. In the present study, the morphology of these specimens was challenging due both to the size and to the fact that many specimens were not well preserved.

We conclude that the optimal fixation of specimens for whole-mounted preparations is as follows: place a living specimen on a slide glass, pour a small amount of fixative on it to kill it gently, put a cover glass on it, bind the two glasses with a white cotton thread to prevent the anterior part of the body from curving ventrally and finally soak the specimen thus sandwiched between the two glasses in fixative for a day or more. Do not kill specimens in hot saline or hot fixative, or the intestinal ceca and uterus of them will be torn to pieces. We recommend fixing specimens with AFA and then staining them with Heidenhain's iron hematoxylin.

The generation of molecular data was important in this study in corroborating the conclusions based on morphology in a study in which the use of the two data sources was partly iterative. The value of molecular data in studies of trematodes such as this is now so well-established that it requires no further comment except to observe that it also here allowed the identification of a juvenile from *Mastacembelus favus* and the definitive identification of the cercaria of *I. eurytremum*. The molecular data also corroborate the uncertainty regarding the identity of the form from Cambodia and point to the fact that the taxonomic analysis of the genus should not be considered complete.

The most important implication of the present findings is the change in our understanding of the genus from comprising a single exceptionally widespread species with somewhat variable biology in different localities to a complex of species. In part, the likelihood that this would prove to be the case is present in some of the informa-

tion about aspects of the life cycle of the various species. In particular, as reported here, *I. tandani* is a parasite of *Tandanus tandanus*, which becomes infected by the ingestion of freshwater atyid shrimps. This is consistent with the diet of this species which rarely includes fish. In contrast, all the northern hemisphere species infect siluriform fishes which are significantly piscivorous, correlating with the numerous reports of juvenile specimens of *Isoparorchis* in the body cavity and tissues of fishes for *I. trisimilitubis*, *I. hypselobagri* and *I. eurytremum*. Those of *I. eurytremum* are sometimes fully gravid adults. In addition, the apparently narrow host ranges of the various species at different localities, when numerous other siluriform species are present at most localities, is also anomalous if just one rather than multiple species is present.

Perhaps, the most challenging aspect of the results presented here is the demonstration that *Wallago attu* harbors two different species in sympatry (and perhaps a third allopatrically) and that *Silurus asotus* harbors two (as far as is known) allopatrically. In the first place, this calls for parasitologists to exercise extreme care in the identification of species in the genus: it is by no means certain that all the species have been discovered, and it is certain that the full geographical distribution of these species is not known. Explanation of the radiation of the genus implied by these hosts and geographical distributions is probably not yet possible, given the incompleteness of the data.

Acknowledgements

We are indebted to Dr. Sven Boström (SMNH), Mr. Jimmy Cassone (MNH), Mrs. Eileen Harris (Department of Life Sciences, NHM), Dr. František Moravec (IPCAS), Dr. Leslie Chisholm (SAM), Dr. Toshiaki Kuramochi (NMNS, Tsukuba) and Dr. Takashi Iwaki (MPM) for the loan of the specimens; Dr. David I. Gibson (Department of Life Sciences, NHM) for observing specimens for us; Dr. Koji Tojo (Department of Mountain and Environmental Science, Interdisciplinary

Graduate School of Science and Technology, Shinshu University, Matsumoto) for laboratory facilities; Dr. Mark J. Grygier (Lake Biwa Museum, Kusatsu, Shiga Prefecture) for helpful suggestions concerning nomenclature; and Dr. Yukifumi Nawa (Department of Parasitology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand) for helpful suggestions about parasitologists in Southeast Asia. Thanks are also due to Dr. Tomáš Scholz (IPCAS) for supplying and lending us his specimens collected in the Indo-Czech expeditions to India in 2009 and 2011 and for reviewing the manuscript and Dr. Anirban Ash (CSIR-Indian Institute of Chemical Biology, Kolkata, West Bengal, India) and Dr. Pradip Kumar Kar (Jhargram Raj College, Paschim Medinipur, West Bengal, India) for attempting to collect additional specimens in India for us.

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