



Sinorhodeus microlepis, a new genus and species of bitterling from China (Teleostei: Cyprinidae: Acheilognathinae)

FAN LI^{1,2,3,5}, TE-YU LIAO², RYOICHI ARAI⁴ & LIANGJIE ZHAO³

¹Institute of Biodiversity Science, Ministry of Education Key Laboratory for Biodiversity Science and Ecological Engineering, Fudan University, Shanghai 200433, P. R. China.

²Department of Oceanography, National Sun Yat-Sen University, Kaohsiung 80424, Taiwan, R. O. China.

³Shanghai Ocean University, Shanghai 200090, P. R. China.

⁴Department of Zoology, University Museum, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo. 113-0033, Japan.

E-mail: arayo@um.u-tokyo.ac.jp

⁵Corresponding author. E-mail: lfaqua@gmail.com

Abstract

A new genus and species, *Sinorhodeus microlepis* **gen. et sp. nov.**, is described from a tributary of the Yangtze River, in Chongqing City, China. *Sinorhodeus* **gen. nov.** can be distinguished from four closely related genera, *Paratanakia*, *Pseudorhodeus*, *Rhodeus*, and *Tanakia*, by the following combination of characters: pharyngeal teeth 0,0,4–4,0,0, longitudinal scales 41–46, white spots on dorsal-fin rays absent, a black blotch on dorsal fin in juvenile absent, and less developed wing-like yolk sac projections in larvae. Phylogenetic analysis of one mitochondrial gene and six nuclear genes supports the establishment of the new genus.

Key words: Cyprinidae, *Rhodeus*, *Tanakia*, phylogeny, Yangtze River, China

Introduction

The subfamily Acheilognathinae, commonly known as the bitterlings, comprise approximately 81 species in five genera and one unnamed clade and are distributed throughout Eurasia (Arai & Akai 1988; Yang *et al.* 2011; Kim *et al.* 2014; Li & Arai 2014). The genera *Acheilognathus* Bleeker with 40 species (Yang *et al.* 2011; Nguyen *et al.* 2013), *Tanakia* *sensu lato* (including *Tanakia* Jordan & Thompson, *Paratanakia* Chang, Chen & Mayden, and *Pseudorhodeus* Chang, Chen & Mayden) with nine species (Arai & Akai 1988; Chang *et al.* 2014; Kim *et al.* 2014), and an unnamed clade with three species (Yang *et al.* 2010; Chang *et al.* 2014), are confined to East Asia (Kottelat 1998; Lin 1998; Kottelat 2001; Hosoya 2002; Chen & Chang 2005; Bogutskaya *et al.* 2009; Kim *et al.* 2014), while the genus *Rhodeus* Agassiz with 19 species, is widespread from East Asia to Western Europe (Bohlen *et al.* 2006; Chang *et al.* 2014; Li & Arai 2014).

The Yangtze River contains a high diversity of acheilognathines, including 13 species of *Acheilognathus*, five species of *Rhodeus*, one species of *Paratanakia*, and three species of the unnamed clade (Miao 1934; Lin 1935; Wu 1964; Lin 1998; Doi *et al.* 1999; Yang *et al.* 2010; Li & Arai 2011; Chang *et al.* 2014; Li & Arai 2014; Zhang *et al.* 2016), among which three species were recently described (Yang *et al.* 2010; Li & Arai 2011; Li & Arai 2014) and more may be waiting to be discovered.

A highly distinctive bitterling was discovered from a tributary of the Yangtze River in Chongqing City in central China. After morphological examination and molecular phylogenetic analysis, this species represents a distinct lineage and is described herein.

Materials and methods

Morphological analyses. Methods for counts and measurements follow Hubbs & Lagler (2004). The last two rays

of the dorsal and anal fins were counted as one ray. Vertebrae and unpaired fin rays of type specimens were counted from radiographs made with the Kodak DXS 4000 system. Vertebral number includes the Weberian complex (as 4) and the terminal urostyle (as 1). The first caudal vertebral centrum is the centrum with a haemal spine. The insertion of the proximal segment of the first pterygiophore in the dorsal and anal fins is expressed according to Arai *et al.* (1995, 2007). Positions of the first dorsal- and anal-fin ray pterygiophore (D-PTG-1 and A-PTG-1, respectively) were examined from radiographs. When the proximal radial of D-PTG-1 is inserted between neural spines of the vertebral centra n th and $(n+1)$ th, the position of D-PTG-1 is expressed as D-PTG-1 = n . When the proximal radial of A-PTG-1 is inserted between haemal spines of the vertebral centra m th and $(m+1)$ th, or in front of the first haemal spine supported by vertebral centrum $(m+1)$, the position of A-PTG-1 is expressed as A-PTG-1 = m . Pharyngeal teeth and gill rakers were observed by dissecting some non-type specimens. Infraorbital bones were observed from cleared and stained specimens. Cephalic sensory canals were observed from cleared and stained and formalin-fixed specimens. The host mussel species was determined based on field and aquarium observations.

Examined specimens in this study are deposited in the following collections: AMNH, American Museum of Natural History, New York; BMNH, The Natural History Museum, London; IHCAS, Institute of Hydrobiology, Chinese Academy of Science, Wuhan; NMW, Naturhistorisches Museum, Wien; NSMT, National Museum of Nature and Science, Tokyo; NTUM, National Taiwan University, Taipei; SMU, Sang Myung University, Seoul; SOU, Shanghai Ocean University, Shanghai; ZUMT, Department of Zoology, University Museum, University of Tokyo, Tokyo.

Molecular phylogenetic analyses. Genomic DNA was extracted from fins preserved in 95% ethanol using a modified phenol-chloroform method (Sambrook & Russell 2001). All primers of the six nuclear markers (recombination activating gene 1 [RAG1], rhodopsin [RH], interphotoreceptor retinoid-binding protein gene 2 [IRBP2], early growth response protein genes [EGR1, 2B, and 3]) and one mitochondrial gene (cytochrome *b*, [Cyt *b*]) were from Chen *et al.* (2003, 2008), López *et al.* (2004) and Chang *et al.* (2014). PCR protocols of all genes followed Chang *et al.* (2014). The PCR products were purified and sequenced using an ABI 3730 analyzer by Sangon Biotech Co., Ltd (Shanghai). The sequences of new species in this study are available on GenBank; the other sequences were obtained from GenBank, primarily from Chang *et al.* (2014). A full list of taxa with corresponding GenBank accession numbers is provided in Table 1.

Seven genes were aligned using MEGA 7 (Kumar *et al.* 2016) and checked by eye. Phylogenetic relationships were estimated using two methods, maximum likelihood (ML) and Bayesian inference (BI), both with partitions based on genes and codon position. *Danio dangila* was designated as the outgroup for tree rooting. RAxML 7.0.4 (Stamatakis 2006) was used for ML analyses. The most appropriate model of sequence evolution (GTR + I + G) was selected under the Akaike Information Criterion (AIC) (Akaike 1974). Bootstrap support was conducted with 1000 reiterations with RAxML (Felsenstein 1985). Bayesian inference was run in MrBayes v3.2 (Ronquist & Huelsenbeck 2003). All parameters except topology and branch length were allowed to vary independently using the unlink command in MrBayes v3.2 (Ronquist & Huelsenbeck 2003). Analyses were conducted with 11 models for 21 partitions as suggested by jModelTest 2 (Darriba *et al.* 2012; Table 2) sampling for six million generations (two simultaneous analyses, nruns = 2; three heated chains, nchains = 4; chain temperature 0.1; sample frequency 1000; burnin = 25%; the average standard deviation of split frequencies of 0.000364). Trees were visualized in FigTree v.1.3.1 (<http://tree.bio.ed.ac.uk/software/figtree>).

Results

Sinorhodeus gen. nov. Li, Liao & Arai

Diagnosis. *Sinorhodeus* can be distinguished from all other genera of Acheilognathinae by the following characters: pharyngeal teeth 0,0,4–4,0,0, longitudinal scales 41–46, transverse scales 16–18, white spots on dorsal-fin rays absent, a black blotch on dorsal fin in juvenile absent, less developed wing-like yolk sac projections in larvae. Similar to *Rhodeus* in absence of barbels and incomplete lateral line, but distinguished from it by absence of white spots on dorsal-fin rays (vs. present), absence of a black blotch on dorsal fin in juvenile (vs. present), and less developed wing-like yolk sac projections in larvae (vs. well developed, Fig. 1A). Similar to *Tanakia* sensu lato (*Tanakia*, *Paratanakia*, and *Pseudorhodeus*) in absence of white spots on dorsal-fin rays, absence of a black blotch

TABLE 1. GenBank accession numbers for sequences of seven genes used in this paper. Abbreviations: Cyt *b*, Cytochrome *b*; RAG1, recombination activation gene 1; RH, Rhodopsin; IRBP2, interphotoreceptor retinoid-binding protein; gene 2; EGR1, 2B, and 3, early growth response protein genes 1, 2B, and 3.

species	GenBank accession number							Reference
	Cyt <i>b</i>	RAG1	RH	IRBP	EGR1	EGR2B	EGR3	
Acheilognathinae								
<i>Sinothodeus microlepis</i> 1	MF186795	MF186810	MF186813	MF186807	MF186798	MF186801	MF186804	This study
<i>Sinothodeus microlepis</i> 2	MF186796	MF186811	MF186814	MF186808	MF186799	MF186802	MF186805	This study
<i>Sinothodeus microlepis</i> 3	MF186797	MF186812	MF186815	MF186809	MF186800	MF186803	MF186806	This study
<i>Acheilognathus macropterus</i>	KF410728	KF417786	KF429389	KF434667	KF442313	KF444589	KF460185	Chang <i>et al.</i> 2014
<i>Acheilognathus meridianus</i>	KF410732	KF417790	KF429393	KF434671	KF442317	KF444593	KF460189	Chang <i>et al.</i> 2014
<i>Acheilognathus rhombeus</i>	KF410738	KF417796	KF429399	KF434677	KF442323	KF444599	KF460195	Chang <i>et al.</i> 2014
<i>Acheilognathus tabira tabira</i>	KF410741	KF417799	KF429402	KF434680	KF442326	KF444602	KF460198	Chang <i>et al.</i> 2014
<i>Acheilognathus typus</i>	KF410746	KF417804	KF429407	KF434685	KF442331	KF444607	KF460203	Chang <i>et al.</i> 2014
" <i>Acheilognathus</i> " <i>striatus</i>	KF410740	KF417798	KF429401	KF434679	KF442325	KF444601	KF460197	Chang <i>et al.</i> 2014
<i>Paratanakia chii</i>	KF410799	KF417857	KF429460	KF434738	KF442384	KF444660	KF460256	Chang <i>et al.</i> 2014
<i>Paratanakia himantegus</i>	KF410804	KF417862	KF429465	KF434743	KF442389	KF444665	KF460261	Chang <i>et al.</i> 2014
<i>Pseudorhodeus tanago</i>	KF410813	KF417871	KF429474	KF434752	KF442398	KF444674	KF460270	Chang <i>et al.</i> 2014
<i>Rhodeus albomarginatus</i>	KF410780	KF417838	KF429441	KF434719	KF442365	KF444641	KF460237	Chang <i>et al.</i> 2014
<i>Rhodeus amarus</i>	KF410751	KF471809	KF429412	KF434690	KF442336	KF444612	KF460208	Chang <i>et al.</i> 2014
<i>Rhodeus atremius</i>	KF410759	KF417817	KF429420	KF434698	KF442344	KF444620	KF460216	Chang <i>et al.</i> 2014
<i>Rhodeus colchicus</i>	KF410762	KF417820	KF429423	KF434701	KF442347	KF444623	KF460219	Chang <i>et al.</i> 2014
<i>Rhodeus fangi</i>	KF410764	KF417822	KF429425	KF434703	KF442349	KF444625	KF460221	Chang <i>et al.</i> 2014
<i>Rhodeus meridionalis</i>	KF410765	KF417823	KF429426	KF434704	KF442350	KF444626	KF460222	Chang <i>et al.</i> 2014
<i>Rhodeus ocellatus kurumeus</i>	KF410782	KF417840	KF429443	KF434721	KF442367	KF444643	KF460239	Chang <i>et al.</i> 2014
<i>Rhodeus ocellatus ocellatus</i>	KF410776	KF417834	KF429437	KF434715	KF442361	KF444637	KF460233	Chang <i>et al.</i> 2014
<i>Rhodeus pseudosericeus</i>	KF410783	KF417841	KF429444	KF434722	KF442368	KF444644	KF460240	Chang <i>et al.</i> 2014
<i>Rhodeus sericeus</i>	KF410785	KF417843	KF429446	KF434724	KF442370	KF444646	KF460242	Chang <i>et al.</i> 2014
<i>Rhodeus shitanensis</i>	KF410787	KF417845	KF429448	KF434726	KF442372	KF444648	KF460244	Chang <i>et al.</i> 2014
<i>Rhodeus sinensis</i>	KF410789	KF417847	KF429450	KF434728	KF442374	KF444650	KF460246	Chang <i>et al.</i> 2014
<i>Rhodeus suigenis</i>	KF410798	KF417856	KF429459	KF434737	KF442383	KF444659	KF460255	Chang <i>et al.</i> 2014
<i>Tanakia lanceolata</i>	KF410807	KF417865	KF429468	KF434746	KF442392	KF444668	KF460264	Chang <i>et al.</i> 2014
<i>Tanakia limbata</i>	KF410809	KF417867	KF429470	KF434748	KF442394	KF444670	KF460266	Chang <i>et al.</i> 2014
Outgroup								
<i>Danio dangila</i>	NC_015525	EU292697	EU409660	EU409662	EU409724	EU409756	EU409788	Chang <i>et al.</i> 2014
<i>Nipponocypris sieboldii</i>	NC_008653	EU292713	FJ197069	FJ197120	FJ531283	FJ531312	FJ531341	Chang <i>et al.</i> 2014

on dorsal fin in juvenile, and less developed wing-like yolk sac projections in larvae (Fig. 1B), but distinguished from them by uninterrupted incomplete lateral line (vs. complete in *Tanakia* and *Paratanakia*, interrupted incomplete in *Pseudorhodeus*) and absence of barbels (vs. present). Distinguished from *Acheilognathus* by incomplete lateral line (vs. complete, except *A. typus* with incomplete lateral line), absence of white spots on fin-rays of dorsal fin (vs. present), and less developed wing-like yolk sac projections in larvae (vs. not developed, Fig. 1C). *Sinorhodeus* also can be further distinguished from all other genera by fewer pharyngeal teeth (0,0,4–4,0,0 vs. 0,0,5–5,0,0) (Table 3).

TABLE 2. Models applied for each codon of seven genes. Numbers refer to codon positions. Refer to Table 1 for abbreviations of genes.

	F81	F81+G	F81+I	GTR+G	GTR+I	GTR+I+G	HKY+I	JC+G	JC+G+I	HKY	HKY+G
Cyt <i>b</i>	1										*
	2										*
	3			*							
EGR1	1										*
	2	*									
	3			*							
EGR2B	1									*	
	2	*									
	3										*
EGR3	1							*			
	2								*		
	3				*						
IRBP2	1						*				
	2		*								
	3										*
RAG1	1	*									
	2				*						
	3			*							
RH	1				*						
	2					*					
	3			*							

TABLE 3. Diagnoses of six genera of the subfamily Acheilognathinae.

Character	<i>Sinorhodeus</i>	<i>Rhodeus</i>	<i>Tanakia</i> / <i>Paratanakia</i>	<i>Pseudorhodeus</i>	<i>Acheilognathus</i>
Morphology of adults					
Lateral line	incomplete	incomplete	complete	interrupted incomplete	complete except <i>A. typus</i>
Barbels	absent	absent	present	present	present or absent
Whitish spots on fin-rays of dorsal fin form two transverse bands	absent	present	absent	absent	present
Pharyngeal teeth	0,0,4–4,0,0	0,0,5–5,0,0	0,0,5–5,0,0	0,0,5–5,0,0	0,0,5–5,0,0
Morphology of juveniles					
A black blotch on dorsal fin	absent	present	absent	absent	present or absent
Morphology of larvae					
Wing-like yolk sac projection	less developed	well developed	less developed	less developed	not developed

Type species. *Sinorhodeus microlepis*.

Etymology. The generic name, *Sinorhodeus*, is derived from the Latin *Sino*, meaning "Chinese", and *Rhodeus*, a genus of bitterling fish, in reference to its distribution in China and morphological similarity to *Rhodeus*. The gender is masculine.

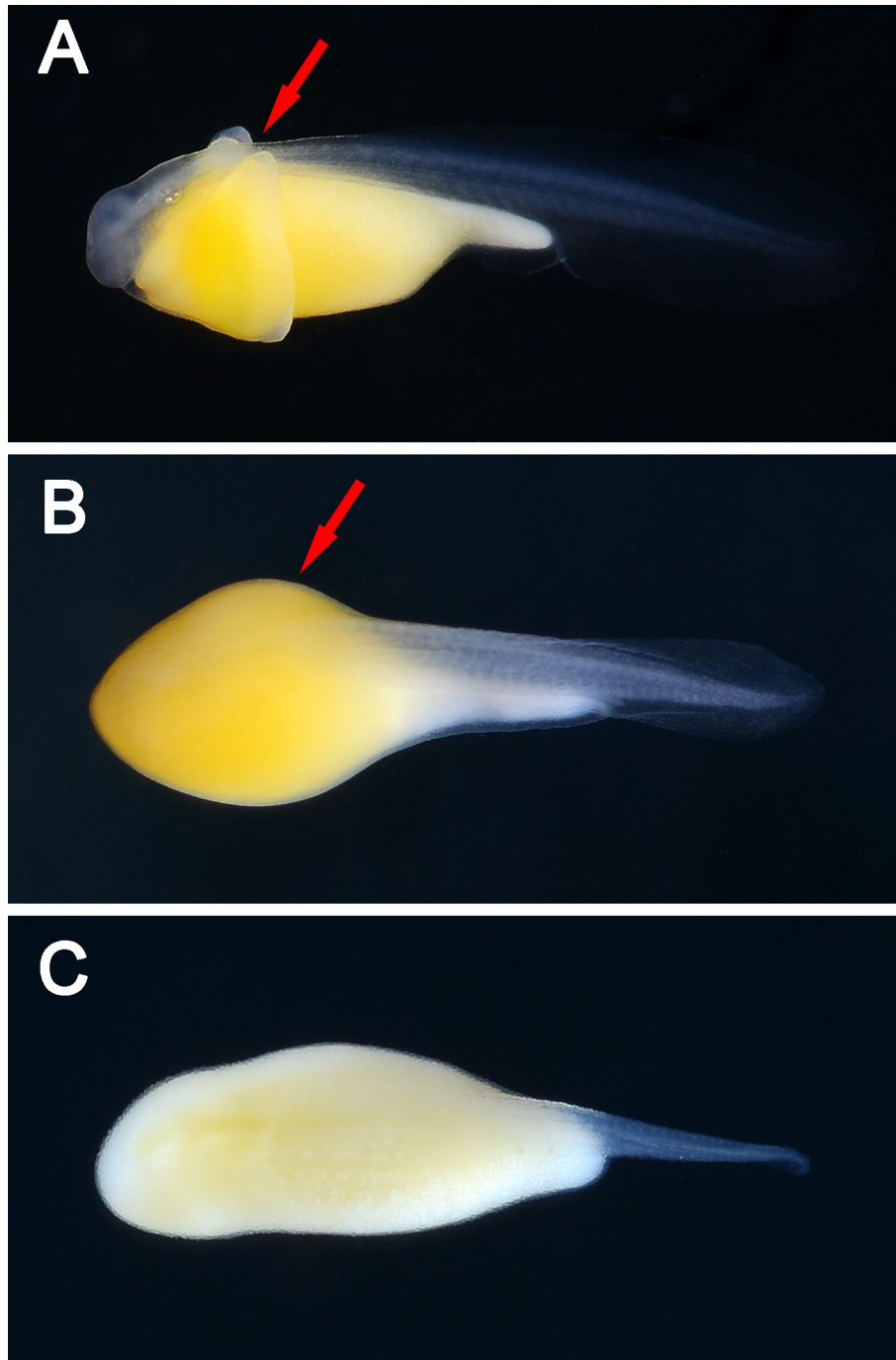


FIGURE 1. Three types of wing-like yolk sac projections. Arrows show a wing-like yolk sac. **A**, well developed in prolarva of *Rhodeus albomarginatus*. **B**, less developed in prolarva of *Tanakia lanceolata*. **C**, not developed in prolarva of *Acheilognathus omeiensis*.

***Sinorhodeus microlepis* sp. nov. Li, Liao & Arai**

(Figs. 2–9; Table 4)

Holotype. SOU 1604001, male, 48.7 mm SL; a tributary of Yangtze River, Banan District, Chongqing City, China; 16 April 2016.

Paratypes. SOU 1604002–1604009, 8 males, 34.2–58.1mm SL; SOU 1604011–1604022, 12 females, 35.7–46.6 mm SL; NSMT-P 130011, male, 42.7 mm SL; NSMT-P 130012, female, 36.3 mm SL; same data as holotype.

Non-type specimen. SOU 1604024, female, 36.5 mm SL, cleared and stained, same data as holotype..

Diagnosis. See generic diagnosis.

Description. Morphometric and meristic data of holotype and paratypes presented in Table 4. Body compressed. Mouth large and subterminal, corner of mouth extending to vertical of anterior margin of orbit. Barbels absent. Pearl organs developed on snout, area between nostril and eye, and top of head in mature males; absent in females. A short ovipositor present in mature females, maximum length approximately 7–10 mm.



FIGURE 2. *Sinorhodeus microlepis*, China: Chongqing City: Banan District. **A**, SOU 1604001, holotype, 48.7 mm SL, male; **B**, SOU 1604011, paratype, 44.3 mm SL, female.

Dorsal fin with 3 simple and 8 branched rays (rarely 6 or 9). Anal fin with 3 simple and 8 branched rays (rarely 7 or 9). First simple ray in dorsal and anal fins very small, hidden under skin. Longest simple ray of dorsal fin strong and stiff, distally segmented; width of basal portion much wider than that of first branched ray; longest simple dorsal-fin ray segmented from area corresponding to second branching point of first branched ray (Fig. 3A). Longest simple ray of anal fin soft and distally segmented; width of basal portion equivalent to that of first branched ray; longest simple anal-fin ray segmented from area corresponding to first branching point of first branched ray (Fig. 3B). Pectoral fin with 1 simple and 12–13 branched rays. Pelvic fin with 1 simple and 6–7 branched rays. Principal caudal rays 19, including branched rays 17 (9 + 8); dorsal procurrent rays 6–7, ventral procurrent rays 5–6.

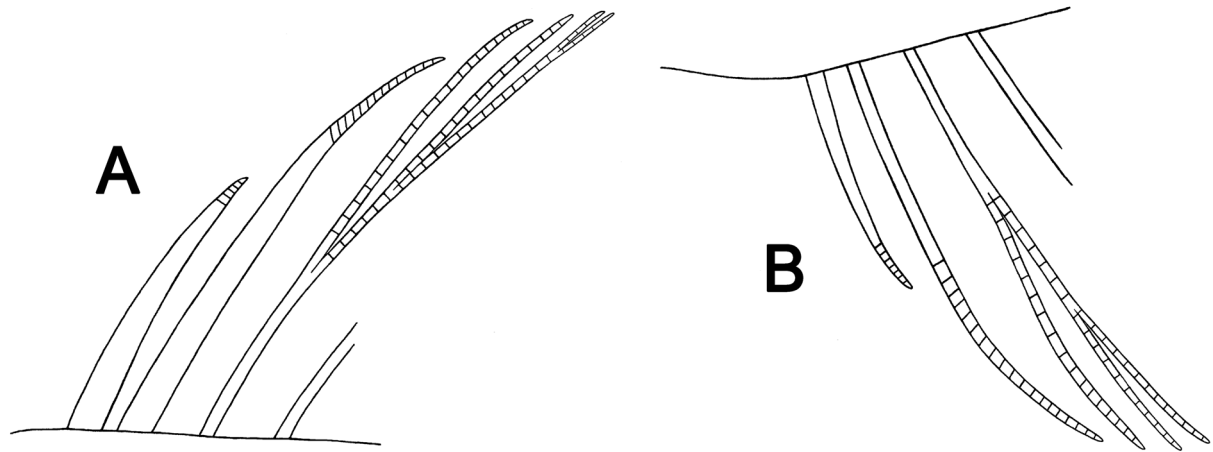


FIGURE 3. *Sinorhodeus microlepis*, SOU 1604001, holotype, male. Simple and first branched rays of dorsal (A) and anal (B) fins.

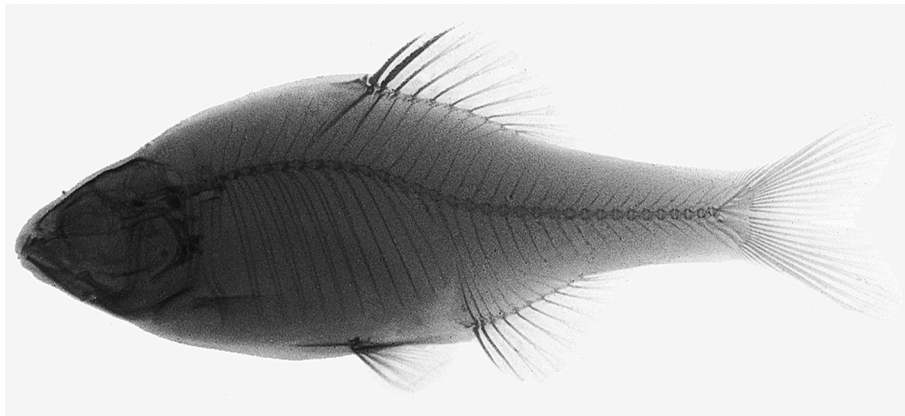


FIGURE 4. *Sinorhodeus microlepis*, SOU 1604001, holotype, 48.7 mm SL, male. Radiograph.

Longitudinal scales 41–46 (40–45 on body, 0–1 on caudal fin). Lateral line incomplete. Pored scales 3–5. Transverse scales 16–18. Predorsal scales 17–20. Circumpeduncular scales 18–20.

Abdominal vertebrae modally 16 (15–17); caudal vertebrae modally 19 (18–20); total vertebrae modally 35 (34–37). Position of first dorsal-fin ray pterygiophore (D-PTG-1) = 10 (between 10th and 11th vertebrae, denoted as 10; range from 9 to 11). Position of first anal-fin ray pterygiophore (A-PTG-1) = 16 (range from 15 to 17) (Fig. 4). Pharyngeal teeth in one row, formula 0,0,4–4,0,0; occlusal grooves developed (six adult specimens dissected, Fig. 5). Gill rakers on external side of first gill arch 7–9.

Four infraorbital bones (io) present. Cephalic sensory canals of adult specimens highly reduced: infraorbital canal interruptedly incomplete, usually separated into 3 (corresponding to io1, io2, and io3) or 4 (corresponding to io1, io2, io3, and io4) parts in males, but 1 (corresponding to io1) or 2 (corresponding to io1 and io3) parts in females; temporal canal present; supratemporal canal absent; supraorbital canal not connecting to infraorbital sensory canal; infraorbital canal not connecting to preopercular canal (Fig. 6). Asymmetry common in cephalic sensory canal system of adult specimens.

Ripe eggs short pear-shaped, length of major axis approximately 2 mm, ratio of major axis to minor axis 1.3–1.4 (Fig. 7A). Larvae with less developed wing-like yolk sac projections (Figs. 7B, 7C).

TABLE 4. Meristic and morphometric measurements of holotype and paratypes in *Sinorhodus microlepis*. Numbers in parentheses are number of specimens with a given count. * br.A minus br.D, number of branched anal-fin rays minus the number of branched dorsal-fin rays.

sex	holotype		paratypes	
	male	n	males	females
n	1	9		13
Standard length (mm)	48.7		34.8–58.1	35.7–46.6
Dorsal-fin rays	iii,8		iii,6 (1); iii,8 (8)	iii,8 (12); iii,9 (1)
Anal-fin rays	iii,8		iii,8 (8); iii,9 (1)	iii,7 (1); iii,8 (12)
br. A minus br. D*	0		0 (7); 1 (1); 2 (1)	-1 (2); 0 (11)
Pectoral-fin rays	i,12		i,12 (6); i,13 (3)	i,12 (10); i,13 (3)
Pelvic-fin rays	i,7		i,6 (3); i,7 (6)	i,6 (3); i,7 (10)
Vertebrae	36		34 (2); 35 (7)	35 (8); 36 (4); 37 (1)
D-PTG-1	10		9 (3); 10 (6)	9 (2); 10 (10); 11 (1)
A-PTG-1	17		15 (1); 16 (6); 17 (2)	16 (11); 17 (2)
Scales in lateral series	45		41 (1); 42 (2); 43 (2); 44 (2); 46 (2)	41 (2); 42 (5); 43 (2); 44 (3); 45 (1)
Scales in transverse series	16		16 (4); 17 (5)	16 (4); 17 (7); 18 (2)
Pored lateral-line scales	5		3 (3); 4 (3); 5 (3)	3 (5); 4 (7); 5 (1)
Scales around caudal peduncle	20		19 (1); 20 (8)	18 (4); 19 (1); 20 (8)
Predorsal scales	19		17 (1); 18 (1); 19 (5); 20 (2)	17 (1); 18 (6); 19 (4); 20 (2)
Morphometry				
% standard length				
Head length	25.3		23.8–25.9	24.5–26.2
Body depth	37.4		31.6–38.9	32.1–37.1
Snout length	5.9		5.0–6.1	4.9–5.9
Orbit diameter	7.4		7.3–8.5	7.6–9.0
Predorsal length	47.0		46.3–50.0	47.3–51.2
Caudal peduncle length	22.4		22.0–25.4	20.3–25.0
Caudal peduncle depth	12.5		11.3–12.9	11.1–13.1

Coloration in life. Adult males are strikingly colorful during breeding season: body color mostly red, with bluish sheen dorsally. A light red vertical band covering 3rd–4th scales in lateral series. Dorsal fin blackish without stripes. Anal fin blackish with a red longitudinal band in center. Inner part of anal fin usually fully filled with red in large males. Pectoral fin, pelvic fin, and basal part of caudal fin reddish. Iris red (Fig. 8A).

In females, all fins hyaline. Iris blackish in small individuals, but reddish-orange in large individuals. Egg tube (ovipositor) whitish (Fig. 8B).

In juveniles, dorsal fin without a black blotch (Fig. 9).



FIGURE 5. Pharyngeal teeth of *Sinorhodeus microlepis*.

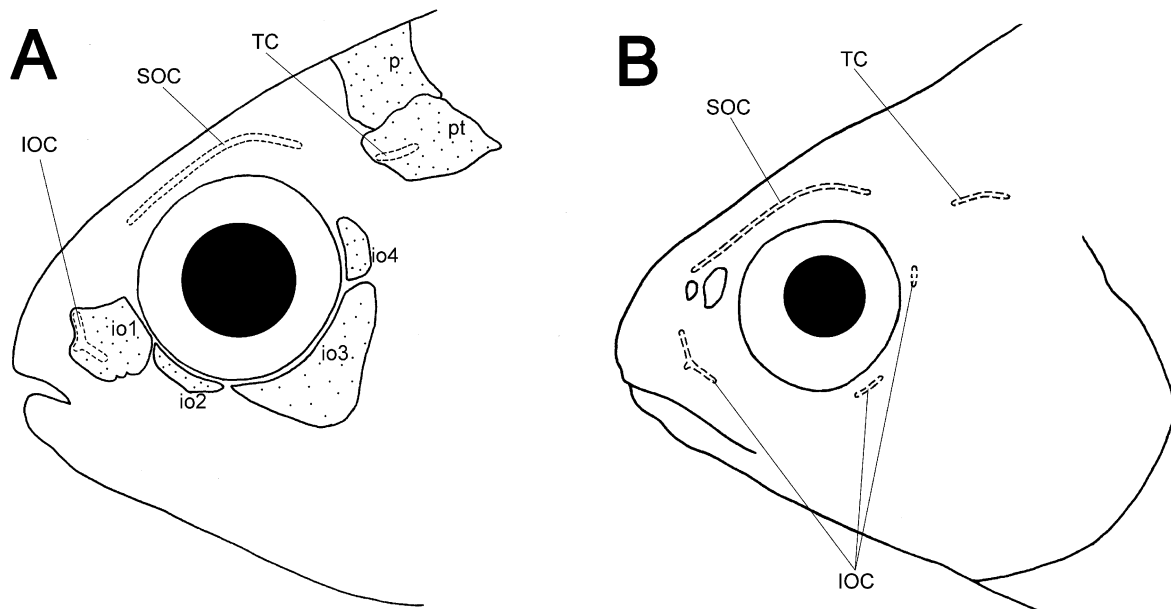


FIGURE 6. Portion of cephalic sensory canal system of *Sinorhodeus microlepis*. **A**, SOU 1604024, 36.5 mm SL, female; **B**, SOU 1604001, holotype, 48.7 mm SL, male. Abbreviations: io1–4, infraorbital bones 1–4; p, parietal bone; pt, pterotic bone; IOC, infraorbital sensory canal; SOC, supraorbital sensory canal; TC, temporal sensory canal.



FIGURE 7. Eggs and larvae of *Sinorhodeus microlepis*, at a water temperature around 23?. **A**, unfertilized ripe eggs; **B**, larvae immediately after hatching; **C**, larvae 3 days after hatching.

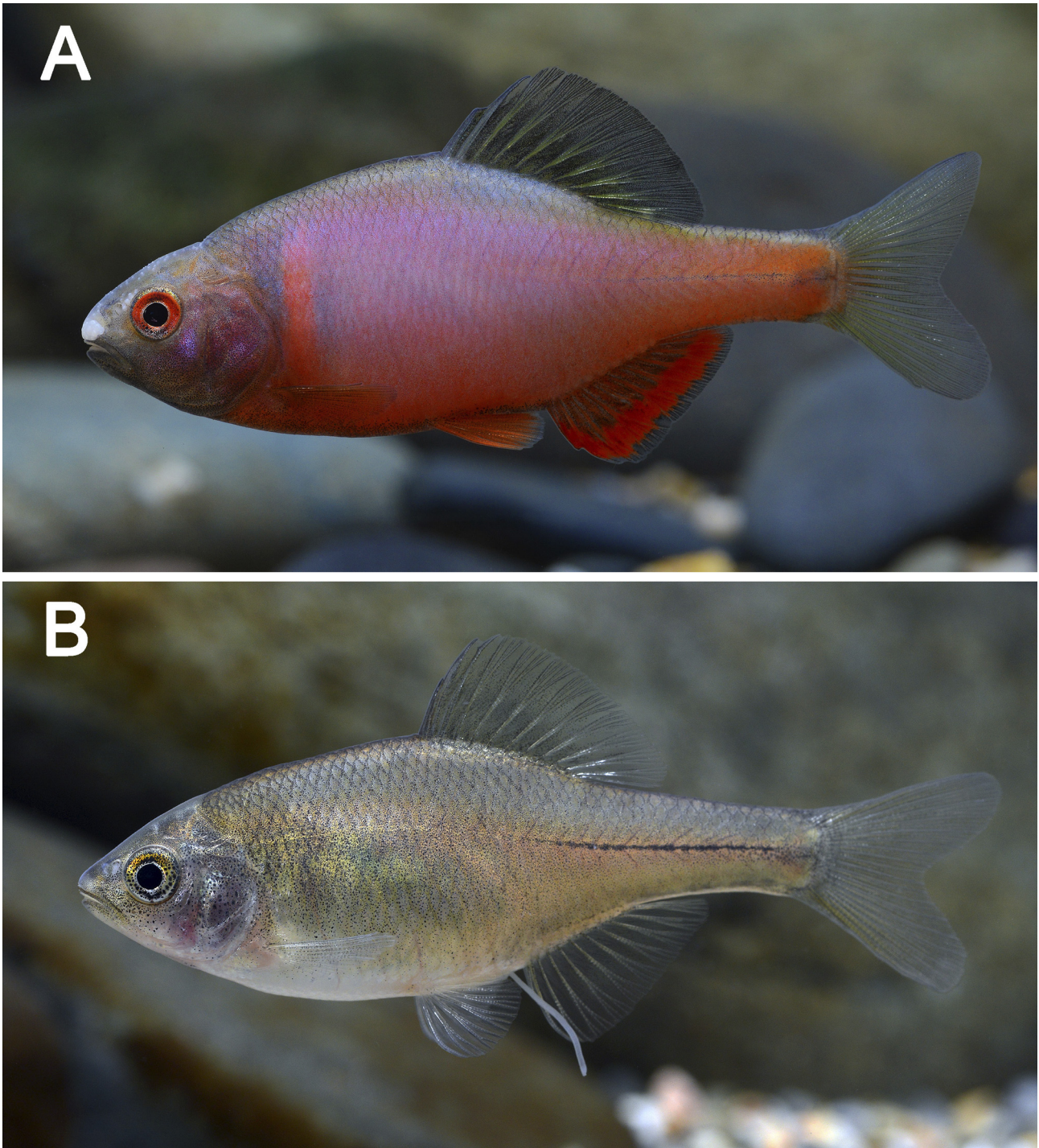


FIGURE 8. *Sinorhodeus microlepis* in breeding season, male (**A**) and female (**B**).

Color in preservative. Ground color brown, darker on dorsal portion of body than on ventral portion of body. Vertical light band present on anterior flank in males; absent in females. Narrow longitudinal stripe on each side of body running from below dorsal fin and ending about 3 scales in front of caudal-fin base. Dorsal fin of males blackish; anal fin whitish with blackish margin. Dorsal and anal fins of females hyaline without white spots on fin rays. (Fig. 2).

Distribution and ecology. Known only from a tributary of Yangtze River, in Banan District, Chongqing City, China (Fig. 10). The type locality was a slow-flowing stream at an altitude of 625 meters. The substrate consisted of mud mixed with gravel. Other syntopic species were *Rhodeus ocellatus*, *Hemiculter leucisculus* and *Pseudorasbora parva*.

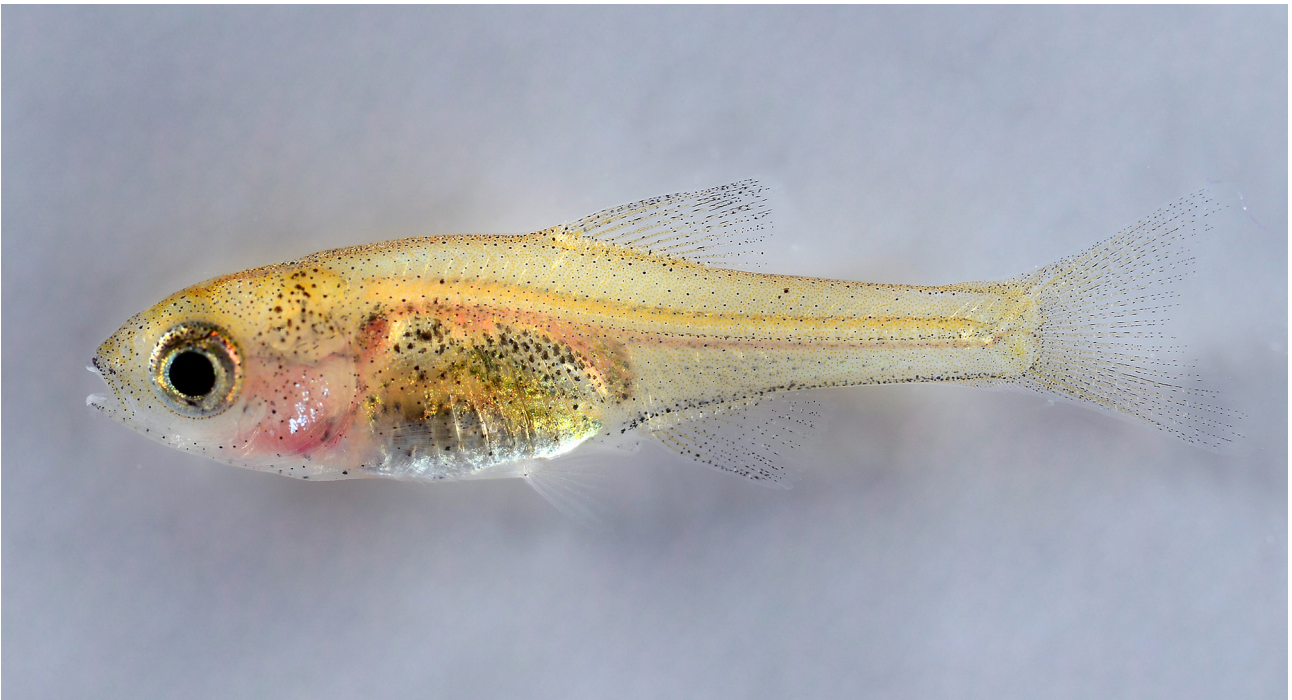


FIGURE 9. *Sinorhodeus microlepis*, juvenile, 11.5 mm SL.

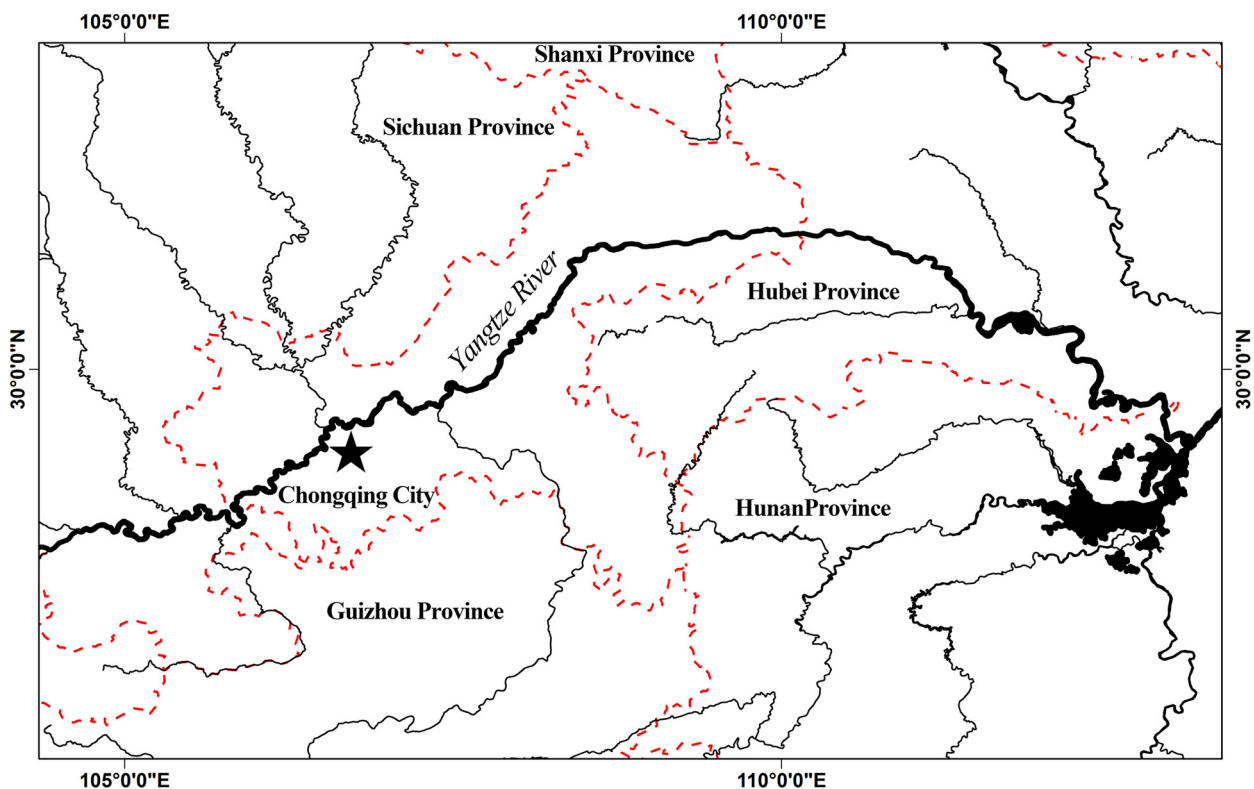


FIGURE 10. Sampling locality of *Sinorhodeus microlepis* (★).

Surveys were also conducted in the streams of peripheral regions in same basin, and only one bitterling, *Rhodeus ocellatus*, was collected. The limited distribution suggests that *S. microlepis* may be rare and stenotopic. Conservation for *S. microlepis* may be necessary.

Sinorhodeus microlepis exhibits a unique host preference. It spawns in the gills of freshwater clam, *Corbicula fluminea* (Figs. 11A, 11B), from March to October, and usually releases 10–15 eggs at one time. The breeding peak time is spring (April to May).

Etymology. The specific name, *microlepis*, is derived from the Greek *micro*, meaning small, and *lepis*, meaning scale, a noun in apposition, in reference to the diagnostic small scales of this species.

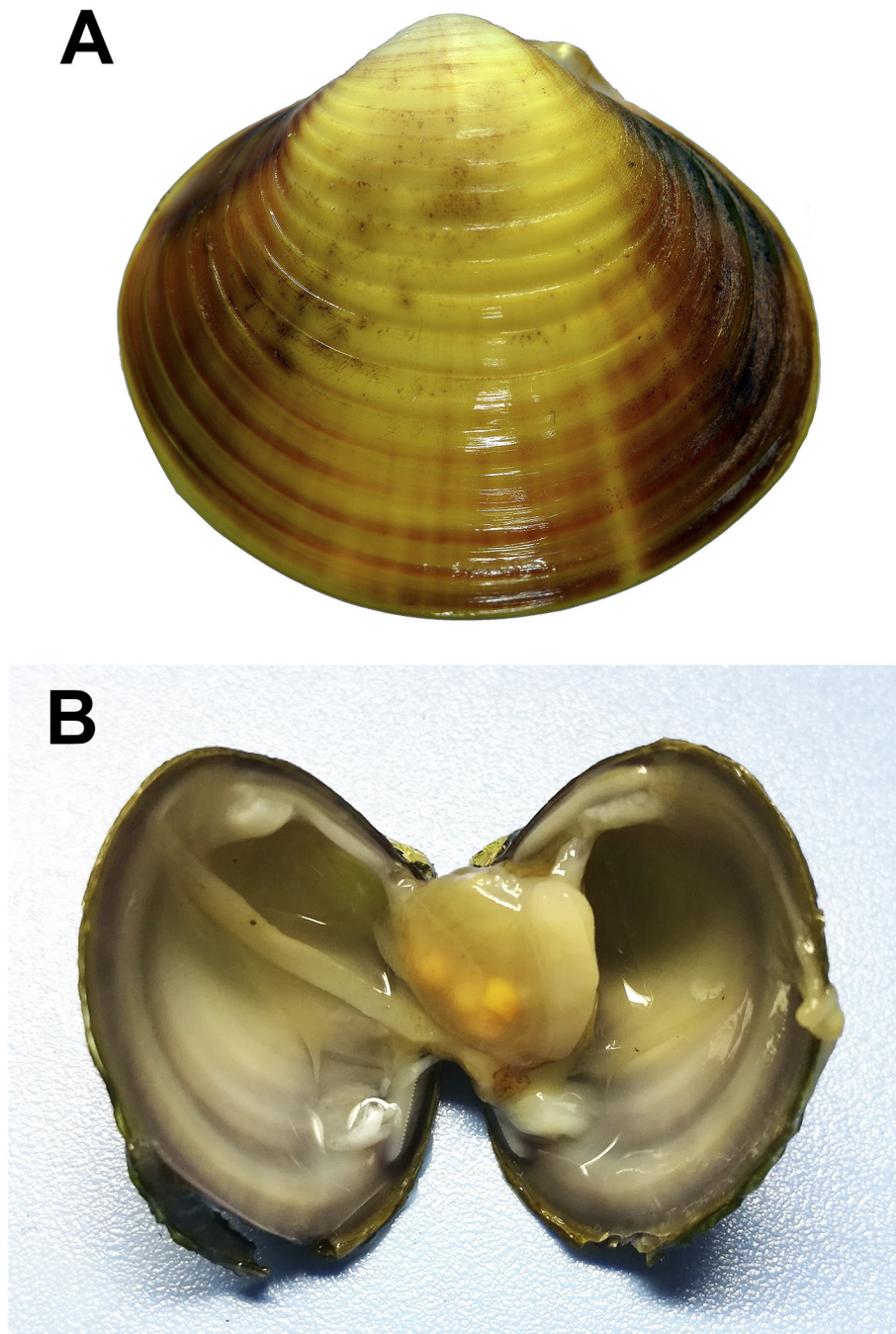


FIGURE 11. Host clam of *Sinorhodeus microlepis*, *Corbicula fluminea*. **A**, shell of *C. fluminea*, 18 mm shell length. **B**, eggs of *S. microlepis* in gills of *C. fluminea* (four yellow blotches in the middle; **B** by Mr. Yi Yu, 15 April 2017).

Molecular phylogenetic analyses

A total of 6207 bps were aligned for the combined seven genes of 25 bitterling and two outgroup taxa. The topological structures of BI and ML trees were highly consistent, except for limited variation in some support values (Fig. 12). BI and ML trees showed strong support for the monophyly of *Sinorhodeus* and the other six clades defined in Chang *et al.* (2014). *Sinorhodeus* forms a monophyletic group with the unnamed-*Rhodeus* clade, and the *Sinorhodeus*-unnamed-*Rhodeus* clade is sister to the *Paratanakia* clade. Topology of our analyses is otherwise

identical to that of Chang *et al.* (2014). The molecular data support the establishment of *Sinorhodeus* as a new genus in the subfamily Acheilognathinae.

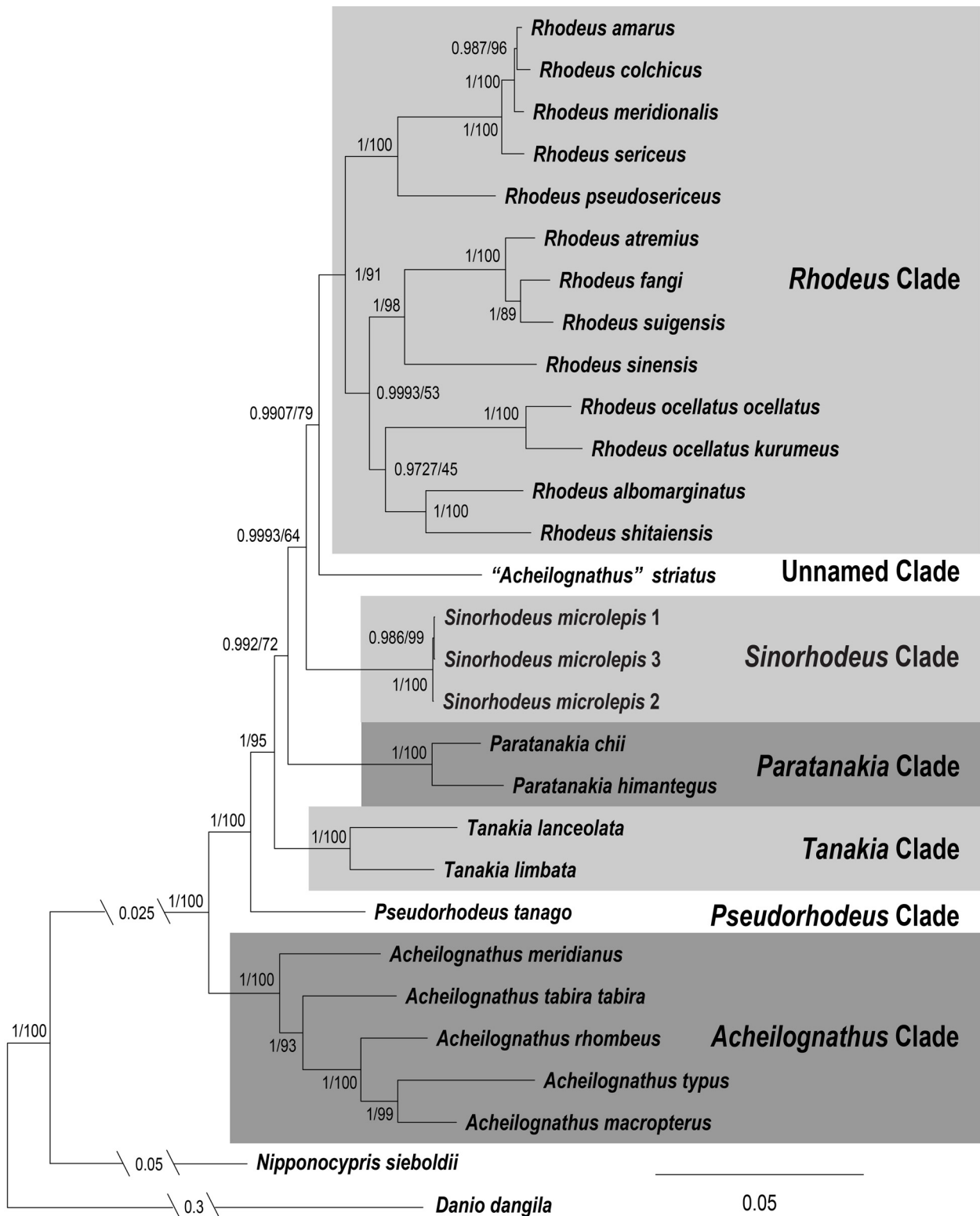


FIGURE 12. A Bayesian inference (BI) tree of the combined dataset (7 genes: 6207 bps) for the Acheilognathinae. The topology from maximum likelihood (ML) analysis is similar to BI tree. Posterior probability value from BI analysis, and the bootstrap confidence values from ML analysis, are given in order at each node.

Discussion

Arai & Akai (1988) classified the species of the subfamily Acheilognathinae into three genera (*Acheilognathus*, *Tanakia*, and *Rhodeus*) with morphological characters and karyotypes, but several molecular phylogenetic studies have indicated that the Acheilognathinae only contain two major clades: *Acheilognathus* clade and *Tanakia-Rhodeus* clade, and the genus *Tanakia* is a paraphyletic group (Okazaki *et al.* 2001; Chang *et al.* 2014; Cheng *et al.* 2014; Kawamura *et al.* 2014). Furthermore, Chang *et al.* (2014) showed the *Tanakia-Rhodeus* clade can be subdivided into five clades, and proposed two new genera, *Pseudorhodeus* and *Paratanakia*, to allocate one (*Tanakia tanago* (Tanaka 1909)) and two (*T. himantegus* (Günther 1868) and *T. chii* (Miao 1934)) species, respectively. However, the diagnosis of *Paratanakia* is poor. Chang *et al.* (2014) referred the diagnostics of *Paratanakia* typified by *Tanakia himantegus* to Arai & Akai (1988), Lin (1998) and Ueda *et al.* (1997, 2006). However, Arai & Akai (1988) only listed *T. himantegus* and *T. chii* as two species of *Tanakia*. No diagnostic character was given to distinguish the two species from other congeners. Lin (1998) placed *T. himantegus* in an unavailable genus, *Paracheilognathus*. The generic name *Tanakia* was not mentioned by Lin (1998), and, again, not a single character to distinguish this species from other species of *Tanakia* was provided. There is indeed no morphological character to distinguish *Paratanakia* from *Tanakia*, and the two genera can only be distinguished from each other by molecular characters in DNA sequences. The morphology of *Paratanakia* needs thorough examination to find diagnostic morphological characters unique from *Tanakia*. Although *Pseudorhodeus* can be distinguished from the *Tanakia* clade (true *Tanakia*) of Chang *et al.* (2014) by an incomplete lateral line (vs. complete), the karyotype that they provided in the diagnosis is problematic. Chang *et al.* (2014) used data from Ojima *et al.* (1973) that *Pseudorhodeus* has a chromosomal constitution of 8M + 20SM + 20ST. This is identical to the karyotype of *T. limbata* (Temminck & Schlegel), the type species of *Tanakia* ($2n=48$: 8M+20SM+20ST) (Sola *et al.* 2003; Arai, 2011), and cannot be considered a diagnostic character for these two genera. Despite the unclear definition of *Pseudorhodeus* and *Paratanakia*, the genus *Sinorhodeus* can be distinguished from these two genera by the absence of barbels (vs. present) and an incomplete lateral line (vs. interrupted incomplete in *Pseudorhodeus*, and complete in *Paratanakia*) (Günther 1868; Tanaka 1909; Miao 1934; Arai & Kato 2003).

In the Acheilognathinae, species with an incomplete lateral line include *Sinorhodeus microlepis*, *Pseudorhodeus tanago*, *Acheilognathus typus* (Bleeker 1863), all species of *Rhodeus* (Arai & Kato 2003; Arai *et al.* 2007; Li & Arai 2011; Li & Arai 2014), and three questionable species, viz. *Rhodeus oryzae* Jordan & Seale 1906, *Pseudoperilampus hondae* Jordan & Metz 1913, and *Acanthorhodeus kristinae* Holcík 1971. Arai & Akai (1988) reported that *R. oryzae* was a juvenile *Tanakia limbata*, the lateral line of which is complete as an adult. Arai & Kato (2003) proposed that *P. hondae* belongs to the genus *Acheilognathus* based on the numbers of pored scales and dorsal- and anal-fin rays. Lin (1998) considered *A. kristinae* as a possible synonym of *Rhodeus fangi* (Miao 1934), but the presence of small barbels indicates that *A. kristinae* may not be a member of the genus *Rhodeus*. *Sinorhodeus microlepis* can be easily distinguished from *R. oryzae*, *P. hondae*, and *A. kristinae* by fewer pharyngeal teeth (0,0,4–4,0,0 vs. 0,0,5–5,0,0), and more longitudinal scales (41–46 vs. no more than 35) (Jordan & Seale 1906; Jordan & Metz 1913; Holcík 1971).

Arai & Kato (2003) reported that the complete lateral line, along with other characters, represent the plesiomorphic states of Acheilognathinae, and speculated that the incomplete lateral line may be derived from a complete state by paedomorphic reduction. *Sinorhodeus* forms a monophyletic group with *Rhodeus* and an unnamed clade, among which the unnamed clade, with one species ("*Acheilognathus*" *striatus* Yang, Xiong, Tang & Liu 2010) herein and three species in Chang *et al.* (2014), is the only group possessing barbels and a complete lateral line. Since a complete lateral line is a plesiomorphic state, it implies that the incomplete lateral lines of *Sinorhodeus* and *Rhodeus* are independently derived from the plesiomorphic state, or, alternatively, the lateral line might have become incomplete in the ancestor of *Sinorhodeus-Rhodeus*-unnamed clade and the plesiomorphic state might have been regained in the unnamed clade. In addition to its remarkable coloration, *S. microlepis* can be distinguished from all 19 species/subspecies of the genus *Rhodeus* by more longitudinal scales (41–46 vs. less than 41), less developed wing-like yolk sac projections in larvae (vs. well developed), the absence of a black blotch on the dorsal fin in juveniles (vs. present), and the absence of white spots on fin-rays of the dorsal fin (vs. present) (Tirant 1883; Arai & Akai 1988; Kottelat 1998; Arai *et al.* 2001; Hosoya 2002; Bogutskaya & Komlev 2001; Li & Arai 2011; Li & Arai 2014).

All other species of Acheilognathinae, except *Acheilognathus typus*, have no more than 41 longitudinal scales

by far (Mai 1978; Lin 1998; Bogutskaya & Komlev 2001; Kottelat 2001; Hosoya 2002; Arai & Kato 2003; Arai *et al.* 2007; Yang *et al.* 2010; Li & Arai 2011; Yang *et al.* 2011; Nguyen *et al.* 2013; Kim *et al.* 2014; Li & Arai 2014). *Acheilognathus typus* is also the type species of the genus *Pseudoperilampus* (Bleeker 1863a) that was synonymized with *Acheilognathus* by Arai & Akai (1988). *Sinorhodeus microlepis* shares features with *A. typus*, including a large number of scales, incomplete lateral line, and absence of barbels, but differs from it by fewer longitudinal scales (41–46 vs. 52–65), fewer transverse scales (16–18 vs. 18–21), fewer pored scales (3–5 vs. 7–14), fewer branched dorsal-fin rays (8 in mode vs. 10), fewer branched dorsal-fin rays (8 in mode vs. 10–12), and absence of white spots on fin-rays of dorsal fin (vs. present) (Bleeker 1863b; Hosoya 2002; Arai & Kato 2003).

Sinorhodeus microlepis resembles *Pseudorhodeus tanago* from Japan in sharing an incomplete lateral line, a similar number of branched dorsal- and anal-fin rays (D. 8 in mode, A. 8 in mode), and similar morphology of the eggs and larvae (Tanaka 1909; Nakamura 1969; Suzuki *et al.* 1986; Hosoya 2002). However, *S. microlepis* differs from *P. tanago* by more longitudinal scales (41–46 vs. 35–37), more transverse scales (16–18 vs. 10–11), absence of barbels (vs. present), absence of a stripe on the dorsal fin (vs. present), and presence of a longitudinal stripe on the flank (vs. absent) (Tanaka 1909; Hosoya 2002).

Sinorhodeus microlepis also resembles *Tanakia signifier* (Berg 1907) (= *Acheilognathus signifier*) from Korea in sharing the number of branched dorsal- and anal-fin rays (vs. D. 8, A. 8) and morphology of eggs and larvae, but differs from *T. signifier* in having an incomplete lateral line (vs. complete), absence of barbels (vs. present) (Berg 1907; Suzuki & Jeon 1988).

Sinorhodeus microlepis exhibits a number of unique characters among species of the subfamily Acheilognathinae. It is the only species possessing pharyngeal teeth in the arrangement of 0,0,4–4,0,0, whereas all other species so far known have pharyngeal teeth 0,0,5–5,0,0 (Lin 1998; Hosoya 2002). Host preference of *S. microlepis* is also unique. All known acheilognathines only use freshwater mussels (Bivalvia: Unionidae and Margaritiferidae) to date (Liu *et al.* 2006; Kitamura *et al.* 2012; Kim *et al.* 2014), whereas *S. microlepis* can use freshwater clams (Bivalvia: Cyrenidae). However, it is not yet known whether *S. microlepis* also utilizes mussels or not. Moreover, the cephalic sensory canal system and infraorbital bones of *S. microlepis* are distinctive among the Acheilognathinae. Arai & Kato (2003) reported the cephalic sensory canals and infraorbital bones of 27 acheilognathines from three genera and indicated that *Rhodeus sinensis* Günther 1868 is the only species in possession of both the most reduced cephalic sensory canals and the lowest number of infraorbital bones (4). *Sinorhodeus microlepis* shares the same number of infraorbital bones and a similar pattern of cephalic sensory canals with *R. sinensis*, but differs from *R. sinensis* in presence of the temporal canal (vs. absent) and an interrupted incomplete infraorbital canal (vs. complete or uninterrupted incomplete) (Arai & Kato 2003).

Comparative material

"Acheilognathus" striatus: IHCAS 0805401–0805402, IHCAS 0805404–0805434, 33 paratypes, 17 males and 16 females, 41.9–61.1 mm SL; China: Jiangxi Province: Wuyuan County; Apr., 2008. SOU 201009501–201009506, 6 specimens, 30.2–41.0 mm SL; China: Jiangxi Province: Wuyuan County; 3 Sep. 2010.

Paratanakia chii: SOU 200909501, 17 specimens, 44.5–55.6 mm SL; China: Shanghai City; Sep. 2009.

Paratanakia himantegus: NTUM 04929, 12 specimens, 32.3–63.2 mm SL; Taiwan: Taipei City; Nov. 1957; SOU 200700501, 2 males and 4 females, 32.3–63.2 mm SL; Taiwan: Taipei City; 2007. SOU 200408501, 5 specimens, 33.4–39.8 mm SL; Taiwan: Taichung City; 8 Aug. 2004.

Rhodeus albomarginatus: SOU 1306001, holotype, male, 53.4 mm SL; China: Anhui Province: Qimen County; 10 May 2013. SOU 1306002, male, 53.6 mm SL; SOU 1306003–1306005, 3 females, 43.3–47.2 mm SL; same data as holotype. SOU 1110001–1110008, 8 males, 44.5–58.4 mm SL; SOU 1110011–1110013, 3 females, 39.8–45.0 mm SL; NSMT-P 114853, male, 43.2 mm SL; NSMT-P 114854, female, 42.6 mm SL; ZUMT 61948, male, 45.8 mm SL; ZUMT 61949, female, 38.8 mm SL; same locality as holotype; 5 Oct. 2011.

Rhodeus amarus: SOU 200406501, 19 specimens, 35.2–57.5 mm SL; Germany: Oldenburg; 26 Jun. 2004.

Rhodeus fangi: SOU 200905501, 9 males and 4 females, 31.4–34.5 mm SL; China: Jiangsu Province: Chinkingang (Zhenjiang) City; May 2009. SOU 200706501, 1 male, 38.5 mm SL; China: Zhejiang Province: Shengzhou City; 9 Jun. 2007. SOU 201005504, 3 males and 2 females, 30.6–45.0 mm SL; China: Anhui Province: Xiuning County; 4 May 2010.

Rhodeus haradai: NTUM 7600, holotype, male, 56.0 mm SL; China: Hainan: Longtang; Jun. 1942.

Rhodeus notatus: AMNH 9654, holotype, male, 33.0 mm SL (x-ray); AMNH 10812, paratypes, 5 males and 1 female, 24.3–29.2 mm SL; China: Shandong Province: Tsinan (Jinan); Apr.–Jul. 1924. SOU 200702501, 2 males and 2 females, 42.6–46.0 mm SL; China: Beijing City; Feb. 2007.

Rhodeus ocellatus: NMW 10837, holotype, male, 51.6 mm SL (x-ray); China: Shanghai City. SOU 200705501, 10 males and 7 females, 41.8–57.9 mm SL; China: Shanghai City; May 2007. SOU 200706501, 3 males and 5 females, 36.8–56.3 mm SL; China: Zhejiang Province: Shengzhou City; 9 Jun. 2007. SOU 200811501, 5 males and 4 females, 44.7–55.6 mm SL; China: Anhui Province: Shitai County; Nov. 2008. SOU 201005504, 1 male and 2 females, 35.8–48.8 mm SL; China: Anhui Province: Xiuning County; 4 May 2010. SOU 201208501, 3 males and 1 female, 39.1–56.6 mm SL; China: Liaoning Province: Zhuanghe City; 14 Aug. 2014.

Rhodeus pseudosericeus: SMU 211, holotype, male, 45.5 mm SL; Korea: Gangwon-do: Hoengsong-gun: Gonggun-myon: Hakdam-ri: Gungye River, tributary of Som River, Namhan River system; S. R. Jeon, 16 Oct. 1999. ZUMT 61149–61151, 3 paratypes, 1 male and 2 females, 41.0–41.9 mm SL; same data as holotype.

Rhodeus sericeus: SOU 201208503, 12 males and 2 females, 68.9–85.5 mm SL; China: Jilin Province: Huichun City; Aug. 2012. SOU 201208504, 11 males and 4 females, 34.9–66.5 mm SL; China: Heilongjiang Province: Mishan City; Aug. 2012.

Rhodeus shitaiensis: SOU 0811001, holotype, male, 59.9 mm SL; China: Anhui Province: Shitai County; Nov. 2008. SOU 0811002–0811004, 4 males, 48.4–53.9 mm SL; same data as holotype. SOU 0905001–0905010, 4 male and 6 females, 41.7–61.5 mm SL; same locality as holotype; May 2009.

Rhodeus sinensis: BMNH 1868.10.19–150, lectotype, male, 51.6 mm SL; China: Chikiang (Zhejiang); 19 Oct. 1868. SOU 201005504, 4 males and 1 female, 35.2–58.2 mm SL; China: Anhui Province: Xiuning County; 4 May 2010. SOU 200705501, 10 males and 4 females, 43.6–58.5 mm SL; China: Shanghai City; May 2007. SOU 200905501, 6 males and 3 females, 32.1–44.7 mm SL; China: Jiangsu Province: Zhenjiang City; May 2009.

Rhodeus spinalis: NSMT-P 31906, neotype, male, 49.7 mm SL; Hainan: Dingan; 27 Mar. 1966. SOU 201312501, 14 specimens, 34.1–51.8 mm SL; China: Hainan Province: Haikou City; 21 Dec. 2013.

Tanakia lanceolata: SOU 201208502, 9 specimens, 32.5–73.1 mm SL; China: Liaoning Province: Fengcheng City; 17 Aug. 2012. SOU 201605501, 3 specimens, 41.7–45.6 mm SL; China: Liaoning Province: Kuandian County; May 2016.

Tanakia limbata: NTUM 07994, 4 specimens, 33.7–47.3 mm SL; Japan: Hiroshima Prefecture: Fukuyama City; 6 Jan. 1980.

Acknowledgements

We thank Can Luo (Chongqing) and Tao Wang (Chongqing) for assistance in collecting type specimens; Chia-Hao Chang (Academia Sinica, Taiwan) and Jörg Bohlen (Academy of Sciences, Czech Republic) for gifts of specimens; Huanzhang Liu (IHCAS) and Yi-Jung Lin (NTUM) for loan of specimens; Wenqiao Tang (SOU) for loan of X-ray imaging machine; Xinyi Huang (Fudan University, Shanghai) for drawing the map; Yi Yu (Southwest University, Chongqing) for photograph of clam; Haoran Zheng (Chongqing) for collecting clams; Jing He (Shanghai) for identifying the clam. This study was supported by grants to TYL from the Ministry of Science and Technology (105-2611-M-110 -008) and Asia-Pacific Ocean Research Center, National Sun Yat-sen University..

References

- Akaike, H. (1974) A new look at the statistical model identification. *IEEE transactions on Automatic Control*, 19, 716–723. <https://doi.org/10.1109/TAC.1974.1100705>
- Arai, R. (2011) *Fish karyotypes: a check list*. Springer, Tokyo/Berlin/Heidelberg/New York, 340 pp. <https://doi.org/10.1007/978-4-431-53877-6>
- Arai, R. & Akai, Y. (1988) *Acheilognathus melanogaster*, a senior synonym of *A. moriokae*, with a revision of the genera of the subfamily Acheilognathinae (Cypriniformes, Cyprinidae). *Bulletin of the National Science Museum, Tokyo, Series A*, 14, 199–213.
- Arai, R. & Kato, K. (2003) Gross morphology and evolution of the lateral line system and infraorbital bone in bitterlings (Cyprinidae, Acheilognathinae), with an overview of the lateral line system in the family Cyprinidae. *The University*

Museum, The University of Tokyo, Bulletin, 40, 1–42.

- Arai, R., Fujikawa, H. & Nagata, Y. (2007) Four new subspecies of *Acheilognathus* bitterling (Cyprinidae: Acheilognathinae) from Japan. *Bulletin of the National Museum of Nature and Science, Tokyo*, Series A, 1 (Supplement), 1–28.
- Arai, R., Jeon, S.R. & Ueda, T. (2001) *Rhodeus pseudosericeus* sp. nov., a new bitterling from South Korea (Cyprinidae, Acheilognathinae). *Ichthyological Research*, 48, 275–282.
<https://doi.org/10.1007/s10228-001-8146-1>
- Arai, R., Xie, Y. & Akai, Y. (1995) Rediscovery of the bitterling, *Tanakia lanceolata*, in China (Pisces, Cyprinidae). *Japanese Journal of Ichthyology*, 42, 196–199.
- Berg, L.S. (1907) Description of a new cyprinoid fish, *Acheilognathus signifer*, from Korea, with a synopsis of all the known Rhodeinae. *Annals and Magazine of Natural History*, Series 7, 19 (110), 159–163.
- Bleeker, P. (1863a) Systema Cyprinoideorum revisum. *Nederlandsch Tijdschrift voor de Dierkunde*, 1, 187–218.
- Bleeker, P. (1863b) Sur une nouvelle espèce de poisson du Japon appartenant à un nouveau genre. *Nederlandsch Tijdschrift voor de Dierkunde*, 1, 382–383.
- Bogutskaya, N.G. & Komlev, A.M. (2001) Some new data to morphology of *Rhodeus sericeus* (Cyprinidae: Acheilognathinae) and a description of a new species, *Rhodeus colchicus*, from West Transcaucasia. *Proceedings of the Zoological Institute, St. Petersburg*, 287, 81–97.
- Bogutskaya, N.G., Naseka, A.M., Shedko, S.V., Vasil'eva, E.D. & Chereshev, I.A. (2009) The fishes of the Amur River: updated check-list and zoogeography. *Ichthyological Exploration of Freshwaters*, 19 (4), 301–366.
- Bohlen, J., Šlechtová, V., Bogutskaya, N. & Freyhof, J. (2006) Across Siberia and over Europe: phylogenetic relationships of the freshwater fish genus *Rhodeus* in Europe and the phylogenetic position of *R. sericeus* from the River Amur. *Molecular Phylogenetics and Evolution*, 40, 856–865.
<https://doi.org/10.1016/j.ympev.2006.04.020>
- Chang, C.-H., Li, F., Shao, K.-T., Lin, Y.-S., Morosawa, T., Kim, S., Koo, H., Kim, W., Lee, J.-S., He, S., Smith, C., Reichard, M., Miya, M., Sado, T., Uehara, K., Lavoué, S., Chen, W.-J. & Mayden, R.L. (2014) Phylogenetic relationships of Acheilognathidae (Cypriniformes: Cyprinoidea) as revealed from evidence of both nuclear and mitochondrial gene sequence variation: evidence for necessary taxonomic revision in the family and the identification of cryptic species. *Molecular Phylogenetics and Evolution*, 81, 182–194.
<https://doi.org/10.1016/j.ympev.2014.08.026>
- Chen, I.-S. & Chang, Y.-C. (2005) *A photographic guide to the inland-water fishes of Taiwan. Vol. 1. Cypriniformes*. Suei Chan Press, Keelung, xx + 280 pp.
- Chen, W.-J., Bonillo, C. & Lecointre, G. (2003) Repeatability of clades as a criterion of reliability: a case study for molecular phylogeny of Acanthomorpha (Teleostei) with larger number of taxa. *Molecular Phylogenetics and Evolution*, 26, 262–288.
- Chen, W.-J., Miya, M., Saitoh, K. & Mayden, R.L. (2008) Phylogenetic utility of two existing and four novel nuclear gene loci in reconstructing Tree of Life of ray-finned fishes: the order Cypriniformes (Ostariophysi) as a case study. *Gene*, 423, 125–134.
<https://doi.org/10.1016/j.gene.2008.07.016>
- Cheng, P., Yu, D., Liu, S., Tang, Q. & Liu, H. (2014) Molecular phylogeny and conservation priorities of the subfamily Acheilognathinae (Teleostei: Cyprinidae). *Zoological Science*, 31, 300–308.
<https://doi.org/10.2108/zs130069>
- Darriba, D., Taboada, G.L., Doallo, R. & Posada, D. (2012) jModelTest2: more models, new heuristics and parallel computing. *Nature Methods*, 9 (8), 772.
<https://doi.org/10.1038/nmeth.2109>
- Doi, A., Arai, R. & Liu, H.-Z. (1999) *Acheilognathus macromandibularis*, a new bitterling (Cyprinidae) from the lower Changjiang basin, China. *Ichthyological Exploration of Freshwaters*, 10 (4), 303–308.
- Felsenstein, J. (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, 39, 783–791.
<https://doi.org/10.1111/j.1558-5646.1985.tb00420.x>
- Günther, A. (1868) *Catalogue of the fishes in the British Museum. Vol. 7*. Printed by order of the Trustees, London, xx + 512 pp.
- Holcík, J. (1971) *Acanthorhodeus kristinae* sp. n., a new bitterling (Pisces, Cyprinidae, Acheilognathinae) from China. *Věstník Československé společnosti zoologické*, 35 (4), 270–274.
- Hosoya, K. (2002) Acheilognathinae. In: Nakabo, T. (Ed.), *Fishes of Japan with pictorial keys to the species, English edition*. Tokai University Press, Tokyo, pp. 255–260.
- Hubbs, C.L. & Lagler, K.F. (2004) *Fishes of the Great Lakes region*, revised edition. University of Michigan Press, Bloomfield Hills, xxxii + 276 pp.
<https://doi.org/10.3998/mpub.17658>
- Jordan, D.S. & Metz, C.W. (1913) A catalog of the fishes known from the waters of Korea. *Memoirs of the Carnegie Museum*, 6 (1), 1–65.
- Jordan, D.S. & Seale, A. (1906) Descriptions of six new species of fishes from Japan. *Proceedings of the United States National Museum*, 30 (1445), 143–148.
<https://doi.org/10.5479/si.00963801.30-1445.143>
- Kawamura, K., Ueda, T., Arai, R. & Smith, C. (2014) Phylogenetic relationships of bitterling fishes (Teleostei: Cypriniformes:

- Acheilognathinae), inferred from mitochondrial cytochrome *b* sequences. *Zoological Science*, 31, 321–329.
<https://doi.org/10.2108/zs130233>
- Kim, D., Jeon, H.-B. & Suk, H.Y. (2014) *Tanakia latimarginata*, a new species of bitterling from the Nakdong River, South Korea (Teleostei: Cyprinidae). *Ichthyological Exploration of Freshwaters*, 25 (1), 59–68.
- Kitamura, J., Nagata, N., Nakajima, J. & Sota, T. (2012) Divergence of ovipositor length and egg shape in a brood parasitic bitterling fish through the use of different mussel hosts. *Journal of Evolutionary Biology*, 25, 566–573.
<https://doi.org/10.1111/j.1420-9101.2011.02453.x>
- Kumar, S., Stecher, G. & Tamura, K. (2016) MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, 33, 1870–1874.
<https://doi.org/10.1093/molbev/msw054>
- Kottelat, M. (1998) Fishes of the Nam Theun and Xe Bangfai basins, Laos, with diagnoses of twenty-two new species (Teleostei: Cyprinidae, Balitoridae, Cobitidae, Coiidae and Odontobutidae). *Ichthyological Exploration of Freshwaters*, 9, 1–128.
- Kottelat, M. (2001) *Fishes of Laos*. WHT Publications, Colombo, 198 pp.
- Li, F. & Arai, R. (2011) *Rhodeus shintaiensis*, a new bitterling from China (Teleostei: Cyprinidae). *Ichthyological Exploration of Freshwaters*, 21 (4), 303–312.
- Li, F. & Arai, R. (2014) *Rhodeus albomarginatus*, a new bitterling (Teleostei: Cyprinidae: Acheilognathinae) from China. *Zootaxa*, 3790 (1), 165–176.
<https://doi.org/10.11646/zootaxa.3790.1.7>
- Lin, R. (1998) Acheilognathinae. In: Chen, Y. (Ed.), *Fauna Sinica, Osteichthyes, Cypriniformes II*. Science Press, Beijing, pp. 413–454, 504–506. [in Chinese, English abstract]
- Lin, S.Y. (1935) Contribution to a study of Cyprinidae of Kwangtung and adjacent provinces. *Lingnan Science Journal*, 14, 249–260.
- Liu, H.-Z., Zhu, Y.-R., Smith, C. & Reichard, M. (2006) Evidence of host specificity and congruence between phylogenies of bitterlings and freshwater mussels. *Zoological Studies*, 45 (3), 428–434.
- López, J.A., Chen, W.-J. & Ortí, G. (2004) Esociform phylogeny. *Copeia*, 3, 449–464.
<https://doi.org/10.1643/CG-03-087R1>
- Mai, D.Y. (1978) *Identification of the freshwater fishes of the North of Vietnam*. Science & Technics Publishing House, Ha Noi, 339 pp. [in Vietnamese]
- Miao, C.-P. (1934) Notes on the fresh-water fishes of the southern part of Kiangsu I. Chinkingang. *Contributions from the Biological Laboratory of the Science Society of China, Zoological Series*, 10 (3), 111–244.
- Nakamura, M. (1969) Cyprinid fishes of Japan: Studies on the life history of cyprinid fishes of Japan. *Special Publications of the Research Institute for Natural Resources, Tokyo*, 4, 1–455. [in Japanese, English abstract]
- Nguyen, H.D., Tran, D.H. & Ta, T.T. (2013) A new species of genus *Acheilognathus* Bleeker, 1859 from the Tien Yen River, Vietnam. *Tap Chi Sinh Hoc*, 35 (1), 18–22. [in Vietnamese, English synopsis]
- Ojima, Y., Ueno, K. & Hayashi, M. (1973) Karyotypes of the acheilognathine fishes (Cyprinidae) of Japan with a discussion of phylogenetic problems. *Zoological Magazine*, 82, 171–177. [in Japanese, English abstract]
- Okazaki, M., Naruse, K., Shima, A. & Arai, R. (2001) Phylogenetic relationships of bitterlings based on mitochondrial 12S ribosomal DNA sequences. *Journal of Fish Biology*, 58, 89–106.
<https://doi.org/10.1111/j.1095-8649.2001.tb00501.x>
- Ronquist, F. & Huelsenbeck, J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19, 1572–1574.
<https://doi.org/10.1093/bioinformatics/btg180>
- Sambrook, J. & Russell, D.W. (2001) *Molecular Cloning: A Laboratory Manual, third edition*. Cold Spring Harbor Laboratory Press, New York, 999 pp.
- Sola, L., Gornung, E., Naoi, H., Gunji, R., Sato, C., Kawamura, K., Arai, R. & Ueda, T. (2003) FISH-mapping of 18S ribosomal RNA genes and telomeric sequences in the Japanese bitterlings *Rhodeus ocellatus kurumeus* and *Tanakia limbata* (Pisces, Cyprinidae) reveals significant cytogenetic differences in morphologically similar karyotypes. *Genetica*, 119, 99–106.
<https://doi.org/10.1023/A:1024446910161>
- Stamatakis, A. (2006) RAXML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*, 22, 2688–2690.
<https://doi.org/10.1093/bioinformatics/btl446>
- Suzuki, N. & Jeon, S.-R. (1988) Development of the bitterling, *Acheilognathus signifer* (Cyprinidae), with a note on minute tubercles on the skin surface. *Korean Journal of Limnology*, 21 (3), 165–179.
- Suzuki, N., Oka, A., Sugon, Y., Yamakawa, K. & Hibiya, T. (1986) Development of the bitterling, *Tanakia tanago* (cyprinidae), with a note on minute tubercles on the skin surface. *Japanese Journal of Ichthyology*, 33 (3), 225–231.
- Tanaka, S. (1909) Descriptions of one new genus and ten new species of Japanese fishes. *Journal of the College of Science, Imperial University, Tokyo, Japan*, 27 (8), 1–27.
- Tirant, G. (1883) Mémoire sur les poissons de la rivière de Hué. *Bulletin de la Société des Études Indo-chinoises, Saigon*, 1883, 80–101.

- Yang, Q., Xiong, B., Tang, Q. & Liu, H. (2010) *Acheilognathus striatus* (Family: Cyprinidae), a new bitterling species from the lower Yangtze River. *Environmental Biology of Fishes*, 88 (4), 333–341.
<https://doi.org/10.1007/s10641-010-9645-6>
- Yang, Q., Zhu, Y., Xiong, B. & Liu, H. (2011) *Acheilognathus changtingensis* sp. nov., a new species of the cyprinid genus *Acheilognathus* (Teleostei: Cyprinidae) from southeastern China based on morphological and molecular evidence. *Zoological Science*, 28 (2), 158–167.
<https://doi.org/10.2108/zsj.28.158>
- Ueda, T., Iijima, K., Naoi, H., Arai, R., Ishinabe, T. & Jeon, S.-R. (2006) Karyotypes of three *Tanakia* bitterlings (Pisces, Cyprinidae) from East Asia. *Cytologia*, 71, 251–255.
<https://doi.org/10.1508/cytologia.71.251>
- Ueda, T., Mashiko, N., Takizawa, H., Akai, Y., Ishinabe, T., Arai, R. & Wu, H. (1997) Ag- NOR variation in chromosomes of Chinese bitterlings, *Rhodeus lighti* and *Tanakia himantegus* (Cypriniformes, Cyprinidae). *Ichthyological Research*, 44, 302–305.
<https://doi.org/10.1007/BF02678709>
- Wu, Q.J. (1964) Acheilognathinae. In: Wu, H.W. (Ed.), *Cyprinid fishes of China. Vol. 1*. Shanghai Science Technology Press, Shanghai, pp. 199–211, 64–67. [in Chinese]
- Zhang, C.-G. & Zhao, Y.-H. (2016) *Species diversity and distribution of inland fishes in China*. Science Press, Beijing, 284 pp. [in Chinese]