Zoosyst. Evol. 100 (2) 2024, 515–529 | DOI 10.3897/zse.100.119520

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Description of a new troglobitic *Sinocyclocheilus* (Pisces, Cyprinidae) species from the upper Yangtze River Basin in Guizhou, South China

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https://zoobank.org/5B410772-DA79-437F-8ABB-A047B4FDC604

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Academic editor: Nicolas Hubert ♦ **Received** 29 January 2024 ♦ **Accepted** 28 March 2024 ♦ **Published** 14 May 2024

Abstract

Sinocyclocheilus guiyang, a new troglobitic species from a subterranean tributary of the upper Yangtze Basin in Guiyang City, Guizhou Province, China is described in the present study. The new species is distinguishable from its congeneric species by a combination of the following characters: tip of maxillary barbel reaching to posterior edge of orbit; forehead horn absent; eye absent (or highly reduced) and tip of pectoral fins not significantly extending beyond the base of the pelvic fin. Molecular evidence, based on the mitochondrial cytochrome *b* (cyt*b*) gene, further supports the validity of the species and also reveals its close relationship with *S. cyphotergous*, *S. multipunctatus*, *S. punctatus* and *S. sanxiaensis*. In addition, the new species faces a high risk of extinction, underscoring the urgency for habitat protection measures within its limited range.

Key Words

cavefish, conservation, morphology, phylogenetic analysis, Yangtze River

Introduction

Sinocyclocheilus Fang, 1936 (golden-line barbel), endemic to south China, is one of the most diversified genera in the family Cyprinidae, consisting of more than seventy species (Jiang et al. 2019; Mao et al. 2022; Xu et al. 2023; Luo et al. 2024). Highly-developed subterranean river systems in this region are a major contributor to its remarkable diversity because *Sinocyclocheilus* is restricted to subterranean river systems and adjacent regions (Ma et al. 2019). To date, sixty-six *Sinocyclocheilus* species are endemic to the Pearl River Basin, with only six species (*S. grahami* Regan, 1904, *S. wumengshanensis* Li, Mao & Lu, 2003, *S. huizeensis* Cheng, Pan, Chen, Li, Ma & Yang, 2015, *S. wui* Li & Li, 2013, *S. sanxiaensis* Jiang, Li, Yang & Chang, 2019 and *S. multipunctatus* Pellegrin, 1931) occurring in the Yangtze River Basin (Eschmeyer et al. 2024). In addition, three of them (*S. grahami*, *S. wumengshanensis* and *S. huizeensis*) are restricted to Yunnan Province which belongs to the Jinshajiang River (a section of the mainstream of upper Yangtze River) System. Only one species, *S. multipunctatus*, is found in the Wujiang River System, a major southern tributary of the upper Yangtze River in Guizhou Province.

However, similar to the Pearl River Basin, the Wujiang River System also exhibits extensive and well-developed

karst landforms (Che and Yu 1985), which has provided good conditions for the formation of subterranean river systems and the subsequent evolution of troglobitic fishes. This can be seen in the high diversity of the hypogean *Triplophysa* which has five described species within this river system (Liu et al. 2022). Moreover, a recent breakthrough study reported *S. sanxiaensis* from the Three Gorges Reservoir which belongs to the mainstream of the upper Yangtze River Basin in west Hubei Province. Molecular phylogenetic analysis has grouped *S. sanxiaensis* with *S. cyphotergous* Dai, 1988, *S. multipunctatus* and *S. punctatus* Lan & Yang, 2017 (Jiang et al. 2019). The *S. cyphotergous – S. multipunctatus* group, herein defined for the abovementioned four species, is characterised by a typically convex dorsal profile, short barbels and high head depth. For a long time, members of this species group were only mainly known from Hongshui River (a section of the mainstream of the Pearl River) System and Liujiang River (north tributary of the Pearl River) System, with only *S. multipunctatus* spanning from Hongshui River to Wujiang River (Wu 1989; Zhao and Zhang 2009). The description of *S. sanxiaensis* greatly expanded the distribution boundary of this species group, providing insights into the potentially underestimated diversity of *Sinocyclocheilus* in the Wujiang River System, situated between the Three Gorges Reservoir and the Hongshui River.

Various morphological features that are adapted to subterranean environments have been found in troglobitic species of *Sinocyclocheilus* including degenerated eyes, reduced (or lost) pigmentation, degenerated scales, elongated fins and horn-like structures (e.g. humpback and horn) (Zhao and Zhang 2009; Ma et al. 2019). In addition, possession of extended barbels in *Sinocyclocheilus* species is common as the long barbels better detect water flow and aid foraging in subterranean water systems which are marked by permanent darkness and food scarcity (Ma et al. 2019). Fewer than one third (twenty-one) of *Sinocyclocheilus* species have short maxillary barbels that do not extend to the posterior edge of the preoperculum (Zhao and Zhang 2009; Lan et al. 2013; Xu et al. 2023) and more than half possess horn-like structures (e.g. *S. anatirostris* Lin & Luo, 1986, *S. aquihornes* Li & Yang, 2007, *S. cyphotergous*, *S. rhinocerous* Li & Tao, 1994 and *S. longicornus* Luo, Xu, Wu, Zhou & Zhou, 2023). Amongst the currently recognised species of *Sinocyclocheilus*, only two, *S. jinxiensis* Zheng, Xiu & Yang, 2013 and *S. sanxiaensis*, possess a combination of short maxillary barbels, degenerated eyes, reduced pigmentation and lack of horn-like structures. These combined characters are unique within *Sinocyclocheilus* and represent exceptional cases for evolutionary studies.

The authors conducted a fish field survey in a subterranean stream within the Wujiang River System in central Guizhou Province, south China. This survey yielded seven specimens characterised by short maxillary barbels, no horn-like structures and absent or highly reduced eyes, traits shared with *S. jinxiensis* and *S. sanxiaensis*. Careful morphological examination revealed that they are, in fact, not conspecific with any other known species of *Sinocyclocheilus* and, thus, represent an unnamed species. Genetic analyses further revealed that these specimens formed a distinct cluster within the *S. cyphotergous – S. multipunctatus* group. The purpose of the present paper is to provide a formal description of this unnamed species, based on multiple lines of evidence including morphological and molecular datasets.

Material and methods

Specimen sampling and preservation

The treatment of experimental animals in this study was consistent with the Chinese animal welfare laws (GB/T 35892–2018). Specimens were collected from central-south Guizhou and north Guangxi from 2019 to 2023. After anaesthesia, specimens were fixed in 10% formaldehyde and then preserved in 75% ethanol for morphological comparison. The right pelvic fin of some specimens was dissected and fixed in 95% ethanol for DNA extraction. Specimens newly collected for this study have been deposited in the Institute of Hydrobiology, Chinese Academy of Sciences (**IHB**), Guangxi University (**GXU**) and Zhejiang Forest Resource Monitoring Center (**ZJFR**). Other comparative materials have been stored in Kunming Institute of Zoology, Chinese Academy of Sciences (**KIZ**).

Morphological analyses

Measurements were taken point-to-point on the left side of the specimens with a Vernier caliper to a precision of 0.1 mm. All measurements, counts and terminologies follow Zhao et al. (2006), with the following exceptions: maxillary barbel in the present study refers to the barbel rooted at the corner of the mouth and rostral barbel refers to the barbel rooted at the rostrum. The major morphometrics are shown in Fig. 1. All morphometric measurements have been transferred to percentage of standard length (SL) and recorded to the closest 0.1%. The caudal peduncle depth (CPD) to caudal peduncle length (CPL) ratios are calculated and recorded to the closest 0.01. For osteological observation, specimens were scanned by micro-computed tomography (micro-CT) (Siemens Somatom Definition X-ray machine). The 3D renderings of the osteological structure of the whole specimen and pharyngeal dentition were created and visualised in VG Studio Max 2.1 (He et al. 2013). Total vertebrae were counted from the first free vertebra to the last half-centrum.

Morphometric measurements were subject to principal component analysis (PCA) to explore the relative contributions of specific variables to morphological variations. PCA was conducted on the Statistical Package for the Social Sciences (SPSS) 19.0 (IBM, Armonk, NY, USA). Prior to PCA, all included measurements were normalised by log transformation. Linear regression analysis for origin data of each character was also computed on SPSS 19.0.

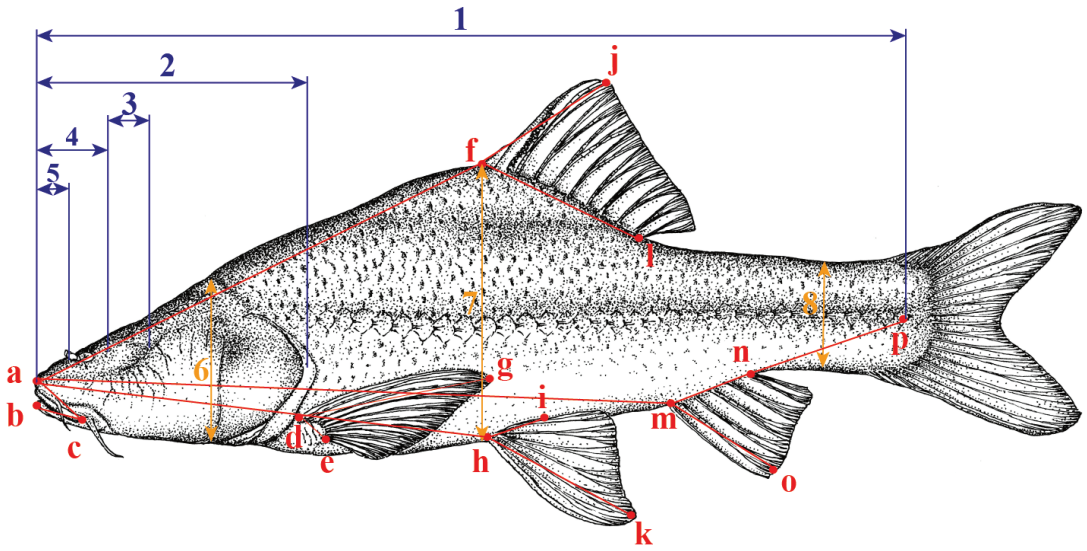

Figure 1. Major morphometrics demonstrated on original drawing of *Sinocyclocheilus guiyang*. standard length (**1**), head length (**2**), eye diameter (**3**), snout length (**4**), pre-nostril length (**5**), head depth (**6**), body depth (**7**), caudal peduncle depth (**8**), upper jaw length (**a–c**), lower jaw length (**b–c**), pre-pectoral length (**a–d**), pre-dorsal length (**a–f**), pre-pelvic length (**a–h**), pre-anal length (**a–m**), pectoral-fin base length (**d–e**), pectoral-fin length (**d–g**), dorsal-fin length (**f–j**), dorsal-fin base length (**f–l**), pelvic-fin base length (**h–i**), pelvic-fin length (**h–k**), anal-fin base length (**m–n**), anal-fin length (**m–o**), caudal peduncle length (**n–p**).

DNA extraction, PCR and sequencing

Genomic DNA was extracted from 95% ethanol-fixed fin tissue using the modified salt-extraction method described by Tang et al. (2008). Fragments containing the mitochondrial cytochrome *b* (cyt*b*) gene were amplified by polymerase chain reaction (PCR) with the primer pairs (L14724 and H15915) (Zhao et al. 2006) in a 30 µl reaction system: 3 µl 10 × PCR buffer, 30–50 ng DNA template, 1 µl primers (each 10 µM), 1.5 µl dNTPs (each 2.5 mM), 2.5 U Taq DNA polymerase and ddH_2O added to reach the final volume. PCR procedures also follow Tang et al. (2008). The PCR products were purified and sequenced in both directions with the corresponding primers by a commercial sequencing company. All newly-generated sequences have been submitted to GenBank.

Molecular data analyses

Phylogenetic analysis was performed, based on nine newly-obtained cyt*b* sequences and an additional 47 sequences downloaded from NCBI GenBank, including 49 *Sinocyclocheilus* species and a single species *Cyprinus carpio* Linnaeus, 1758 as outgroup (Table 1). The sequences were revised manually and then aligned using ClustalW in MEGA7.0 (Kumar et al. 2016). Both Maximum Likelihood (ML) and Bayesian Inference (BI) methods were utilised to reconstruct the phylogenetic relationship. The optimal nucleotide substitution model was selected in ModelFinder (Kalyaanamoorthy et al. 2017) according to Akaike Information Criterion. Maximum Likelihood analysis was run in IQ-TREE 1.6.8 (Nguyen et al. 2015), with the selected TIM3+F+I+G4 model and 1,000 non-parametric bootstrap replicates. Bayesian Inference was performed in MrBayes 3.2.6 (Ronquist et al. 2012) under the selected GTR+F+I+G4 model, using the MCMC method (four chains simultaneously run for 20,000,000 generations) to calculate posterior probability, with tree sampling frequency set to 1 per 1000 cycles and the initial 25% of the sampled data discarded as burn-in. The convergence of BI analysis was reached when the average standard deviation of split frequencies was less than 0.01. Uncorrected pairwise genetic distances (p-distance), based on cyt*b*, were computed in MEGA 7.0.

Results

***Sinocyclocheilus guiyang* sp. nov.**

https://zoobank.org/67337E27-2D91-4C21-B557-AA36ECABDAA1

Fig. 2, Table 2

Type materal. ***Holotype*.** IHB 202012250001, 124.0 mm SL; China: Guizhou Province: Guiyang City: Qingzhen County: a subterranean stream tributary of the Wujiang System in the upper Yangtze River Basin, 26°50'26"N, 106°16'37"E, 1250 m elevation; Jia-Jun Zhou, Dec 2020.

***Paratypes*.** IHB 201911140001, 1 specimen, 57.5 mm SL; Zhi-Xuan Zeng, Nov 2019; other data same as holotype. IHB 202012250002, 1 specimen, 86.4 mm SL; collected with holotype. IHB 202207260001, GXU 202207260002–04, 4 specimens, 124.3–174.1 mm SL; Jia-Jun Zhou, Jul 2022; other data same as holotype.

Diagnosis. *Sinocyclocheilus guiyang* is distinguishable from all other congeners by a combination of the

Table 1. GenBank accession numbers for molecular phylogenetic analysis. n/a, not available.

Taxon	Voucher specimen	Locality	Accession No.	Source
Sinocyclocheilus sanxiaensis	KIZ 2019000001	Hubei, Yangtze River	MN106258	NCBI
Sinocyclocheilus grahami	XH0701	Yunnan, Yangtze River	AY854694	NCBI
Sinocyclocheilus wumengshanensis	YNUSM20160817008	Yunnan, Yangtze River	MG021442	NCBI
Sinocyclocheilus guiyang 01	IHB 202012250001	Guizhou, Yangtze River	OR141734	This study
Sinocyclocheilus guiyang 02	IHB 202012250002	Guizhou, Yangtze River	OR141735	This study
Sinocyclocheilus guiyang 03	IHB 202207260001	Guizhou, Yangtze River	OR141736	This study
Sinocyclocheilus multipunctatus 01	IHB 202302080001	Guizhou, Yangtze River	OR141737	This study
Sinocyclocheilus multipunctatus 02	n/a	Guizhou, Pearl River	MG026730	NCBI
Sinocyclocheilus cyphotergous 01	IHB 202302080002	Guizhou, Pearl River	OR141738	This study
Sinocyclocheilus cyphotergous 02	IHB 202207280010	Guizhou, Pearl River	OR141739	This study
Sinocyclocheilus punctatus 01	ZJFR 2311001	Guangxi, Pearl River	PP112594	This study
Sinocyclocheilus punctatus 02	ZJFR 2311004	Guizhou, Pearl River	PP112595	This study
Sinocyclocheilus punctatus 03	ZJFR 2312002	Guangxi, Pearl River	PP112596	This study
Sinocyclocheilus punctatus 04	GZNU 20150811002	Guizhou, Pearl River	MK610341	NCBI
Sinocyclocheilus longibarbus	XH2901	Guizhou, Pearl River	AY854714	NCBI
Sinocyclocheilus longicornus	GZNU 20210503016	Guizhou, Pearl River	MZ634123	NCBI
Sinocyclocheilus zhenfengensis	GZNU 20150112021	Guizhou, Pearl River	MK610342	NCBI
Sinocyclocheilus bicornutus	XH8301	Guizhou, Pearl River	AY854730	NCBI
Sinocyclocheilus angularis	GZNU 202001332	Guizhou, Pearl River	MW362289	NCBI
Sinocyclocheilus xingyiensis	GZNU SLS202008180	Guizhou, Pearl River	ON573221	NCBI
Sinocyclocheilus guanyangensis	n/a	Guangxi, Pearl River	OQ718399	NCBI
Sinocyclocheilus lateristriatus	XH1601	Yunnan, Pearl River	AY854707	NCBI
Sinocyclocheilus malacopterus	XH0901	Yunnan, Pearl River	AY854697	NCBI
Sinocyclocheilus angustiporus	XH1203	Yunnan, Pearl River	AY854702	NCBI
Sinocyclocheilus hyalinus	XH4701	Yunnan, Pearl River	AY854721	NCBI
Sinocyclocheilus rhinocerous	XH3901	Yunnan, Pearl River	AY854720	NCBI
Sinocyclocheilus tingi	YNUST 201406180002	Yunnan, Pearl River	MG323567	NCBI
Sinocyclocheilus guishanensis	XH5401	Yunnan, Pearl River	AY854722	NCBI
Sinocyclocheilus maculatus	n/a	Yunnan, Pearl River	MF325010	NCBI
Sinocyclocheilus maitianheensis	XH2301	Yunnan, Pearl River	AY854710	NCBI
Sinocyclocheilus anophthalmus	XH3002	Yunnan, Pearl River	AY854716	NCBI
Sinocyclocheilus qiubeiensis	n/a	Yunnan, Pearl River	MF324998	NCBI
Sinocyclocheilus qujingensis	XH3801	Yunnan, Pearl River	AY854719	NCBI
Sinocyclocheilus purpureus	IHB 2006637	Yunnan, Pearl River	EU366194	NCBI
Sinocyclocheilus lunanensis	XH0302	Yunnan, Pearl River	AY854686	NCBI
Sinocyclocheilus huaningensis	XH3701	Yunnan, Pearl River	AY854718	NCBI
Sinocyclocheilus oxycephalus	XH0201	Yunnan, Pearl River	AY854685	NCBI
Sinocyclocheilus yangzongensis	XH6102	Yunnan, Pearl River	AY854726	NCBI
Sinocyclocheilus macrocephalus	XH0110	Yunnan, Pearl River	AY854684	NCBI
Sinocyclocheilus yishanensis	n/a	Guangxi, Pearl River	MK387704	NCBI
Sinocyclocheilus macrophthalmus	XH8401	Guangxi, Pearl River	AY854733	NCBI
Sinocyclocheilus xunlensis	IHB 04050268	Guangxi, Pearl River	EU366187	NCBI
Sinocyclocheilus lingyunensis	XH0502	Guangxi, Pearl River	AY854691	NCBI
Sinocyclocheilus donglanensis	ASIZB 94746	Guangxi, Pearl River	AB196440	NCBI
Sinocyclocheilus ronganensis	n/a	Guangxi, Pearl River	KX778473	NCBI
Sinocyclocheilus macrolepis	XH8201	Guangxi, Pearl River	AY854729	NCBI
Sinocyclocheilus anatirostris	XH1901	Guangxi, Pearl River	AY854708	NCBI
Sinocyclocheilus anshuiensis	n/a	Guangxi, Pearl River	KR069120	NCBI
Sinocyclocheilus microphthalmus	XH0402	Guangxi, Pearl River	AY854687	NCBI
Sinocyclocheilus tianeensis	XH3403	Guangxi, Pearl River	AY854717	NCBI
Sinocyclocheilus furcodorsalis	XH2202	Guangxi, Pearl River	AY854709	NCBI
Sinocyclocheilus altishoulderus	XH5801	Guangxi, Pearl River	AY854724	NCBI
Sinocyclocheilus jiuxuensis	XH8501	Guangxi, Pearl River	AY854736	NCBI
Sinocyclocheilus jii	XH8101	Guangxi, Pearl River	AY854727	NCBI
Sinocyclocheilus yimenensis	IHB 2006645	Yunnan, Red River	EU366192	NCBI
Cyprinus carpio (outgroup)	n/a	n/a	MK088487	NCBI

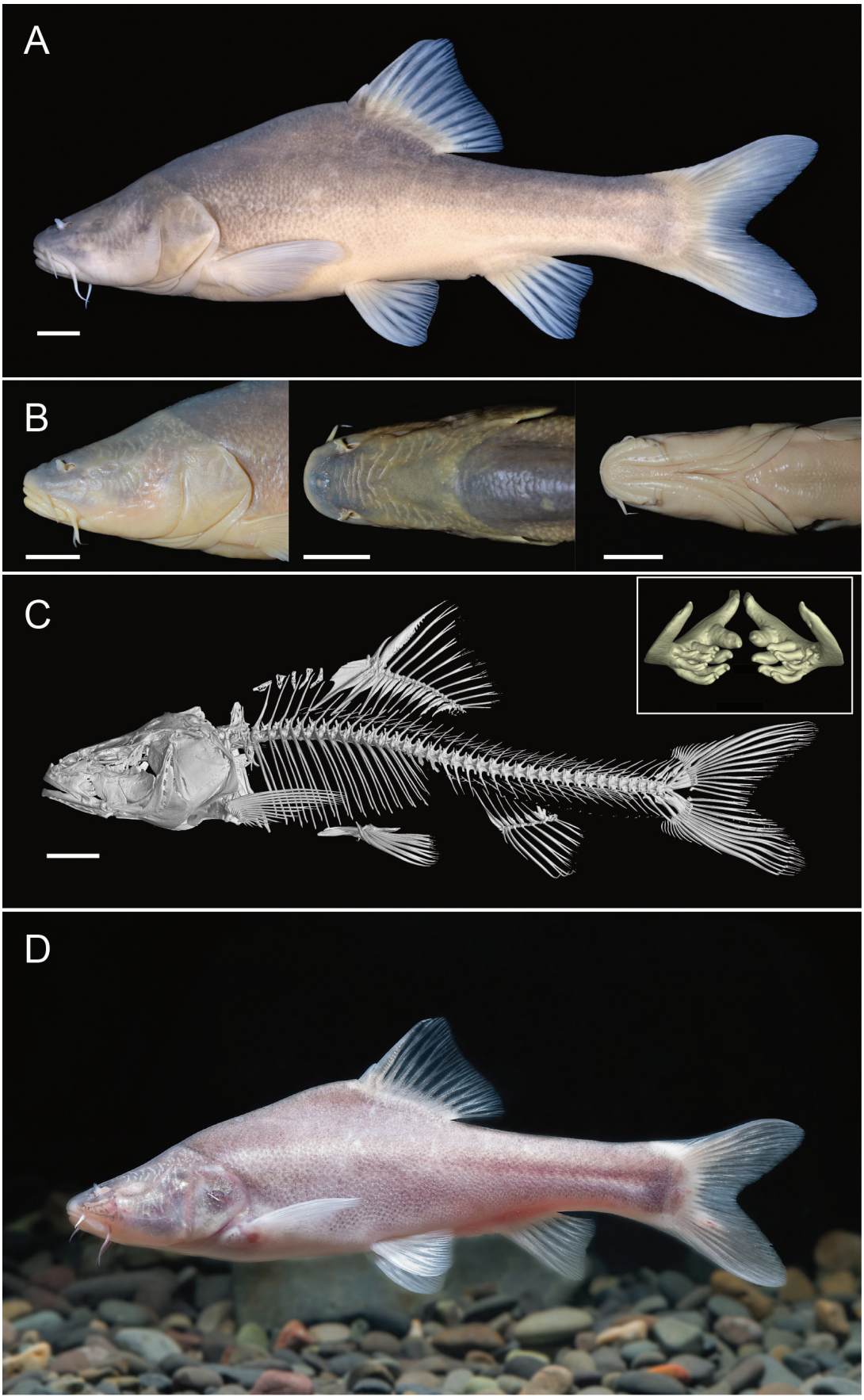

Figure 2. *Sinocyclocheilus guiyang*, IHB 202012250001, holotype, 124.0 mm SL; China: Guizhou Province: Guiyang City: Qingzhen County: Yangtze River Basin. **A.** Lateral view; **B.** Lateral, dorsal and ventral view of head; **C.** Micro-CT graph and reconstructed pharyngeal dentition; **D.** Live photo. Scale bar: 1 cm.

following characters: tip of maxillary barbel not reaching to posterior edge of preoperculum, horn-like structure in forehead absent, eye absent or highly reduced, pectoral fin not significantly extending beyond base of pelvic fin. The major diagnostic characters for *S. guiyang* and related species are summarised in Table 3.

Description. Morphometric measurements of type specimens have been transferred to percentage of standard length (SL), as summarised in Table 2. Body laterally compressed; maximum body depth positioned at insertion of dorsal-fin. Dorsal profile convex from snout tip to dorsal-fin base end and slightly concave after dorsal-fin base. Ventral profile of pre-anal part slightly convex and slightly concave after anal-fin origin.

Head slightly compressed, conical in lateral view. Eyes absent (5) or highly reduced and partially covered with skin (2). Eye orbits located in dorsal anterior part of head, filled with soft tissue. Nostrils located at midway between snout tip and anterior margin of orbit; anterior nostril with rim forming an oblique tube, posteriorly thickening and elongating; posterior nostril open and elliptical. Snout blunt in dorsal view and slightly pointed in lateral view. Mouth subterminal and arched; with two pairs of barbels; rostral pair positioned anterior to anterior nostril, extending to the insertion of anterior margin of orbit, being 6.2% (4.9–7.1%) of SL; maxillary pair positioned at corners of mouth, extending to the posterior margin of orbit, being 7.1% (5.8–8.3%) of SL. Gill opening large; opercular membranes not connected at isthmus. Joints of dentary-angulars not close at isthmus. Ten outer rakers (1) on first gill arch. Pharyngeal teeth pattern 1,3,4–4,3,0 (1); tooth tip pointed and compressed. Vertebrae 36 (2) (Fig. 2C).

Dorsal fin with 3 unbranched and 8 (5) or 9 (2) branched rays, with last one divided at base; dorsal-fin length being 20.2% (17.6–24.5%) SL; origin closer to snout tip than to caudal-fin base; distal margin slightly concave, last unbranched ray strong, with serration on posterior edge; last unbranched ray split to base. Pectoral fin with 1 unbranched and 14 (6) or 15 (1) branched rays; tip extending to pelvic-fin insertion; pectoral-fin length being 21.5% (20.8–22.2%) of SL. Pelvic fin with 1 unbranched and 7 branched rays; inserted slightly posterior to dorsal-fin origin; tip not reaching to anus. Anal fin with 3 unbranched and 5 branched rays, last one divided at base; distal margin slightly concave; origin closer to pelvic-fin insertion than to caudal-fin base. Caudal fin deeply forked, with 17 (6) or 18 (1) branched rays; upper and lower lobes pointed.

Table 2. Morphometric characters of *Sinocyclocheilus guiyang*.

Character	Holotype	Holotype + Paratypes (n = 7)		
		Range	Mean	SD
Standard length (mm)	124.0	57.5–144.1		
In Percentage of SL (%)				
Body depth	29.8	26.7–33.1	29.4	2.1
Predorsal length	55.4	53.6–59.6	55.9	2.1
Dorsal-fin base length	16.8	15.3–16.8	15.8	0.8
Dorsal-fin length	18.8	17.6–24.5	20.2	2.2
Pre-anal length	71.4	70.8–75.7	73.2	1.8
Anal-fin base length	9.8	7.5–10.2	9.2	1.0
Anal-fin length	16.6	16.0–18.3	17.1	0.9
Prepectoral length	31.3	30.7–35.7	32.5	1.6
Pectoral-fin base length	4.4	4.0–4.6	4.3	0.2
Pectoral-fin length	21.3	20.8–22.2	21.5	0.5
Prepelvic length	50.6	50.6–55.3	52.9	1.6
Pelvic-fin base length	5.7	4.5–5.7	5.2	0.4
Pelvic-fin length	16.5	15.4–19.8	17.3	1.4
Caudal peduncle length (CPL)	19.5	16.5–20.7	18.5	1.5
Caudal peduncle depth (CPD)	11.8	10.0–13.2	11.2	1.2
Head length	31.5	30.2–34.1	32.2	1.4
Head depth	19.0	16.8–21.0	19.14	1.3
Head width	16.0	10.4–17.6	15.3	2.4
Snout length	10.9	9.9–11.0	10.5	0.5
Eye diameter	3.5	3.5–5.9	4.6	1.2
Interorbital width	10.4	8.3–10.5	9.7	0.8
Prenostril length	5.7	4.5–6.0	5.6	0.5
Width between posterior nostrils	7.2	5.8–7.4	6.6	0.6
Upper jaw length	10.2	10.1–10.3	10.2	0.1
Lower jaw length	9.4	8.9–9.5	9.2	0.2
Mouth width	8.3	6.8–8.3	7.5	0.6
Rostral barbel length	5.2	4.9–7.1	6.2	0.9
Maxillary barbel length	5.9	5.8–8.3	7.1	0.9
CPD to CPL ratio	0.60	0.55–0.67	0.61	0.04

Table 3. Major diagnostic characters for *Sinocyclocheilus guiyang* and its close congeners. n/a, not available.

Characters	*S. guiyang*	*S. multipunctatus*	*S. punctatus*	*S. sanxiaensis*	*S. jinxiensis*	*S. jiuxuensis*	*S. mashanensis*	*S. brevibarbatus*	*S. yangzongensis*
Eye	Absent or highly reduced	Normal	Normal	Absent	Absent	Normal	Normal	Normal	Normal
Tip of maxillary barbel	Reaching to posterior edge of orbit	Reaching to posterior edge of preoperculum	Reaching to anterior edge of orbit	Not reaching to anterior edge of orbit	Reaching to posterior edge of orbit	Reaching or extending to posterior edge of orbit	Extending to anterior edge of orbit	Not reaching to anterior edge of orbit	Extending to anterior edge of orbit
Pectoral-fin extending to pelvic-fin insertion	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	No
Gill rakers	10	n/a	7–8	7	13–14	8–10	7–9	8–9	8–11
Lateral-line scales	45–47	53–60	48–60	41	38–41	42–51	47–50	49–51	71–81
Degenerated body scales above and below lateral line	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes
Black blotches on body	Absent	Present	Present	Absent	Absent	Absent	Absent	Absent	Present

Body covered with small scales, partially embedded subcutaneously; scales on lateral line slighter larger than other. Lateral line complete and horizontal, with 45 (4), 46 (2) or 47 (1) perforated scales. Scale rows above lateral line 20 (1), 21 (3), 22 (2) or 24 (1); below 13 (2) or 14 (5). Circumpeduncular scales 32 (1), 33 (1), 34 (2), 35 (2) or 36 (1).

All original morphometric measurements and meristic counts are available in Suppl. material 1.

Colouration. In freshly collected individuals (Figs 2D, 3), head and body generally pinkish, with or without pigments dorsally. A pair of dark stripes present on dorsal-posterior part of head, extending to dorsal mid-point of nape; a gold stripe extending along dorsal mid-line from nape to dorsal-fin origin. All fins transparent.

In preserved specimens (Fig. 2A, B), body and head slightly yellowish, with or without pigments dorsally. Abovementioned dark stripes and gold stripe faded. All fins transparent.

Distribution and habitat. This species is presently only known from a subterranean stream flowing into the Wujiang River in the upper Yangtze River Basin in Qingzhen County, Guiyang City, Guizhou Province, China (Fig. 4). The species inhabits pools of subterranean stream with gravel substrate (Fig. 5). Video record of *Sinocyclocheilus guiyang* in situ is available in Suppl. material 2.

Etymology. The location of the subterranean stream where this new species was first collected: Guiyang City, the capital of Guizhou Province, is directly utilised as a specific epithet. The common name proposed for the new species is '贵阳金线鲃' (Guiyang Golden-line Barbel).

Morphometric comparisons. Principal component analysis for *Sinocyclocheilus guiyang*, *S. punctatus*, *S. multipunctatus* and *S. sanxiaensis*, based on 29 log-transformed characters, showed that 95.23% of total variance was explained by the first three components, including 87.24% by PC1, 4.79% by PC2 and 3.20% by PC3, respectively. In the PC1 vs. PC3 scatter plot, *S. guiyang* and *S. punctatus* form a distinct cluster from the other two congeners on the PC3 axis (Fig. 6A). The characters with major loading on PC3 included maxillary barbel length, rostral barbel length, eye diameter, width between posterior nostrils and pectoral-fin base length (Table 4). Further PCA in *S. guiyang* and *S. punctatus* demonstrated that the first three components explained 97.26% of total variance, in which PC1, PC2 and PC3 explained 93.31%, 2.29% and 1.66%, respectively. *Sinocyclocheilus guiyang* is separated from *S. punctatus* on the PC2 axis in the PC1 vs. PC2 scatter plot (Fig. 6B). Eye diameter, maxillary barbel length, width between posterior nostrils, rostral barbel length and snout width are major loading characters on PC2 (Table 4). Linear regression analysis also support *S. multipunctatus* as distinct from *S. guiyang* and *S. punctatus* by shorter maxillary (7.2–13.3% SL vs. 5.8–8.3% in *S. guiyang*, 4.9–9.4% in *S. punctatus*) and rostral barbel lengths (6.0–10.7% SL vs. 4.9–7.1% in *S. guiyang*, 4.9–

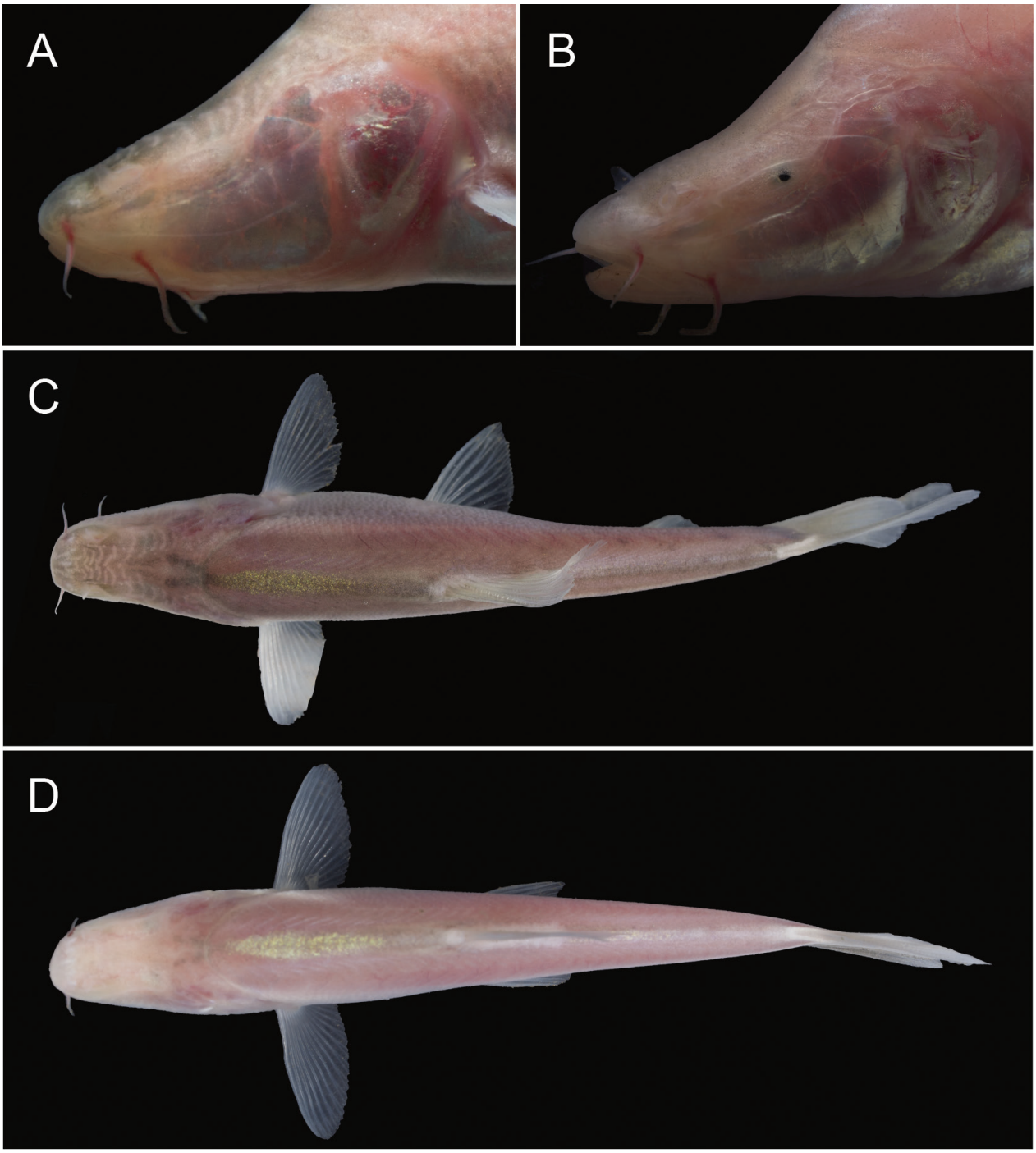

Figure 3. Intraspecific morphological variations of *Sinocyclocheilus guiyang*. **A.** Individual with no eyes; **B.** Individual with highly reduced eyes, partially covered with skin; **C.** Individual with dorsal pigment; **D.** Individual without pigment. Note that individuals of both colouration types share the presence of dark stripes on the dorsal-posterior part of the head and a gold stripe along the midline from the nape to the dorsal-fin origin.

6.7% in *S. punctatus*) (Fig. 6C, D), whereas *S. guiyang* further differed from *S. punctatus* by shorter prenostril length (4.5–6.0% of SL vs. 5.8–7.6%) and higher caudal peduncle depth to caudal peduncle length ratio (0.55–0.67 vs. 0.45–0.56) (Fig. 6E, F).

Molecular data analyses. A total of 1134 bps were included in the aligned dataset of cyt*b* gene, with 661 conservative sites, 473 variable sites, 390 parsimony informative sites and 83 singleton sites. The mean frequency of four nucleotides in the sequences of *Sinocyclocheilus guiyang* is A = 29.7%, G = 14.2%, C = 26.2% and T = 29.9%. The phylogenetic trees, reconstructed by ML and BI methods, are identical in topology (Fig. 7). The monophyletic lineage of *Sinocyclocheilus guiyang* is robustly supported by 100% posterior probabilities and 99% bootstrap supports and is sister to *S. punctatus*. The lineage of the two spe-

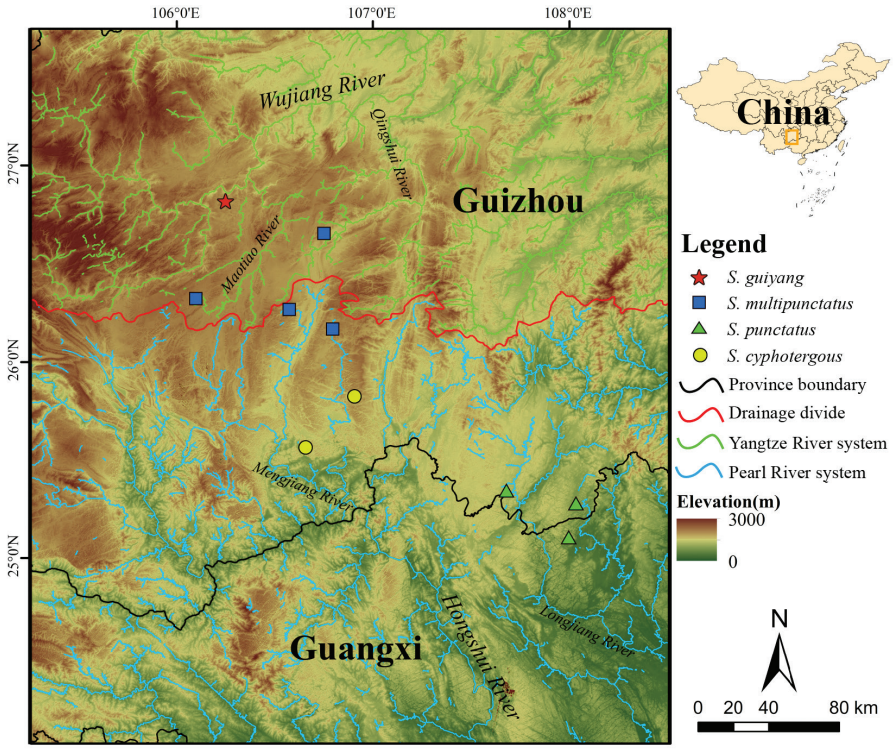

Figure 4. Sampling sites of *Sinocyclocheilus guiyang* and related species in this study.

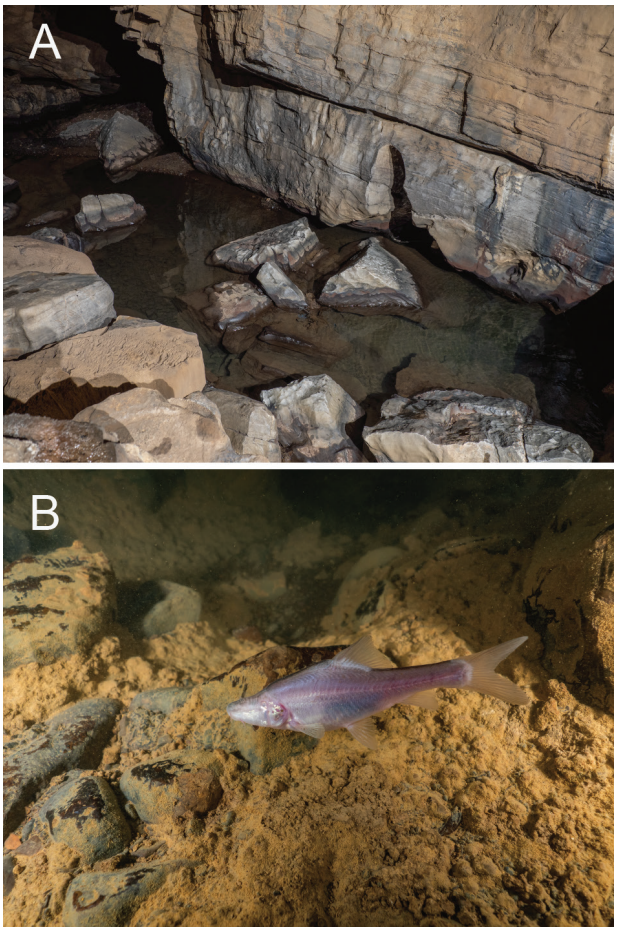

Figure 5. Habitat of *Sinocyclocheilus guiyang*. **A.** The pool of a subterranean stream where *S. guiyang* was collected; **B.** *S. guiyang* in situ.

cies clustered with the lineage comprising sequences of *S. multipunctatus*, *S. cyphotergous* and *S. sanxiaensis*. Additionally, the topology of phylogenetic reconstruction in the present study supports the monophyly of the *S. cyphotergous* – *S. multipunctatus* species group. Average genetic distances derived from cyt*b* sequences of *Sinocyclocheilus* species distributed in Guizhou Province or the Yangtze River Basin are given in Table 5. The intraspecific distance of *S. guiyang* is 0.1% and the mean distances between the new species and other congeners range from 2.3% (vs. *S. punctatus*) to 13.8% (vs. *S. wumengshanensis*).

Discussion

The new species is the first described troglobiotic species of genus *Sinocyclocheilus* in the Wujiang River of upper Yangtze River Basin in Guizhou Province. Three characters are useful for distinguishing *Sinocyclocheilus guiyang* from all other *Sinocyclocheilus* species, except *S. jinxiensis* and *S. sanxiaensis* (Fig. 8): short maxillary barbel not reaching posterior edge of preoperculum, absence of horn-like structure and degenerated (lost or highly reduced) eye. It is distinct from *S. jinxiensis* and *S. sanxiaensis* in having shorter (vs. longer) pectoral fins, just reaching (vs. significantly extending beyond) the base of pelvic fin. It further differs from *S. jinxiensis* in possessing degenerated body scales (20–24 scale rows above lateral line vs. 8–9), and from *S. sanxiaensis* in having shorter (vs. longer) snout (length 9.9–11.0% of SL vs. 16.4%) and longer (vs. shorter) maxillary barbel, reaching to posterior edge of orbit, length 5.8–8.3% of SL (vs. not reaching to anterior edge of orbit, 4.2%).

Table 4. PCA loadings of the first three principal components extracted from 29 morphometric data for *Sinocyclocheilus guiyang* and related species.

	S. guiyang*, *S. punctatus*, *S. multipunctatus*, *S. sanxiaensis			***S. guiyang*, *S. punctatus***		
	PC1	**PC2**	**PC3**	**PC1**	**PC2**	**PC3**
Standard length	0.991	-0.062	0.028	0.995	0.026	-0.045
Body depth	0.948	-0.034	-0.165	0.966	-0.049	-0.204
Predorsal length	0.991	-0.035	-0.022	0.996	0.008	0.050
Dorsal-fin base length	0.978	-0.090	0.030	0.989	-0.011	-0.027
Dorsal-fin length	0.928	0.027	0.019	0.895	-0.115	0.376
Pre-anal length	0.986	-0.058	0.037	0.995	0.020	-0.015
Anal-fin base length	0.967	-0.107	-0.041	0.974	0.020	-0.104
Anal-fin length	0.962	-0.032	-0.075	0.985	0.073	0.101
Prepectoral length	0.990	-0.029	-0.071	0.992	-0.024	0.074
Pectoral-fin base length	0.712	0.655	0.193	0.963	0.161	-0.080
Pectoral-fin length	0.978	-0.011	-0.043	0.970	-0.092	0.132
Prepelvic length	0.991	-0.040	0.020	0.996	-0.005	-0.008
Pelvic-fin base length	0.732	0.636	0.097	0.954	-0.019	-0.228
Pelvic-fin length	0.976	0.002	-0.134	0.987	0.097	0.056
Caudal peduncle length	0.954	-0.074	0.049	0.958	0.092	-0.170
Caudal peduncle depth	0.957	-0.019	-0.065	0.956	-0.107	-0.230
Head length	0.990	-0.035	-0.072	0.997	0.008	0.034
Head depth	0.991	-0.053	-0.046	0.996	-0.014	0.000
Head width	0.972	-0.080	0.048	0.970	-0.104	0.006
Snout length	0.966	0.183	-0.094	0.983	-0.056	0.029
Eye diameter	0.716	0.389	0.408	0.758	0.632	0.056
Interorbital width	0.982	0.010	-0.086	0.979	-0.147	-0.107
Prenostril length	0.961	0.052	-0.036	0.954	0.150	0.045
Width between posterior nostrils	0.956	0.009	-0.199	0.959	-0.183	-0.170
Upper jaw length	0.962	-0.116	-0.179	0.983	-0.040	0.108
Lower jaw length	0.964	-0.118	-0.156	0.986	-0.008	0.103
Mouth width	0.958	0.031	-0.052	0.954	0.155	-0.003
Rostral barbel length	0.731	-0.440	0.492	0.956	-0.161	0.081
Maxillary barbel length	0.764	-0.300	0.524	0.938	-0.185	0.169

Table 5. Average uncorrected pairwise genetic distance (p-distance, %) derived from cyt*b* in 15 species of *Sinocyclocheilus* distributed in Guizhou Province or the Yangtze River Basin. Bold numbers, intraspecific distances; regular numbers, interspecific distances; n/a, not available.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
1. *S. guiyang*	**0.1**														
2. *S. multipunctatus*	2.5	**0.4**													
3. *S. punctatus*	2.3	2.6	**0.7**												
4. *S. cyphotergous*	2.8	1.1	3.1	**0.8**											
5. *S. sanxiaensis*	3.2	1.0	2.9	1.6	**n/a**										
6. *S. longibarbus*	10.9	11.0	11.2	11.2	11.6	**n/a**									
7. *S. grahami*	11.7	11.6	11.8	11.9	12.2	11.5	**n/a**								
8. *S. wumengshanensis*	12.3	11.8	12.1	12.1	12.3	11.7	4.2	**n/a**							
9. *S. angustiporus*	11.4	11.1	11.5	11.3	11.6	10.4	7.7	7.1	**n/a**						
10. *S. macrolepis*	11.5	11.4	11.9	11.7	12.0	11.1	12.7	12.6	11.5	**n/a**					
11. *S. bicornutus*	10.8	10.9	11.4	11.1	11.4	10.3	12.2	12.4	11.1	11.3	**n/a**				
12. *S. zhenfengensis*	10.9	10.8	11.2	10.9	11.3	10.0	12.0	12.4	11.3	11.2	2.9	**n/a**			
13. *S. angularis*	11.2	11.2	11.1	11.5	11.6	9.6	11.8	12.3	11.2	11.6	2.8	2.4	**n/a**		
14. *S. longicornus*	11.9	11.9	11.8	11.9	12.4	10.8	12.3	12.5	11.3	11.8	5.8	6.5	5.9	**n/a**	
15. *S. xingyiensis*	11.1	11.1	11.5	11.3	11.5	10.1	12.6	12.8	11.3	12.0	2.3	2.9	1.9	6.3	**n/a**

Although *Sinocyclocheilus guiyang* displays a high degree of eye degeneration, there is intraspecific variations in the eyes, ranging from absence of eye to highly reduced eyes partially covered by skin (Fig. 3A, B). Similar variations have been recorded in observations of its congeners. The eyes of *S. cyphotergous* have been described as 'very small' in its re-description (Huang et al. 2017) and it is the same case with some of our newly-collected specimens (Fig. 8E); however, some specimens are presented as eyeless (See Suppl. material 3). *Sinocyclocheilus bicornutus*

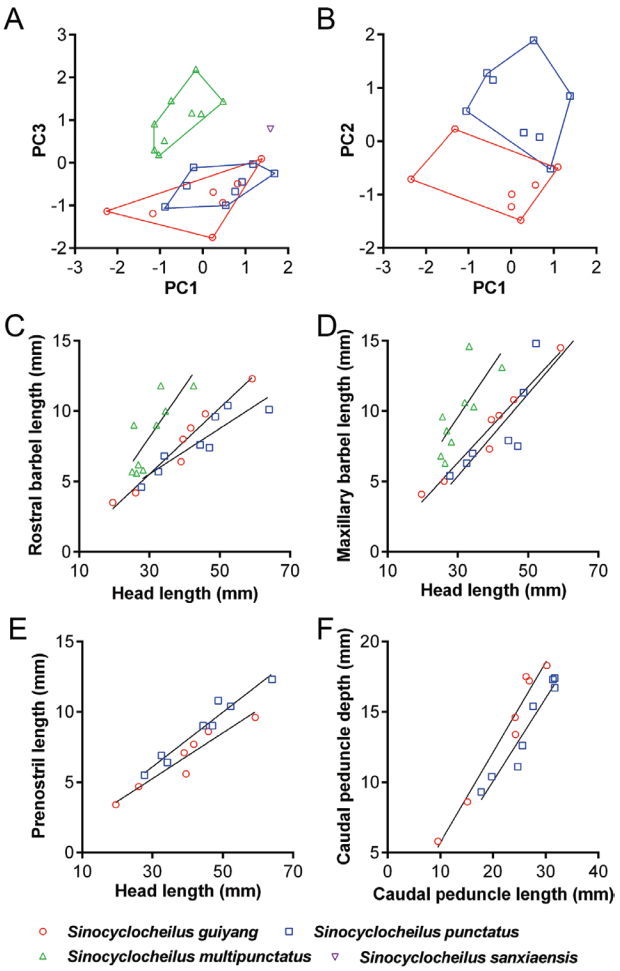

Figure 6. A. Scatter plot of 1st and 3rd principal components for *Sinocyclocheilus guiyang*, *S. punctatus*, *S. multipunctatus* and *S. sanxiaensis*; **B.** Scatter plot of 1st and 2nd principal components for *S. guiyang* and *S. punctatus*; relationships between **C.** Rostral barbel length and head length, **D.** Maxillary barbel length and head length for *S. guiyang*, *S. punctatus* and *S. multipunctatus*; relationships between **E.** Prenostril length and head length, **F.** Caudal peduncle depth and caudal peduncle length for *S. guiyang* and *S. punctatus*.

Wang & Liao, 1997, a cave-dwelling species, also demonstrated polymorphism in eye mode (Wen et al. 2023). Variations in such regressive characters in troglobitic fishes are common, indicating that eye degeneration might not be a totally reliable character in morphology species delineation of *Sinocyclocheilus*. Accordingly, it is of vital importance to also compare the morphology of this new species with other congeneric species possessing a short maxillary barbel, no horn-like structure and normal eyes, including *S. jiuxuensis* Li & Lan, 2003, *S. brevibarbatus* Zhao, Lan & Zhang, 2009, *S. mashanensis* Wu, Liao & Li, 2010, *S. yangzongensis* Chu & Chen, 1977, *S. multipunctatus* and *S. punctatus*. Amongst these species, *S. multipunctatus* and *S. punctatus* showed close phylogenetic relationships with *S. guiyang*, which all belong to the *S. cyphotergous* – *S. multipunctatus* group. *Sinocyclocheilus guiyang* can be further differentiated from *S. jiuxuensis*, *S. brevibarbatus* and *S. mashanensis* by the following characters: pectoral fin just reaching pelvic fin insertion (vs. pectoral fin highly developed, significantly reaching beyond pelvic fin insertion), pelvic-fin origin posterior to dorsal-fin origin (vs. anterior to dorsal-fin origin) (Zhao and Zhang 2009). In addition, *S. guiyang* further differs from *S. yangzongensis* in having a deeper body (depth 26.7–33.1% SL vs. 21.3–28.5%), longer dorsal fin base (length 15.3–16.8% SL vs. 8.0–12.6%) and less lateral line scales (45–47 vs. 71–79) (Zhao and Zhang 2009).

Sinocyclocheilus guiyang is undoubtedly a member of the *S. cyphotergous* – *S. multipunctatus* species group, as evidenced by both morphology and phylogenetic results in this study. The monophyly of the *S. cyphotergous* – *S. multipunctatus* group has also been confirmed in previous works (Wen et al. 2022; Jiang et al. 2023; Xu et al. 2023). Excluding *S. sanxiaensis*, which has been mentioned above, *S. guiyang* differs from *S. cyphotergous* by the absence of a humpback, from *S. multipunctatus* in having developed pectoral fins extending (vs. not extending) to pelvic-fin insertion and fewer lateral line scales (45–47 vs. 53–60), from *S. punctatus* in having longer maxillary barbels reaching to posterior edge of orbit (vs. reaching to anterior edge of orbit). The principal component analysis of measurement characters also confirms the morphological distinctness of *S. guiyang* from other members of the *S. cyphotergous* – *S. multipunctatus* group lacking horn-like structures (Fig. 6A).

As sister to one another, the pairwise distance between *Sinocyclocheilus guiyang* and *S. punctatus* is 2.3%, exceeding the 2% mitochondrial DNA threshold which is often indicative of valid species in most groups (Avies and Walker 1999) and coincides with distances used for currently described species in *Sinocyclocheilus*. The genetic distances between sibling species being comparatively low might be a trait common across the genus *Sinocyclocheilus*, which has undergone recent divergence in extreme subterranean environments (Mao et al. 2022). In addition, *S. guiyang* and *S. punctatus* display allopatric distribution patterns as *S. punctatus* is confined in the Longjiang River (a tributary of Liujiang River in the Pearl River Basin), while *S. guiyang* only occurs in Wujiang River that belongs to the upper Yangtze River Basin. The species validity of *S. guiyang* can be confirmed under an integrative framework combining numerous lines of evidence, comprised of morphological distinctness, molecular phylogeny and geographical range. Moreover, samples of *S. multipunctatus* formed a paraphyletic entity in the phylogenetic tree. It is evident that geographic divergence has occurred as the Yangtze River population showed close affinities to *S. sanxiaensis*, while the Pearl River population is located at the base of the paraphyly (Fig. 7), implying the existence of cryptic species diversity within *S. multipunctatus* that warrants taxonomic revision in the future.

The fish diversity in the Yangtze River Basin of Guiyang City, the most urbanised area of Guizhou, has long been underestimated (Zeng and Liu 2020). The increasing discoveries of narrowly distributed species in this area have raised concerns for the conservation of these species

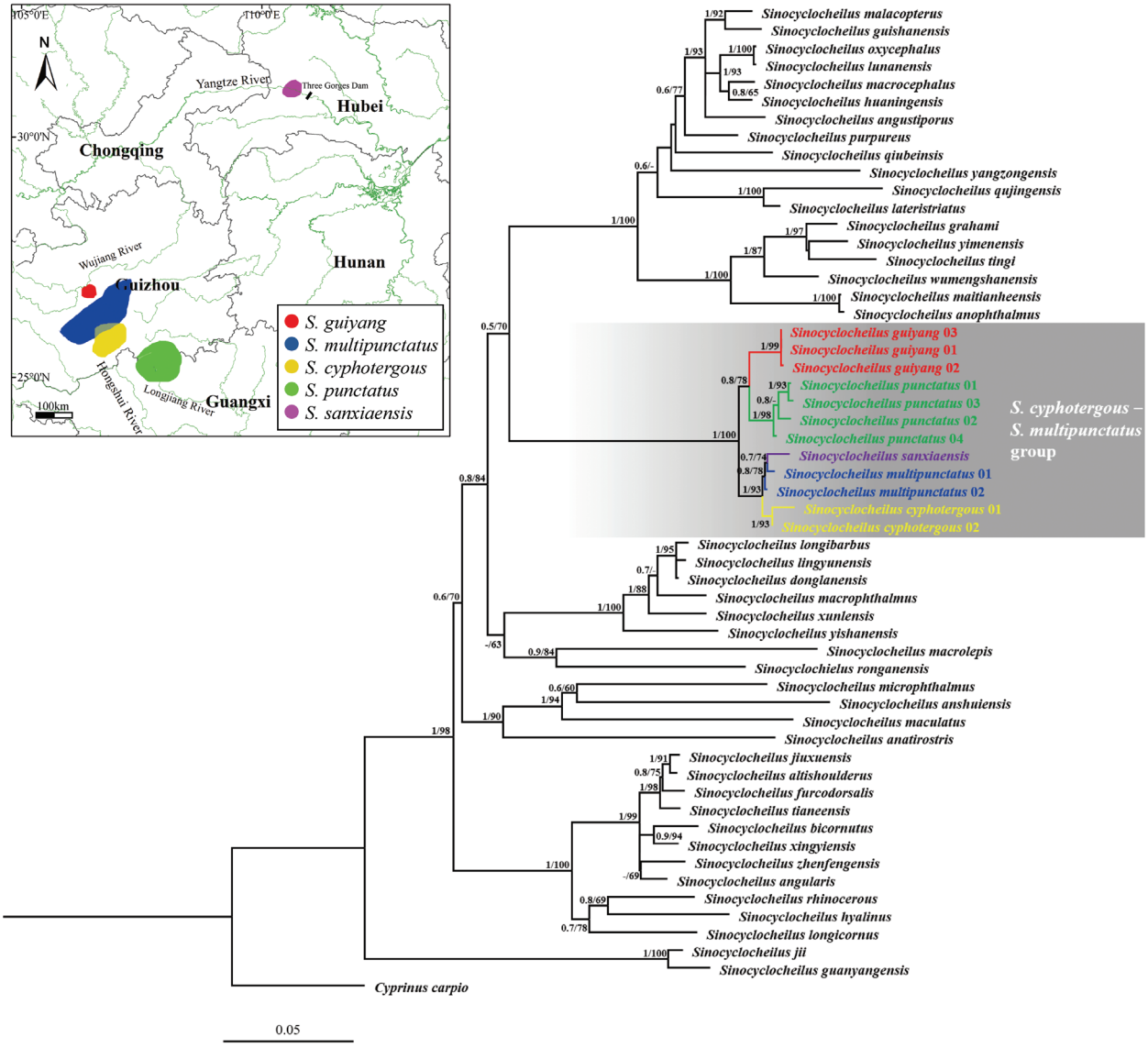

Figure 7. Phylogenetic tree of *Sinocyclocheilus* species inferred from cyt*b* using Bayesian Inference and Maximum Likelihood methods. Node values show posterior probabilities/bootstrap supports if greater than 50%. Currently known distribution of species of the *S. cyphotergous* – *S. multipunctatus* group are shown on map.

(Liu et al. 2022; Zeng et al. 2022). Human disturbance to the habitat and climate change have exacerbated the risk of extinction of cave species (Shu et al. 2013; Mouser et al. 2022). Recent years have witnessed a dramatic increase in land use, fishing pressure, cave tourism and invasion of a large amount of *Procambarus clarkii* Girard, 1852 in the type locality of *Sinocyclocheilus guiyang* (See Suppl. material 4), as well as frequent onset of droughts. Moreover, the estimated population size of the new species is extremely small, as only 25 individuals in total were recorded during our surveys and has suffered a population decline in the recent 2 years (Table 6). *S. guiyang* will be automatically assigned to the list of 2nd Class of the national protected animals after its description as the whole *Sinocyclocheilus* species are within this list since 2021. Construction of a small conservation area with limited human disturbance in the existing habitats of this new species under extreme threat should be considered in priority.

Table 6. Number of individuals of *Sinocyclocheilus guiyang* in type locality recorded each year during the survey.

Year	2019	2020	2021	2022	2023	2024
Number	5	7	5	6	1	1

Material examined

Sinocyclocheilus cyphotergous: IHB 202207280006–12, 7 specimens, 54.2–82.0 mm SL; a tiankeng of the Mengjiang River System in the Pearl River Basin at Pingtang County, Qiannan Prefecture, Guizhou Province, China. IHB 202302080002–03, 2 specimens, 71.5–84.6 mm SL; a subterranean tributary of the Mengjiang River System in the Pearl River Basin at Luodian County, Qiannan Prefecture, Guizhou Province, China.

Sinocyclocheilus multipunctatus: IHB 2014040001, 1 specimen, 96.4 mm SL; a vauclusian spring of the Mengjiang River System in the Pearl River Basin at

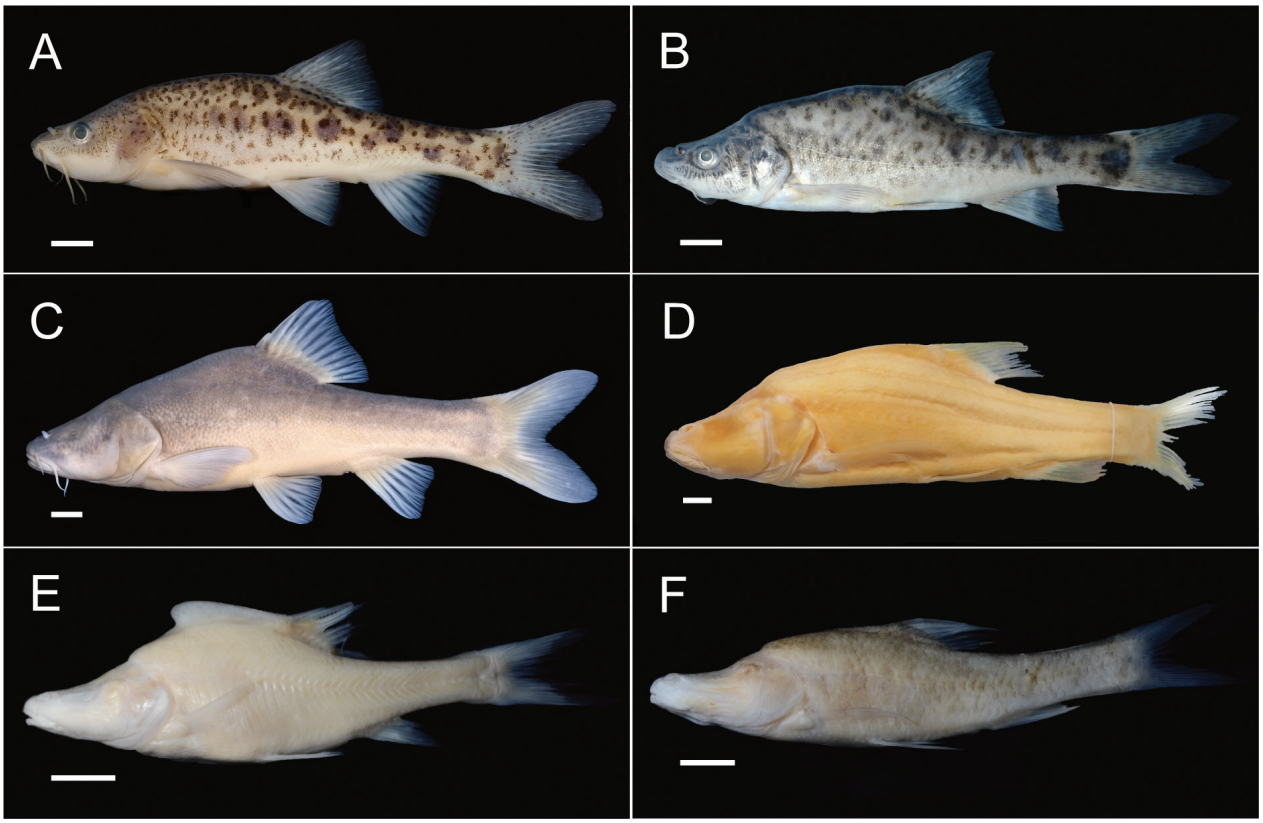

Figure 8. Lateral view of **A.** *Sinocyclocheilus multipunctatus*, IHB 202302080001, 110.3 mm SL; China: Yangtze River Basin; **B.** *S. punctatus*, ZJFR 2311004, 110.1 mm SL; China: Pearl River Basin; **C.** *S. guiyang*, IHB 202012250001, holotype, 124.0 mm SL; China: Yangtze River Basin; **D.** *S. sanxiaensis*, KIZ 2019000001, holotype, 164.1 mm SL; China: Yangtze River Basin; **E.** *S. cyphotergous*, IHB 202207280011, 71.3 mm SL; China: Pearl River Basin; **F.** *S. jinxiensis*, GXU 23070020, 85.8mm SL; China: Pearl River Basin. Scale bar: 1 cm.

Huaxi District, Guiyang City, Guizhou Province, China. IHB 2019070001, IHB 202302080001, 2 specimens, 87.6–110.3 mm SL; a vauclusian spring of the Wujiang River System in the Yangtze River Basin at Wudang District, Guiyang City, Guizhou Province, China. IHB 202307067001–02, 2 specimens, 83.6–88.0 mm SL; Bawang River of the Mengjiang River System in the Pearl River Basin at Huishui County, Qiannan Prefecture, Guizhou Province, China. ZJFR 2306003–05, ZJFR 2312003, 4 specimens, 110.3–137.7 mm SL; a vauclusian spring of the Wujiang River System in the Yangtze River Basin at Xixiu District, Anshun City, Guizhou Province, China.

Sinocyclocheilus punctatus: IHB 20210697587, IHB 202311064952, ZJFR 2311004, 3 specimens, 109.4–152.3 mm SL; a subterranean tributary of the Liujiang River System in the Pearl River Basin at Libo County, Qiannan Prefecture, Guizhou Province, China. IHB 20222936A–38A, ZJFR 202311001, ZJFR 202312002, 5, 129.4–226.0 mm SL; a subterranean tributary of the Liujiang River System in the Pearl River Basin at Huanjiang County, Hechi City, Guangxi Province, China.

Sinocyclocheilus sanxiaensis: KIZ 2019000001, holotype, 164.1 mm SL; near the north bank of the Three Gorges Reservoir in the mainstream of Yangtze River at Zigui County, Yichang City, Hubei Province, China.

Sinocyclocheilus jinxiensis: GXU 23070020, 1 specimen, 85.8mm SL; a vauclusian spring of the Yujiang River System in the Pearl River Basin at Jingxi County, Baise City, Guangxi Province, China.

Conflict of interest

The authors declare that they have no conflict of interest.

Funding

This work was partially funded by a grant from the Ecological Monitoring and Restoration in Dongfeng Lake and Liuchong River Basin (No: 2023-1).

Authors' contributions

Zhi-Xuan Zeng and Jia-Jun Zhou designed the study and revised the manuscript. Guang-Yuan Cheng launched the surveys. Xiao-Long Lu extracted the genomic DNA and performed the molecular analysis. Wei-Han Shao examined the specimens and prepared the manuscript. All authors read and approved the final version of the manuscript.

Acknowledgements

We are grateful to Rui Min (Kunming Natural History Museum of Zoology, KIZ) and Ye-Wei Liu, Cheng-Hai Fu (College of Forestry, Guangxi University) for providing comparative specimens; to Ji-Yong Wang (Guiyang Qianren Ecological Conservation Center) for assistance in the field survey; and to Zheng-Meng Yang for drawing of the new species.

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Supplementary material 1

Measurements of the type specimens of *Sinocyclocheilus guiyang*

Authors: Wei-Han Shao, Guang-Yuan Cheng, Xiao-Long Lu, Jia-Jun Zhou, Zhi-Xuan Zeng
Data type: xlsx

Link: https://doi.org/10.3897/zse.100.119520.suppl1

Supplementary material 2

Video of *Sinocyclocheilus guiyang* in situ

Authors: Wei-Han Shao, Guang-Yuan Cheng, Xiao-Long Lu, Jia-Jun Zhou, Zhi-Xuan Zeng
Data type: mp4

Link: https://doi.org/10.3897/zse.100.119520.suppl2

Supplementary material 3

Eyeless specimen of *Sinocyclocheilus cyphotergous*

Authors: Wei-Han Shao, Guang-Yuan Cheng, Xiao-Long Lu, Jia-Jun Zhou, Zhi-Xuan Zeng
Data type: tif
Explanation note: IHB 202207280010, 82.0 mm SL; China: Qiannan Prefecture: Pingtang County: Pearl River Basin.

Link: https://doi.org/10.3897/zse.100.119520.suppl3

Supplementary material 4

Human disturbance and alien species invasion to the type locality of *Sinocyclocheilus guiyang*

Authors: Wei-Han Shao, Guang-Yuan Cheng, Xiao-Long Lu, Jia-Jun Zhou, Zhi-Xuan Zeng
Data type: tif

Link: https://doi.org/10.3897/zse.100.119520.suppl4