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***Saurogobio punctatus* sp. nov., a new cyprinid gudgeon (Teleostei: Cypriniformes) from the Yangtze River, based on both morphological and molecular data**

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A new cyprinid gudgeon, *Saurogobio punctatus* sp. nov., is described based on specimens collected from the Yangtze River, China. The new species can be distinguished from its congeners by differences in both morphology and the cytochrome *b* (*cytb*) gene sequence. Numerous minute blackish spots are scattered on dorsal and caudal fins in *S. punctatus* sp. nov. v. absent in the other seven valid *Saurogobio* species. The new species can be further distinguished from its congeners by the following unique combination of characters: a dorsal fin with eight branched rays; absence of scales in chest area before pectoral origin; upper and lower lips thick, covered with papillae; and a papillose mental pad approximately triangular. Morphologically, the new species most resembles the Chinese lizard gudgeon *Saurogobio dabryi*, but the new species lays yellowish adhesive eggs v. white pelagic eggs in *S. dabryi*. A phylogenetic analysis of all *Saurogobio* species based on *cytb* gene sequences indicated that *S. punctatus* sp. nov. was distinctly separated from its congeners, with mean sequence divergence ranging from 12.6 to 21.0%. Therefore, molecular data further supported the distinctiveness of the new species.

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Key words: morphological difference; new species; phylogenetic analysis; *Saurogobio*; sequence divergence.

INTRODUCTION

The order Cypriniformes is the most diverse clade of freshwater fishes in the world, with >4200 recognized species (Mayden *et al.*, 2009; Conway *et al.*, 2010; Nelson *et al.*, 2016). Belonging to the speciose subfamily Gobioninae (the gudgeons) of the family Cyprinidae, the genus *Saurogobio* Bleeker 1870 is endemic to East Asia including China, Russia, the Korean Peninsula and northern Vietnam (Yue, 1998). *Saurogobio*

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species are small to medium-sized cyprinids, with a maximum standard length (L_S) ranging from 117 to 270 mm as adults (Froese & Pauly, 2016) and inhabit the bottoms of streams, rivers and lakes. They can be distinguished from other cyprinids by the following combination of characters: body elongate and slender; ventrally flat; inferior mouth horseshoe shaped; lips usually with papillae; dorsal-fin origin closer to tip of snout than to caudal-fin base, with seven or eight branched rays; anterior chamber of air bladder enclosed in a bony capsule; and two median fontanelles on the roof of the skull (Bănărescu & Nalbant, 1973; Luo *et al.*, 1982; Yue, 1998; Dai & Yang, 2002).

Eighteen species have been described since the genus was first established and presently only seven species are recognized as valid: *Saurogobio dabryi* Bleeker 1871, *Saurogobio dumerili* Bleeker 1871, *Saurogobio gracilicaudatus* Yao & Yang 1977, *Saurogobio gymnocheilus* Lo, Yao & Chen 1998, *Saurogobio immaculatus* Koller 1927, *Saurogobio xiangjiangensis* Tang 1980 and *Saurogobio lissilabris* Bănărescu & Nalbant 1973 (Froese & Pauly, 2016). All seven species can be found in China (Bănărescu & Nalbant, 1973; Luo *et al.*, 1982; Yue, 1998; Dai & Yang, 2002; Yang *et al.*, 2003) and all but one occur in the Yangtze River basin except for *S. immaculatus*, which is limited to Hainan Island in China.

Over the past 10 years *Saurogobio* species have been collected from 25 localities, primarily in the Yangtze River basin and nearly all valid *Saurogobio* species have been sampled. With this extensive collection, the necessary comparative material has been made available to investigate the species diversity of this genus. In this study, a new species, which is morphologically similar to *S. dabryi*, was discovered through combined analysis of morphological and molecular data. The specific aims of this study were to describe a new *Saurogobio* species based on morphological character data; to analyse the sequence variation and phylogenetic position of the new species; to construct an identification key for the species of *Saurogobio*.

MATERIALS AND METHODS

SAMPLE COLLECTION

Saurogobio specimens used in this study were collected from 25 localities including 23 sites along the Yangtze River basin, one in Hainan Island and one from the Amur River. In addition, one sequence of molecular data was also obtained from *S. dabryi* in South Korea (Fig. 1; Table I). All samples were identified following the diagnostic characters described by Bănărescu & Nalbant (1973), Luo *et al.* (1982) and Yue (1998).

Among the seven valid species of *Saurogobio*, *S. lissilabris* has not been mentioned since its initial description (Bănărescu & Nalbant, 1973). Some authors suspected its validity (Luo *et al.*, 1982; Yue, 1998) or considered it as a junior synonym of *S. gymnocheilus* without giving any explanation (Dai *et al.*, 2014). To test the validity of *S. lissilabris*, four specimens recognized as *S. lissilabris* following Bănărescu & Nalbant (1973) in this study were compared with the type specimens of *S. lissilabris* from the American Museum of Natural History (lots AMNH 29693 and 29698). *Saurogobio lissilabris* has several characters that distinguish it from *S. gymnocheilus*: chest in front of pectoral origin naked (v. chest in front of pectoral origin with a few scales); an obvious notch in front of nostril (v. head flat without obvious notch in front of nostril); paired fins pointed and long with pectoral fin close to ventral-fin origin (v. paired fins short with pectoral fin far from ventral-fin origin). Therefore, it is reasonable to consider *S. lissilabris* as a valid species and include it in the present analysis.

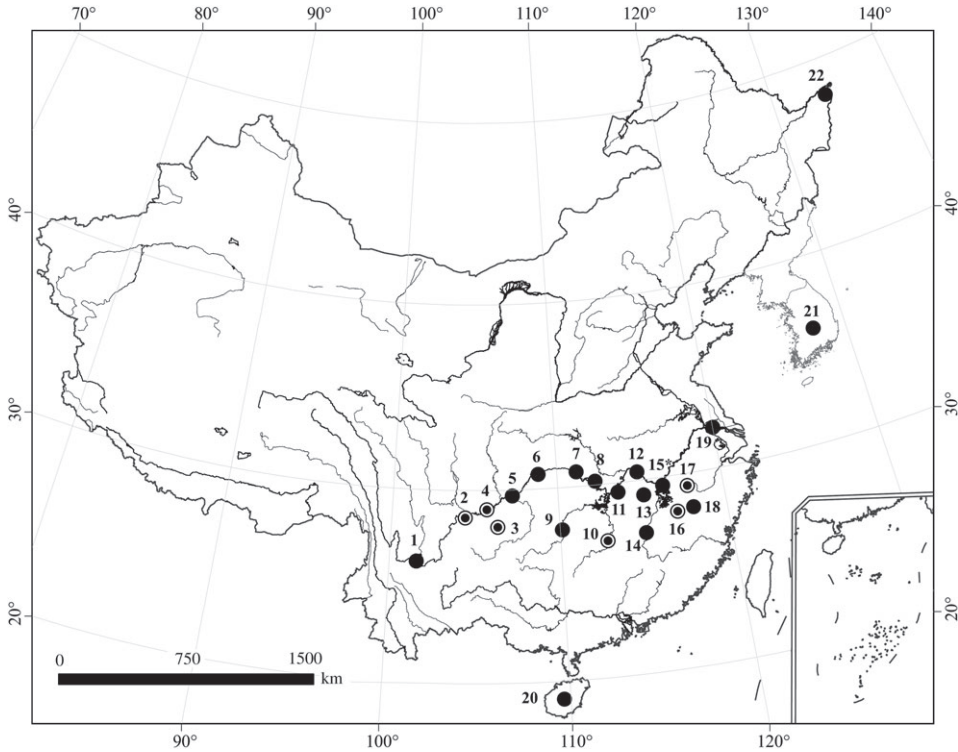


FIG. 1. Sampling localities for *Saurogobio* species in the present molecular study and distribution of *Saurogobio punctatus* sp. nov. ●, sampling sites of *Saurogobio* species; ○, distribution of *S. punctatus* sp. nov. 1, Panzhihua, Sichuan Province (PZH); 2, Yibin, Sichuan Province (YB); 3, Chishui, Guizhou Province (CS); 4, Hejiang, Sichuan Province (HJ); 5, Mudong, Chongqing City (MD); 6, Wanzhou, Chongqing City (WZ); 7, Zigui, Hubei Province (ZG); 8, Yichang, Hubei Province (YC); 9, Yuanling, Hunan Province (YL); 10, Xiangjiang, Hunan Province (XJ); 11, Yueyang, Hunan Province (YY); 12, Wuhan, Hubei Province (WH); 13, Wuning, Jiangxi Province (WN); 14, Ganjiang, Jiangxi Province (GJ); 15*, five nearby localities along Poyang Lake in Jiangxi Province: Hukou (HK), Xinzi (XZ), Duchang (DC), Yugan (YG), Poyang (PY); 16, Yingtan, Jiangxi Province (YT); 17, Wuyuan, Jiangxi Province (WY); 18, Guangfeng, Jiangxi Province (GF); 19, Changshu, Jiangsu Province (JS); 20, Hainan Province (HN); 21, Korea, South Korea; 22, Zhuajiang, Heilongjiang Province (HLJ).

For the morphological study, all valid species in *Saurogobio* were compared with the new species by examining specimens or consulting published descriptions when comparative material was not available. A total of 206 *Saurogobio* samples were examined for this study, including 65 individuals of *S. dabryi* and 62 of the new species. Among the 206 samples, 82 individuals representing all the valid *Saurogobio* species including the new species were also used in the molecular phylogenetic analysis (Table I). Two individuals of *Pseudogobio vaillanti* (Sauvage 1878), species closely related to *Saurogobio*, were collected and used as out groups. The detailed information including catalogue numbers of all samples is listed in Supporting Information Table S1. Fin and muscle tissues were preserved using 95% ethanol and used for DNA extraction. Most of the voucher specimens used for the morphological analysis were preserved in 7–10% formalin, while some smaller specimens were preserved in 95% ethanol. All specimens were deposited in the collection of the Institute of Hydrobiology, Chinese Academy of Sciences (IHCAS).

TABLE I. Summary of sample details for *Saurogobio* species and out-group species used in the present molecular study. Sampling locality, sample size (n) in each site and total sample size (N_T) of each species are given

Species	Locality	n	Code*	Drainage	N_T		
<i>S. gymnocheilus</i>	Wanzhou, Chongqing City	1	SgyWZ	Upper Yangtze River	11		
	Yichang, Hubei Prov.	2	SgyYC	Middle Yangtze River			
	Wuhan, Hubei Prov.	3	SgyWH	Middle Yangtze River			
	Poyang, Jiangxi Prov.	2	SgyPY	Middle Yangtze River			
	Yingtang, Jiangxi Prov.	3	SgyYT	Middle Yangtze River			
<i>S. lissilabris</i>	Wuhan, Hubei Prov.	3	SliWH	Middle Yangtze River	3		
<i>S. immaculatus</i>	Hainan Province	2	SimHN	Hainan Island	2		
<i>S. dumerili</i>	Wuhan, Hubei Prov.	2	SduWH	Middle Yangtze River	4		
	Changshu, Jiangsu Prov.	2	SduJS	Lower Yangtze River			
<i>S. gracilicaudatus</i>	Wuhan, Hubei Prov.	5	SgrWH	Middle Yangtze River	5		
<i>S. xiangjiangensis</i>	Yuanling, Hunan Prov.	3	SxiYL	Middle Yangtze River	4		
	Guangfeng, Jiangxi Prov.	1	SxiGF	Middle Yangtze River			
<i>S. punctatus</i> sp. nov.	Hejiang, Sichuan Prov.	2	SpuHJ	Upper Yangtze River	13		
	Yibin, Sichuan Prov.	2	SpuYB	Upper Yangtze River			
	Chishui, Guizhou Prov.	3	SpuCS	Upper Yangtze River			
	Duchang, Jiangxi Prov.	1	SpuDC	Middle Yangtze River			
	Wuyuan, Jiangxi Prov.	1	SpuWY	Lower Yangtze River			
	Wuning, Jiangxi Prov.	1	SpuWN	Middle Yangtze River			
	Xiangjiang, Hunan Prov.	1	SpuXJ	Middle Yangtze River			
	Ganjiang, Jiangxi Prov.	2	SpuGJ	Middle Yangtze River			
	<i>S. dabryi</i>	South Korea	1	SdaKorea			40
Chishui, Sichuan Prov.		3	SdaCS	Upper Yangtze River			
Yibin, Sichuan Prov.		3	SdaYB	Upper Yangtze River			
Hejiang, Sichuan Prov.		3	SdaHJ	Upper Yangtze River			
Panzhihua, Sichuan Prov.		3	SdaPZH	Upper Yangtze River			
Wanzhou, Chongqing City		2	SdaWZ	Upper Yangtze River			
Mudong, Chongqing City		2	SdaMD	Upper Yangtze River			
Zigui, Hubei Prov.		2	SdaZG	Middle Yangtze River			
Yichang, Hubei Prov.		3	SdaYC	Middle Yangtze River			
Wuhan, Hubei Prov.		2	SdaWH	Middle Yangtze River			
Yueyang, Hunan Prov.		2	SdaYY	Middle Yangtze River			
Xiangjiang, Hunan Prov.		2	SdaXJ	Middle Yangtze River			
Duchang, Jiangxi Prov.		2	SdaDC	Middle Yangtze River			
Xinzi, Jiangxi Prov.		2	SdaXZ	Middle Yangtze River			
Poyang, Jiangxi Prov.		2	SdaPY	Middle Yangtze River			
Hukou, Jiangxi Prov.		2	SdaHK	Middle Yangtze River			
Yugan, Jiangxi Prov.		2	SdaYG	Middle Yangtze River			
Zhuaji, Heilongjiang Prov.		2	SdaHLJ	Amur River			
Out group							
<i>Pseudogobio vaillanti</i>		Wuyuan, Jiangxi Prov.	1		Lower Yangtze River	2	
	Guilin, Guangxi Prov.	1		Pearl River			

*Code is composed of species abbreviation (e.g. Sgy for *S. gymnocheilus*) followed by locality abbreviation (e.g. WZ for Wanzhou).

MORPHOLOGICAL ANALYSIS

Measurements were made point-to-point with a dial calliper and recorded to 0.1 mm. Measurements and counts were taken on the left side of specimens whenever possible following the methods of Hubbs & Lagler (2004). Five meristic characters were recorded including the number of branched rays of dorsal, pectoral, pelvic and anal fins and lateral line scales. When counting the number of branched dorsal or anal-fin rays, the last two branched fin rays, which are fused at the base, were counted as one, following Wu (1982). Some terms used to describe the oral lip structure of *Saurogobio* species were based on the works of Bănărescu & Nalbant (1973) and Dai *et al.* (2014).

Because the new species morphologically resembles *S. dabryi*, morphological variation between samples of the two species was analysed further by using additional morphological characters (Table II). Morphological data were collected from 65 specimens of *S. dabryi* and 62 of the new species.

MOLECULAR PHYLOGENETIC ANALYSIS

Total DNA was extracted from fin or muscle tissues using a salt-extraction method following the procedure of Tang *et al.* (2008). Part of the mitochondrial cytochrome *b* (*cytb*) gene was amplified using the polymerase chain reaction with the primers L14724 and H15915 adopted from Xiao *et al.* (2001). Amplified DNA was purified and sequenced using the same primers as above by commercial sequencing companies. All sequences have been deposited in GenBank (Accession numbers: KR362919–KR363002; Table S1, Supporting Information).

Multiple sequence alignments were performed using the ClustalX 2.1 (Thompson *et al.*, 1997; Larkin *et al.*, 2007) alignment editor with the default settings. All the aligned sequences were translated into amino acid residues in MEGA 6.0 (Tamura *et al.*, 2013) to test for sequence editing errors. The pairwise DNA sequence differences between species were calculated based on the best evolutionary model determined by jModelTest 2.1.7 (Guindon & Gascuel, 2003; Darriba *et al.*, 2012) with s.e. estimated using 1000 bootstrap replicates in MEGA.

Phylogenetic relationships were estimated using Bayesian inference (BI) and maximum likelihood (ML) methods. The best-fit model of nucleotide evolution for the data was identified using the Bayesian information criterion as estimated in jModelTest. Partitioned Bayesian analysis was carried out using MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003). The data were partitioned by codon position. Two independent analyses with four simultaneous Markov chain Monte-Carlo (MCMC) runs of 6 000 000 generations were made sampling every 1000 generations, with a total of 6001 trees each. After testing for convergence of the MCMC algorithm, the first 2000 trees were discarded as burn-in. A 50% majority-rule consensus tree was obtained from the remaining 4001 trees and nodal support was assessed by calculating the mean posterior probability (PP) value of each node of the resulting consensus tree. ML analysis was conducted using RAxML 8.1.13 (Stamatakis, 2014). The best scoring ML tree was identified using a nucleotide substitution model of GTR + I + G. The support of each node was estimated using a rapid bootstrap analysis with 1000 replicates.

RESULTS

DESCRIPTION OF *SAUROGOBIO PUNCTATUS* SP. NOV.

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[Fig. 2(a), (b), (c)]

Holotype

IHCAS 2014070313, 159.6 mm standard length (L_S), Chishui City (28° 36' N; 105° 42' E) Chishui River, a tributary in the upper Yangtze River, Guizhou Province, south-western China; X. B. Li, F. Liu & X. Wang, October 2014 [Fig. 2(a), (b)].

TABLE II. Meristic and morphometric data for *Saurogobio punctatus* sp. nov. and its related species *S. dabryi*. Significant differences ($P < 0.05$) are shown in bold

Characters	<i>S. punctatus</i> sp. nov. ($n = 30$ for meristic data, $n = 62$ for morphometric data)				<i>S. dabryi</i> ($n = 30$ for meristic data, $n = 65$ for morphometric data)		
	Holotype	Range	Mean	S.D.	Range	Mean	S.D.
Lateral line scales	47	44–49			48–50		
Branched dorsal–fin rays	8	8			8		
Branched anal–fin rays	6	6			6		
Branched pectoral–fin rays	12	11–15			11–14		
Branched pelvic–fin rays	7	6–8			5–7		
Standard length, L_S (mm)	159.6	98.3–161.4	124.0	18.8	48.5–175.5	126.7	29.6
Head length, L_H (mm)	35.7	19.7–38.5	28.0	4.6	11.6–36.0	26.8	5.6
% L_S							
Body depth, D_B	15.6	12.5–18.2	15.4	1.4	11.6–21.5	16.4	2.1
Head depth, D_H	22.3	18.7–25.4	22.6	1.3	17.3–24.8	21.4	1.4
Snout length, L_{SN}	8.8	6.7–12.7	9.3	1.1	7.0–11.0	9.1	0.9
Orbital diameter, D_E	5.2	4.5–7.2	5.8	0.6	3.6–6.9	5.6	0.7
Predorsal length, L_{PD}	35.8	36.3–44.0	40.3	1.7	37.9–44.3	39.9	1.5
Prepectoral length, L_{PtB}	24.9	19.9–26.7	24.0	1.4	20.0–33.9	23.6	1.9
Prepelvic length, L_{PPV}	49.7	44.1–51.4	47.9	1.5	43.8–54.4	49.0	2.1
Pre-anal length, L_{PA}	77.4	72.1–79.6	75.8	1.5	73.3–84.9	77.4	2.3
D_{PtPv}	25.5	20.8–28.9	24.6	1.6	21.9–31.7	26.0	2.2
D_{PvAf}	27.9	22.5–29.9	18.4	2.3	20.8–30.7	26.4	2.1
D_{PvAn}	4.8	3.0–6.9	5.1	0.9	4.5–9.0	6.5	1.0
Caudal peduncle length, L_{CP}	14.1	11.7–22.9	18.4	2.3	12.9–20.6	15.7	1.8
Caudal peduncle depth, D_{CP}	6.1	4.5–6.7	5.8	0.4	5.3–7.9	6.2	0.6
% L_H							
Head width, W_H	51.8	40.1–64.5	52.9	4.5	46.1–69.5	57.2	6.0
Snout length, L_{SN}	39.2	31.5–50.8	41.0	3.3	33.3–52.4	42.4	3.8
Orbital diameter, D_O	23.3	18.7–33.3	25.6	2.8	18.9–32.7	26.3	3.0
Interorbital width, W_{IO}	22.4	14.7–30.6	22.2	3.2	14.5–39.8	22.6	5.2
% L_{CP}							
D_{CP}	43.5	24.4–48.6	32.2	5.3	29.2–56.9	40.4	6.5

D_{PtPv} , distance between pectoral origin and pelvic origin; D_{PvAf} , distance between end of pelvic–fin base to origin of anal–fin base; D_{PvAn} , distance between end of pelvic–fin base to anus.

Paratypes

IHCAS 2014070185–7, 2014070192–3, 2014070196, 2014070198–9, 2014070203, 2014070273, 2014070275, 2014070277, 2014070279, 2014070281, 2014070297, 2014070339, 2014070350, 2014070352, 2014070356, 20137329, 20137337, 21 specimens, 102.1–155.4 mm L_S , same data as the holotype; IHCAS 20136433, 20 137 158, two specimens, 105.6–134.8 mm L_S , Xuyong County (27° 43' N; 105° 34' E) Chishui River, in the upper Yangtze River, Sichuan Province; collected with holotype. IHCAS 2014070111, 2014070229, 2014070233–4, 2014070236–7, 20 137 245, seven specimens, 117.5–161.4 mm L_S , Hejiang County (28° 42' N; 105° 43' E) in the upper Yangtze River, Sichuan Province; same collectors and collecting time as the holotype.

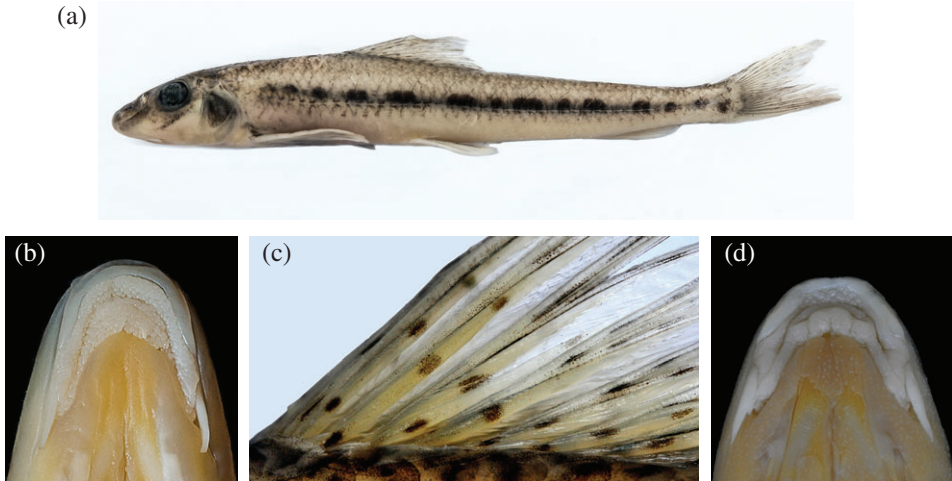


FIG. 2. *Saurogobio punctatus* sp. nov., IHCAS 2014070313, holotype, 159.6 mm standard length (L_S): (a) lateral view and (b) ventral view of the head; (c) *S. punctatus* sp. nov., IHCAS 2014070229, paratype, 161.4 mm L_S , showing spotted dorsal fin; (d) ventral view of the head in *Saurogobio dabryi*, IHCAS 2014070235, 147.5 mm L_S .

Diagnosis

Presence of numerous minute blackish spots scattered on dorsal and caudal fins in *S. punctatus* sp. nov. [Fig. 2(a), (c)] v. absent in all seven other valid species. The new species can be further distinguished from its congeners by the following unique combination of characters: a dorsal fin with eight branched rays v. seven rays in *S. dumerili*, *S. lissilabris*, *S. immaculatus* and *S. gymnocheilus*; chest in front of pectoral origin naked v. completely scaled in *S. dumerili* or with few scales in *S. gymnocheilus*; both upper and lower lips thick and developed, densely covered with developed papillae v. thin and smooth or with reduced papillae in *S. lissilabris* and *S. gymnocheilus*; anterior fold of lower lip present, discontinued from lateral lobe v. absent in *S. gracilicaudatus* or present but continued with lateral lobe in *S. dumerili*, *S. lissilabris* and *S. gymnocheilus*; lateral lobe continued with central pad of lower lip v. discontinued in *S. dumerili*, *S. lissilabris* and *S. gymnocheilus*; a notch anterior to nostrils v. a flat head and no obvious notch in *S. gymnocheilus* or a slight notch in *S. dumerili*; eye diameter usually larger than interorbital width v. smaller than interorbital width in *S. gracilicaudatus* and *S. xiangjiangensis*; lateral line scales 44–49 v. 55–61 in *S. dumerili*, 52–54 in *S. xiangjiangensis* and 40–42 in *S. immaculatus*; a relatively short and deep caudal peduncle v. a slender peduncle in *S. gracilicaudatus* and *S. xiangjiangensis*.

Description

Meristic and morphometric data are listed in Table II. Dorsal-fin rays III, 8; anal-fin rays III, 6. Pharyngeal teeth one row, 5–5 in formula (three samples). Lateral line scales 44–49. Body small or medium-sized, slender, somewhat cylindrical, dorsally rounded and ventrally flattened; dorsal profile convex from tip of snout to dorsal origin, where the maximum body depth is attained. Caudal peduncle slender, slightly compressed, least caudal peduncle depth near to caudal-fin base. Head slightly deeper than wide.

Snout bluntly pointed and longer or shorter than postorbital distance; an obvious notch anterior to nostrils. Eye large, superolateral. Mouth inferior and horseshoe-shaped; a pair of short barbels present at the corner of mouth, much shorter than eye diameter. Both lips thick and developed, densely covered with developed papillae; anterior fold of lower lip present, discontinued from lateral lobe; lateral lobes anteriorly connected with mental pad; mental pad papillose, approximately triangular in shape and anteriorly separated from anterior fold of lower lip by a short arched shallow groove; lateral lobes of lower lip and posterior edge of mental pad entirely separated from mental region by an arched deep groove [Fig. 2(b)]. Oral lip structure of the new species is distinct from its sibling species *S. dabryi* in shape of the mental pad, approximately triangular v. more square in *S. dabryi* [Fig. 2(d)].

Dorsal fin without ossified rays, its origin nearer to tip of snout than to caudal-fin base. Edge of dorsal and anal fins emarginate; caudal forked, in most specimens upper lobe longer than lower lobe, lobes equal in a few specimens. Paired fins horizontal; pectoral fin extending almost to pelvic-fin origin with a distance of 1–3 scales; end of pelvic fin reaching halfway or slightly greater than halfway to anal-fin origin; anus much nearer to posterior end of pelvic-fin base than to anal-fin origin, distance from posterior of pelvic-fin base to anal-fin origin about 4–6 times distance from posterior end of pelvic-fin base to anus; anal-fin origin slightly nearer to caudal-fin base than to posterior end of pelvic-fin base.

Lateral line complete with 44–49 perforated scales, running straight in middle of caudal peduncle.

Colouration in life

Yellow brownish above, silvery yellowish below; fins a little reddish orange or yellowish. Top of head brown blackish. Scales on back and both sides with dark chromatophores along its edge forming a faint, dusky and crescentic mark. In some samples, scales on anterior half of back showing lightly green bluish iridescence. Many minute blackish spots on dorsal and caudal fins, surface of pectoral and pelvic fins having irregular melanin pigments but some individuals also having minute blackish spots, anal fin white.

Colouration in preservative

Preserved in formalin, body colour fades to greyish black; dorsal surface and both sides greyish black, scales bordered with minute slight blackish spots; ventral yellow-white; top of head black; operculum with a large irregular spot; black above origin of pectoral base; a faint grey longitudinal stripe above lateral line from upper angle of gill opening to root of caudal, 9–13 large round or elongate dark spots along this stripe. Dorsal and caudal fins spotted, pectoral and pelvic fins light greyish black but some individuals also having minute blackish spots, anal fin grey-white.

Distribution

Saurogobio punctatus sp. nov. is distributed in the upper Yangtze River mostly in Chishui drainage, also in Yibin and Hejiang City located in the main stream of the Yangtze River. Only a few specimens were found in tributaries of the middle or lower Yangtze River, such as the rivers flowing into Dongting Lake in the Hunan Province or Poyang Lake in the Jiangxi Province, China (Fig. 1).

Ecology

Saurogobio punctatus sp. nov. usually lives on the bottom of fast flowing water. It attains sexual maturity at the age of 1 year. The spawning period occurs from March to May when the water temperature is 12–20° C in the Chishui drainage. Their eggs are yellowish and adhesive (X. B. Li., unpubl. data).

Etymology

The specific epithet *punctatus* (from the Latin *punctum*, meaning spot), refers to the diagnostic characters of dark spots scattered on the dorsal and caudal fins and elongate round spots above the lateral line. It is to be treated as a noun in apposition. Its Chinese common name is 'bandian shejiu' and English common name is spotted lizard gudgeon.

MORPHOLOGICAL COMPARISON BETWEEN SAMPLES OF *S. DABRYI* AND THE NEW SPECIES

For both meristic and morphometric characters, samples of *S. dabryi* and the new species displayed little variation. Both species have a stable number of branched dorsal (8) and anal-fin rays (6). For the number of branched pectoral and pelvic-fin rays, they have different, but overlapping ranges (pectoral fin: 11–14 in *S. dabryi* v. 11–15 in the new species; pelvic fin: 5–7 v. 6–8), which is similar to the number of lateral line scales (48–50 v. 44–49) (Table II).

Among 20 morphometric characters, most show overlapping range and similar mean, but four characters related to body shape and three related to position of pelvic fins, anal fin and anus, exhibit different variation ranges although large overlaps still exist (Table II). The new species has a more slender body shape with a shallower body depth ($D_B:L_S$: 12.5–18.2% v. 11.6–21.5%), caudal peduncle depth [$D_{CP}:L_S$: 4.5–6.7% v. 5.3–7.9%; $D_{CP}:L_{CP}$ (caudal-peduncle length): 24.4–48.6% v. 29.2–56.9%] and narrower head width [$W_H:L_H$ (head length): 40.1–64.5% v. 46.1–69.5%] than *S. dabryi*. The position of the pelvic and anal-fin origins and anus in the new species is more anterior with a shorter distance between the pectoral and pelvic origins ($D_{PiPv}:L_S$: 20.8–28.9% v. 21.9–31.7%), shorter pre-anal length ($L_{PA}:L_S$: 72.1–79.6% v. 73.3–84.9%) and shorter distance between end of pelvic-fin base and anus ($D_{PvAn}:L_S$: 3.0–6.9% v. 4.5–9.0%) (Table II).

SEQUENCE VARIATION AND PHYLOGENETIC POSITION OF THE NEW SPECIES

Partial *cytb* gene sequences for 84 individuals (including out groups) were obtained. After alignment, sequences were trimmed and 1100 bp of the *cytb* gene were used for analysis. Among the 1100 bp, 411 sites were variable and 351 were parsimony informative. The pairwise sequence divergence within *Saurogobio* species ranged from 8.5% (between *S. gracilicaudatus* and *S. xiangjiangensis*) to 21.9% (between *S. immaculatus* and *S. gracilicaudatus*) (Table III). Based on the *cytb* gene sequences, two inference methods (BI and ML) yielded, for the most part, a congruent topology, although some of the more shallow nodes varied between the methods. Here, only the BI tree is shown with node support values displayed from the two inference methods (Fig. 3).

All *Saurogobio* species formed a clade with high bootstrap (BP) support and PP (BP = 100 and PP = 1.00), including two reciprocally monophyletic groups (clade A

TABLE III. Pairwise mean genetic distance (below the diagonal) and s.e. (above the diagonal) for the *Saurogobio* species and the out group *Pseudogobio vaillanti*, based on Tamura-Nei model with γ -shape parameter ($G = 1.360$)

Clade	Species	Code	Code								
			1	2	3	4	5	6	7	8	9
Clade A	<i>S. lissilabris</i>	1		0.012	0.014	0.014	0.017	0.016	0.018	0.016	0.018
	<i>S. gymnocheilus</i>	2	0.116		0.012	0.015	0.018	0.016	0.017	0.016	0.020
	<i>S. immaculatus</i>	3	0.149	0.128		0.015	0.018	0.017	0.019	0.017	0.019
	<i>S. dumerili</i>	4	0.145	0.149	0.153		0.016	0.015	0.017	0.015	0.018
Clade B	<i>S. dabryi</i>	5	0.182	0.195	0.204	0.173		0.011	0.013	0.013	0.017
	<i>S. xiangjiangensis</i>	6	0.176	0.175	0.199	0.167	0.092		0.010	0.013	0.016
	<i>S. gracilicaudatus</i>	7	0.191	0.185	0.219	0.171	0.124	0.085		0.015	0.020
	<i>S. punctatus</i> sp. nov.	8	0.182	0.173	0.210	0.168	0.137	0.126	0.151		0.016
Out group	<i>Pseudogobio vaillanti</i>	9	0.230	0.246	0.235	0.220	0.215	0.197	0.224	0.195	

and clade B). Clade A contained four species with seven branched dorsal-fin rays, *S. dumerili*, *S. lissilabris*, *S. immaculatus* and *S. gymnocheilus* and their pairwise sequence difference ranged from 11.6% (between *S. lissilabris* and *S. gymnocheilus*) to 15.3% (between *S. dumerili* and *S. immaculatus*) (Fig. 3; Table III). Clade B was composed of the other four *Saurogobio* species with eight branched dorsal-fin rays. Thirteen samples of the new species *S. punctatus* formed a monophyletic group and clustered within clade B. This clade was distinctly separated from the other three species (*S. dabryi*, *S. gracilicaudatus* and *S. xiangjiangensis*) in Clade B. The pairwise sequence difference between the new species and the other three species was 13.7, 15.1 and 12.6%, respectively, which was much higher than the divergences between some other species, such as 8.5% between *S. gracilicaudatus* and *S. xiangjiangensis* (Table III). The interspecific relationships of the four species in clades A and B are presented in Fig. 3.

KEY TO THE SPECIES OF *SAUROGOBIO*

Eight *Saurogobio* species have been recognized. The morphological differences among them are listed in Table IV and a key to identify them is as following.

- 1a. Dorsal fin with seven branched rays 2
- 1b. Dorsal fin with eight branched rays 5
- 2a. Chest completely scaled; lateral line scales 55–61; eye much smaller than interorbital width *S. dumerili*
- 2b. Chest in front of pectoral origin naked or with a few scales; lateral line scales less than 50; eye equal or slightly larger than interorbital width 3
- 3a. Lips thick with small papillae; no spots above lateral line *S. immaculatus*.
- 3b. Lips thin and smooth or with reduced papillae; several round spots above lateral line or confluent as a faint stripe 4
- 4a. Chest in front of pectoral origin with a few scales; head flat without obvious notch in front of nostril; paired fins short with pectoral fin far from ventral fin origin ...
..... *S. gymnocheilus*

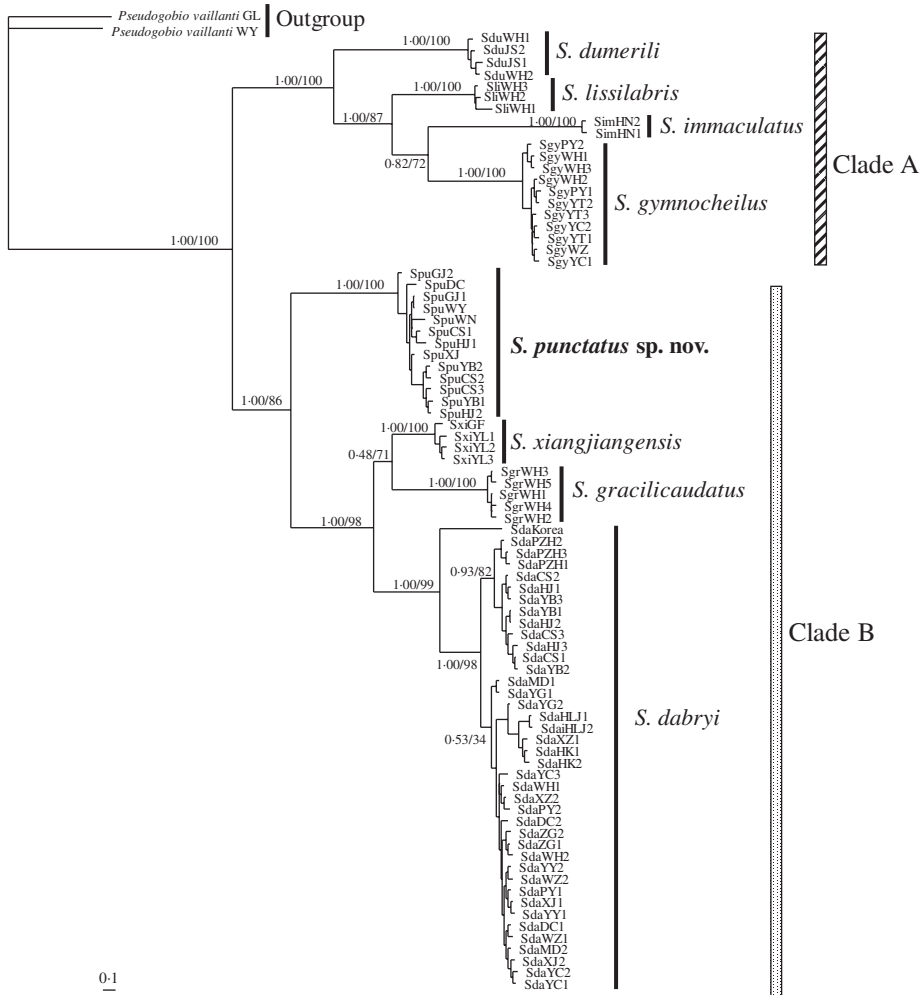


FIG. 3. Bayesian 50% majority rule consensus tree of the genus *Saurogobio* based on mitochondrial cytochrome *b* gene sequences. Values at the nodes correspond to the support values for Bayesian inference and maximum likelihood (BI/ML) methods.

- 4b. Chest in front of pectoral origin naked; an obvious notch in front of nostril; paired fins pointed and long with pectoral fin close to ventral fin origin *S. lissilabris*
- 5a. Many minute black spots on dorsal and caudal fins *S. punctatus*
- 5b. Fins without spots 6
- 6a. Snout bluntly pointed, usually equal or slightly longer than postorbital distance; caudal peduncle relatively short and deep *S. dabryi*
- 6b. Snout long and blunt, evidently longer than postorbital distance; caudal peduncle slender 7
- 7a. Lateral line scales 52–54 *S. xiangjiangensis*
- 7b. Lateral line scales 44–46 *S. gracilicaudatus*

TABLE IV. Morphological differences among *Saurogobio* species

Character	<i>S. fissilabris</i> ^a	<i>S. gymnocheilus</i> ^b	<i>S. immaculatus</i> ^b	<i>S. dumerilii</i> ^b	<i>S. dabryi</i> ^b	<i>S. xiangjiangensis</i> ^b	<i>S. gracilicaudatus</i> ^b	<i>S. punctatus</i> sp. nov.
Number of branched dorsal fin rays	7	7	7	7	8	8	8	8
Chest in front of pectoral origin	Naked	With few scales ^c	Naked	Completely scaled	Naked	Naked	Naked	Naked
Number of lateral line scales	43–46	40–45 usually 42–43	40–42	55–61	47–50	52–54	44–46	44–49
Lips	Thin, smooth or with reduced papillae	Thin, smooth or with reduced papillae	Thick with small papillae	Thick with small papillae	Thick with lots of globular papillae	Thick and developed with lots of globular papillae	Thick and developed with lots of brush-like and conical papillae	Thick with lots of globular papillae
Anterior fold of lower lip	Present, continued with lateral lobe	Present, continued with lateral lobe	Present, discontinued from lateral lobe	Present, continued with lateral lobe	Present, discontinued from lateral lobe	Present, discontinued from lateral lobe	Absent	Present, discontinued from lateral lobe
Mental pad	Smooth	Smooth	With small or reduced papillae	Smooth or with reduced papillae	With small papillae	With developed papillae	With not very obvious papillae	With small papillae
Snout	Bluntly pointed, shorter than postorbital distance	A slightly triangular, shorter than postorbital distance	Bluntly pointed, usually equal or longer than postorbital distance	Bluntly pointed, usually shorter than postorbital distance	Bluntly pointed, usually equal or longer than, occasionally shorter than postorbital distance	Long and blunt, obvious longer than postorbital distance	Long and blunt, obvious longer than postorbital distance	Bluntly pointed, longer, or shorter than postorbital distance
Notch in front of nostril	Obvious	Head flat, no obvious notch	Obvious	Slightly	Obvious	Obvious	Obvious	Obvious
Size of eye diameter	Equal or slightly larger than interorbital width	Equal or slightly larger than interorbital width	Equal or slightly larger than interorbital width	Much smaller than interorbital width	Slightly larger than interorbital width	Smaller than interorbital width	Smaller than interorbital width	Larger than interorbital width
Spots or stripe above lateral line	A faint black stripe occasionally with several slightly dark spots	A slightly black stripe usually with 12–13 dark spots	No spots, with a faint stripe	No obvious spots, some with a very faint stripe	A faint stripe, some individuals with round or elongate black spots	8–9 obvious large elongate dark spots	A very faint dusky stripe without spots	A faint stripe with 9–13 large round or elongate black spots

TABLE IV. Continued

Character	<i>S. issilabris</i> ^a	<i>S. gymnocheilus</i> ^b	<i>S. immaculatus</i> ^b	<i>S. dumeril</i> ^b	<i>S. dabryi</i> ^b	<i>S. xiangjiangensis</i> ^b	<i>S. gracilicaudatus</i> ^b	<i>S. punctatus</i> sp. nov.
Minute spots on fins	No	No	No	No	No	No	No	Many minute black spots on dorsal and caudal fins
Distribution	Dongting Lake in Hunan Prov., Wuhan city in Hubei Prov., China	Upper and middle Yangtze River	Endemic to Hainan Island, China	Middle and lower Yangtze River, Yellow River and Liaohe River	Widely in East Asia	Xiangjiang River and Yuanjiang River in Hunan Prov., Minjiang River in Fujian Prov., China	Middle Yangtze River	Mostly in the upper Yangtze River mainly in Chishui drainage, also in Yibin and Hejiang City, a few in the middle Yangtze River including in Jiangxi and Hunan Provinces

^aFrom Bănărescu & Nalbant (1973); ^b from Luo *et al.* (1982) and Yue (1998); ^c from this study.

DISCUSSION

In the present study, the recognition of *S. punctatus* sp. nov. is supported by both molecular and morphological evidence. This new species has a large genetic difference from its congeners with mean *cytb* sequence divergence ranging from 12.6–21.0%. This level of genetic differentiation is higher than the *cytb* sequence divergence between some other congeners, such as 8.5% between *S. xiangjiangensis* and *S. gracilicaudatus*, 9.2% between *S. xiangjiangensis* and *S. dabryi* and 11.6% between *S. lissilabris* and *S. gymnocheilus*. Furthermore, phylogenetic analyses indicated that all sampled individuals of *S. punctatus* formed a monophyletic group, assigned to a clade restricted to all species having eight branched dorsal-fin rays. *Saurogobio punctatus* was resolved as the sister taxon of the clade formed by *S. xiangjiangensis*, *S. gracilicaudatus* and *S. dabryi*, distinctly separated from all other *Saurogobio* species.

Morphologically, *S. punctatus* can be easily distinguished from four valid *Saurogobio* species in clade A (*S. dumerili*, *S. lissilabris*, *S. immaculatus* and *S. gymnocheilus*) by the number of branched dorsal-fin rays (eight in *S. punctatus* v. seven in the other four species). In cyprinid species, the number of branched dorsal-fin rays is variable in many groups but can also be very stable. In *Saurogobio* species, the current study and previous research have showed this number to be stable (Luo *et al.*, 1982; Yue, 1998). Therefore, the number of branched dorsal-fin rays is a good diagnostic character for *Saurogobio* species.

Although the remaining three species, *S. xiangjiangensis*, *S. gracilicaudatus* and *S. dabryi*, all possess eight branched dorsal-fin rays, these species can be easily distinguished from each other. *Saurogobio xiangjiangensis* and *S. gracilicaudatus* have well-developed lips, longer snouts and much more slender caudal peduncles (Luo *et al.*, 1982; Yue, 1998) than either *S. dabryi* or *S. punctatus*. A cursory examination suggests that the new species *S. punctatus* is very similar to *S. dabryi*; however, some differences in several characters still exist between them. For example, the new species has a more slender body shape with more anterior pelvic fins, anal-fin and anus, which may be an adaptation to faster flowing water. In addition, comparative analysis of the oral-lip structure indicated that the shape of the mental pad in *S. punctatus* [Fig. 2(b)] is approximately triangular v. more square in *S. dabryi* [Fig. 2(d); Dai *et al.*, 2014]. The two species also differ in their colour patterns. *Saurogobio punctatus* has many black spots on the dorsal and caudal fins, with more obvious large round or elongate black spots present above the lateral line [Fig. 2(a), (c)]. In the original description by Bleeker (1871), where *S. dabryi* was first established, minute black spots were not mentioned on any fins. Subsequently, several new species were reported, but later synonymized with *S. dabryi*, such as *Pseudogobio drakei* Abbott 1901, *Pseudogobio productus* Peters 1881, *Saurogobio longirostris* Wu & Wang 1931 and *Saurogobio dabryi chenghaiensis* Dai & Yang 2002. None of these species have any black spots on their fins (Abbott, 1901; Tchang, 1931; Wu & Wang, 1931; Dai & Yang, 2002). Therefore, many minute black spots on the dorsal and caudal fins can be considered as one of the distinctive characters separating the new species from *S. dabryi* and its synonyms.

In addition to differences in morphology and colour pattern, *S. punctatus* and *S. dabryi* can be differentiated based on egg characteristics and geographic distributions. *Saurogobio punctatus* lays yellowish adhesive eggs, while *S. dabryi* lays white pelagic eggs (He *et al.*, 1996; X. B. Li., unpubl. data). *Saurogobio dabryi* has a continuous distribution in the Yangtze River basin, whereas *S. punctatus* is mainly distributed in

the Chishui drainage and the Hejing and Yibin sections of the upper Yangtze River. Only a few specimens were found in tributaries of the middle or lower Yangtze River, such as the rivers flowing into Dongting Lake in the Hunan Province or Poyang Lake in the Jiangxi Province. No samples were discovered in the main stream of the Yangtze River downstream of Hejiang (Three Gorge Reservoir) to upstream of Poyang Lake.

In conclusion, based on morphological and genetic analyses, a new cyprinid species *S. punctatus* is erected here. Furthermore, the morphological differences among all valid *Saurogobio* species are listed and an identification key is presented.

COMPARATIVE MATERIALS

Saurogobio dumerili

IHCAS uncatalogued, two ex., 209.8–237.1 mm L_S , Wuhan in the middle Yangtze River, Hubei Province, China; IHCAS 2008053, 2008060, 2008125–6, 2008160, five ex., 106.8–174.9 mm L_S , Changshu in the lower Yangtze River, Jiangsu Province, China. Additional data from Bănărescu & Nalbant (1973) and photos of type specimen from Muséum National d'Histoire Naturelle, Paris (MNHN), MNHN 5048.

Saurogobio immaculatus

IHCAS 20010039, 20010041, two ex., 126.1–127.0 mm L_S , Hainan Island, Hainan Province, China. Additional data from Luo *et al.* (1982) and Yue (1998).

Saurogobio gymnocheilus

IHCAS 53.3.604–606, 53.3.632–636, syntypes, eight ex., 79.1–97.2 mm L_S , Yichang in the middle Yangtze River, Hubei Province, China; IHCAS 73IV0381–0395, syntypes, 15 ex., 76.3–90.2 mm L_S , Yueyang in the middle Yangtze River, Hunan Province, China; IHCAS uncatalogued, four ex., 82.1–98.2 mm L_S , Wanzhou in the upper Yangtze River, Chongqing City, China; IHCAS 200525216, 200525259, two ex., 76.3–81.5 mm L_S , Yichang in the middle Yangtze River, Hubei Province, China; IHCAS 200908888, 200908858, uncatalogued, three ex., 73.7–80.9 mm L_S , Wuhan in the middle Yangtze River, Hubei Province, China; IHCAS 200805503, 200805505–507, 200805623–624, six ex., 74.6–81.8 mm L_S , Yingtan in the middle Yangtze River, Jiangxi Province, China; IHCAS 200908425, 200906152, two ex., 76.8–83.7 mm L_S , Poyang in the middle Yangtze River, Jiangxi Province, China. Additional data from Luo *et al.* (1982).

Saurogobio lissilabris

IHCAS uncatalogued, 200000124–126, four ex., 73.13–100.33 mm L_S , Wuhan in the middle Yangtze River, Hubei Province, China. Additional data from Bănărescu & Nalbant 1973 and photos of paratypes from the American Museum of Natural History (AMNH), AMNH 29693, 29698.

Saurogobio gracilicaudatus

IHCAS 63VI2598, 63VI2745–2748, syntype, five ex., 84.3–131.0 mm L_S , Yichang in the middle Yangtze River, Hubei Province, China; IHCAS 200908071, 200908742,

200908769, 200908807, 200908908, 2008050, 2008052, 2008114, 2008122–123, 10 ex., 85.4–118.0 mm L_S , Wuhan in the middle Yangtze River, Hubei Province, China. Additional data from Luo *et al.* (1982).

Saurogobio xiangjiangensis

IHCAS 200000127–129, three ex., 140.3–162.7 mm L_S , Yuanling in the middle Yangtze River, Hunan Province, China; IHCAS uncatalogued, one ex., 161.8 mm L_S , Guangfeng in the middle Yangtze River, Jiangxi Province, China. Additional data from Tang (1980) and Yue (1998).

Saurogobio dabryi

IHCAS 20123008, 20 123 021, 20 123 024, three ex., 91.1–124.8 mm L_S , Panzhihua in the upper Yangtze River, Sichuan Province, China; IHCAS 200805384, 200805391, 20102320, 2014070110, 2014070112, 2014070115, 2014070221–224, 2014070226–227, 2014070230–232, 2014070235, 2014070238, 2014070241, 20136329, 20136436, 20137244, 20 ex., 96.6–171.8 mm L_S , Chishui in the upper Yangtze River, Guizhou Province, China; IHCAS 200805575, 200805585, 2014011731, 2014070103, 2014070117–118, 2014070121, 2014070156–157, 2014070168, 2014070216, 2014070254, 2014070288, 2014070299, 2014070318, 15 ex., 91.1–175.5 mm L_S , Hejiang in the upper Yangtze River, Sichuan Province, China; IHCAS 200805438, 200805461, 200805463, three ex., 48.5–116.5 mm L_S , Yibin in the upper Yangtze River, Sichuan Province, China; IHCAS 200805485, 200805494, two ex., 106.8–124.9 mm L_S , Mudong in the upper Yangtze River, Chongqing City, China; IHCAS 200703001, 200703009, two ex., 89.8–104.9 mm L_S , Wanzhou in the upper Yangtze River, Chongqing City, China; IHCAS 200525347–348, 200 525 366, three ex., 100.0–118.5 mm L_S , Yichang in the upper Yangtze River, Hubei Province, China; IHCAS 2008144, 2008146, two ex., 110.1–135.2 mm L_S , Wuhan in the middle Yangtze River, Hubei Province, China; IHCAS uncatalogued, 200 702 881, two ex., 67.0–128.8 mm L_S , Zigui in the middle Yangtze River, Hubei Province, China; IHCAS 201007176, 201 007 178, two ex., 109.2–114.4 mm L_S , Yueyang in the middle Yangtze River, Hunan Province, China; IHCAS 200805644–645, two ex., 63.5–75.1 mm L_S , Xiangjiang in the middle Yangtze River, Hunan Province, China; IHCAS 201008901, 201008497, two ex., 119.1–120.1 mm L_S , Hukou in the middle Yangtze River, Jiangxi Province, China; IHCAS 201008876, 201008951, two ex., 96.0–101.4 mm L_S , Duchang in the middle Yangtze River, Jiangxi Province, China; IHCAS 201008369, 201 008 388, two ex., 97.8–109.5 mm L_S , Poyang in the middle Yangtze River, Jiangxi Province, China; IHCAS 201007150, 201008033, two ex., 78.0–129.7 mm L_S , Xinzi in the middle Yangtze River, Jiangxi Province, China; IHCAS 201008408, 201 008 500, two ex., 101.7–125.2 mm L_S , Yugan in the middle Yangtze River, Jiangxi Province, China; IHCAS 200205041, 200205042, two ex., 140.5–151.9 mm L_S , Zhuaji in Amur River, Heilongjiang Province, China. Additional data from Bleeker (1871), Tchang (1933), Bănărescu & Nalbant (1973) and photos of type specimen MNHN 5040.

Saurogobio drakei

Synonym of *S. dabryi*; data from Abbott (1901), Tchang (1931, 1933).

Saurogobio longirostris

Synonym of *S. dabryi*; data from Wu & Wang (1931).

Saurogobio dabryi chenghaiensis

Synonym of *S. dabryi*; data from Dai & Yang (2002).

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Supporting Information

Supporting Information may be found in the online version of this paper:

TABLE S1. The detailed information of all samples used for the present study of *Saurogobio* species. Sampling locality, sample size (n) in each site, total sample size (N_T) of each species, museum catalogue number, and GenBank accession number are given.

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