Zoological Studies **65:**05 (2026)
doi:10.6620/ZS.2026.65-05

Zoological Studies

Open Access

Descriptions of Two New Species of the Cyprinid Genus *Hongshuia* (Pisces: Cypriniformes) from the Pearl River Basin, Southwest China, with a Re-evaluation of the Taxonomic Status of *H. paoli*

Zhi-Xuan Zeng[1], Cheng-Jiang Tan[2], Ben-Lin Zhang[3], and E Zhang[4,*]

[1]*Department of Endocrinology, Branch of National Clinical Research Center for Metabolic Diseases, Hubei Clinical Medical Research Center for Endocrinology and Metabolic Diseases, Tongji Hospital, Huazhong University of Science and Technology, Wuhan 430030, Hubei Province, China. E-mail: 985801524@qq.com (Zeng)*
[2]*Management Department of Maolan Nature Reserve in Guizhou, Libo 558400, Guizhou Province, China. E-mail: maolantchj@163.com (Tan)*
[3]*Guizhou Jinnong Technology Co., Ltd., Guiyang 550006, Guizhou Province, China. E-mail: 1194661336@qq.com (Zhang)*
[4]*Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, Hubei Province, China.*
**Correspondence: E-mail: zhange@ihb.ac.cn (Zhang)*

urn:lsid:zoobank.org:pub:DF663F1D-1830-42E2-A457-47FFA66EDC71
Received 10 May 2025 / Accepted 20 January 2026 / Published 12 February 2026
Communicated by Felipe Ottoni

Two new species of the labeonin cyprinid genus *Hongshuia*, *H. boulobos* and *H. wangi*, are here described from tributaries of the Pearl River Basin, Southwest China. Both share with *H. brevibarba* the presence of a lower lip with a sector-shaped median lobe (the maximum width positioned at anterior potion of median lobe), distinguishing them from *H. microstomata* and *H. megalophthalmus*, which have a roughly rounded median lobe. *Hongshuia wangi* is distinct from *H. brevibarba* and *H. boulobos* in having wider median lobe of lower lip (width 47.8–57.6% of head width vs. 40.5–43.4% in *H. brevibarba* and 38.5–44.0% in *H. boulobos*). *Hongshuia boulobos* further differs from all other congeners in having a lower lip with larger lateral lobes (width more vs. less than one-third of the median lobe width). The study also considers *H. paoli* as a junior synonym of *H. megalophthalmus* based on morphological and molecular evidence.

Keywords: Biogeography, Cyprinidae, Morphology, Speciation, Taxonomy

BACKGROUND

Southwest China, characterized by well-developed karst landforms (Guo et al. 2010), harbors the greatest diversity of Chinese labeonin cyprinid fishes (Zheng et al. 2016). The genus *Hongshuia* Zhang, Qiang & Lan 2008 includes four small-sized species, characterized by a median lobe of lower lip modified into a round fleshy plate, peripherally greatly protruded to form a ring-like fold, centrally sunken to form a round, flat, fleshy pad, and posteromedially continuous with the mental region (Zhang et al. 2008). Some of the species dwell in underground waters within the karst areas of this region. Four species of *Hongshuia* are accepted under molecular scrutiny, including *H. brevibarba* Zeng, Shao, Jin & Zhang, 2022, *H. megalophthalmus* (Chen, Yang & Cui, 2006), *H. microstomata* (Wang & Chen, 1989), and *H. paoli* Zhang, Qiang & Lan, 2008, currently known mainly from the Pearl River Basin and also from the upper Yangtze River Basin (Zhang et al. 2008 2020; Zeng et al. 2022).

Although *Hongshuia* does not have a complex taxonomic history, questions regarding species identification persists. Following the synonymization of *H. banmo* with *H. megalophthalmus* (Zeng et al. 2022), the validity of *H. paoli*, is also raised. This species was

Citation: Zeng ZX, Tan CJ, Zhang BL, Zhang E. 2026. Descriptions of two new species of the cyprinid genus *Hongshuia* (Pisces: Cypriniformes) from the Pearl River Basin, Southwest China, with a re-evaluation of the taxonomic status of *H. paoli*. Zool Stud **65:**05. doi:10.6620/ZS.2026.65-05.

described based on morphological data of specimens from the Pangyang River, a tributary of the Hongshui River (the middle section of the Pearl River) at Paoli village, Fengshan County, and the specimens from Lingyun County, herein named as *H.* aff. *paoli*, were listed as non-types (Zhang et al. 2008). Previously, only DNA sequences from Lingyun County (voucher specimen: KIZDLN20080003) were available in GenBank (Zheng et al. 2010). Our recent field survey in the type locality of *H. paoli* yielded some specimens, and for the first time this species is included in a more comprehensive study. Additionally, specimens from some tributaries of the Caodu River and the Longjiang River in the Pearl River Basin did not conform to any of the described species in the genus, thus representing two new species. The aims of the present study are to (1) provide a formal description of the two undescribed species and (2) clarify the taxonomic confusion around *H. paoli*.

MATERIALS AND METHODS

The care and use of experimental animals in present study complied with animal welfare laws of Chinese Laboratory Animal Welfare and Ethics. All Specimens were captured with a dipnet and anaesthetized with MS-222 and were thereafter fixed in 10% formalin and later preserved in 75% ethanol for morphological comparisons and permanent curation, or fixed in 95% ethanol for DNA extraction. All specimens were stored at the Museum of Aquatic Organisms, Institute of Hydrobiology (IHB), Chinese Academy of Sciences, Wuhan, China.

Point-to-point morphometric measurements were made with digital calipers to 0.1 mm from the left side of individuals whenever possible. Major measurements and counts followed Kottelat (2001), with some modifications. The head width was measured at the corners of the mouth. The median lobe width was measured as the maximum transverse diameter of the median lobe of lower lip, the lateral lobe width was measured as the maximum transverse width of the lateral lobe of lower lip, and the connection width represented the transverse width of direct connection between the median lobe of lower lip and the mental region (Fig. 1). Additionally, the last two branched rays of dorsal and anal fins were counted as one. Head length and all body measurements were given as a percentage of the standard length (SL), snout length, eye diameter, and interorbital width as a percentage of head length (HL), median lobe of lower lip width and lateral lobe of lower lip width as percentage of head width, and maxillary and rostral barbels length as percentage of eye diameter. For osteological observation, specimens were scanned using micro-computed tomography (micro-CT; Siemens Somatom Definition X-ray machine) and 3D renderings of the osteological structure of the whole specimen and pharyngeal dentition were created and visualized in VG Studio Max 2.1. Abdominal, caudal, and total number of vertebrae were counted following Roberts (1989), and the pharyngeal teeth were counted and presented following Hubbs and Lagler (1947).

A morphometric comparison of *Hongshuia* species was analyzed using principal component analysis (PCA) in Statistical Package for the Social Sciences version 19.0 (SPSS 19.0) based on 23 log-transformed morphometric measurements. A linear regression analysis for origin data of each character was also computed using SPSS 19.0.

Total genomic DNA was extracted from a small portion of fin tissue with the TIANamp Genomic DNA Kit (Tiangen Biotech). Fragments of the mtDNA cytochrome *b* (Cyt *b*) gene were amplified by polymerase chain reaction (PCR) with the following Cyt *b* gene primers: L14724 (5'-GACTTGAAAAACCACCGTTG-3') and H15915 (5'-CTCCGATCTCCGGATTACAAGAC-3') (Xiao et al. 2001). For the cytochrome *c* oxidase subunit I (*COI*) gene, the following primers were used: LCOIa (5'-CCTACCTGTGGCAATCACRCGC-3') and (HCOI 5'-GTAAATAGGGGRAATCARTG-3'), modified from

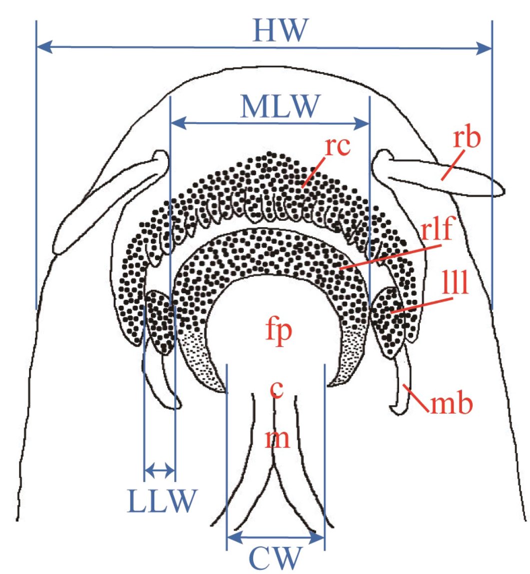

Fig. 1. Oromandibular structure demonstrated on original drawing of *Hongshuia* (shown in *H. wangi*). rc, rostral cap; rb, rostral barbel; lll, lateral lobe of lower lip; mb, maxillary barbel; fp, fleshy pad of median lobe of lower lip; rlf, ring-like fold of median lobe of lower lip; c, connection between median lobe of lower lip and mental region; m, mental region. HW: head width; MLW: median lobe width; LLW: lateral lobe with; CW: connection width.

Liu et al. (2012). The PCR was conducted in a 25 μL volume system consisting of 12.5 μL 2 × GS Taq PCR Mix, 30–50 ng DNA template, 1 μL primers (each 10 μM), and ddH_2O to reach the final volume. The PCR was performed in the following steps: pre-denaturing at 95°C for 3 min; 35 cycles of denaturing at 94°C for 25 s, annealing at 53°C (Cyt *b*) or 56°C (*COI*) for 25 s and extension at 72°C for 30 s; and a final extension step of 72°C for 5 min. The PCR products were purified and sequenced in both directions by the commercial company.

The phylogenetic analysis was performed based on the Cyt *b* and *COI* fragments of the newly generated sequences and GenBank-retrieved sequences from six specimens representing the topotypes of *H. megalophthalmus* and *H. microstomata*, *H.* aff. *paoli* from Lingyun County, and sequences of the following outgroups: *Pseudocrossocheilus bamaensis* (Fang 1981), *Discogobio tetrabarbatus* Lin 1931, *Linichthys laticeps* (Lin & Zhang, 1988), *Ageneiogarra imberba* (Garman, 1912) and *Pseudogyrinocheilus prochilus* (Sauvage & Dabry de Thiersant, 1874) (Table 1). These species were selected as outgroups due to their close phylogenetic relationships with *Hongshuia* (Zheng et al. 2010; Zeng et al. 2022). The sequences were aligned using ClustalW in MEGA7 (Kumar et al. 2016). The best partitioning scheme and evolutionary models were selected in PartitionFinder2 (Lanfear et al. 2017) based on the corrected Akaike information criterion for small sample sizes (AICc), in which an evolution model was defined for each gene codon (Table S1). The phylogenetic tree was inferred with maximum likelihood (ML) and Bayesian inference (BI) methods. The ML analysis was run in IQ-tree version 2.4.0 (Nguyen et al. 2015), with the selected models and 1,000 nonparametric bootstrap replicates. The BI analysis was conducted in MrBayes version 3.2 (Ronquist et al. 2012) with the selected model. Two independent runs of Markov Chain Monte Carlo (MCMC) models using four chains were run over 1,000,000 generations, sampling one tree per 100 replicates for each run. The first 25% of the sampled trees were discarded as burn-in and the remaining trees were used to construct a consensus tree and calculate the posterior probabilities. The intraspecific and interspecific genetic distances based on the *COI* were computed using the Kimura-2-parameter (K2P) model (Kimura 1980). The automatic barcoding gap discovery (ABGD) method (Puillandre et al. 2012) was performed for species delimitation based on the *COI* gene and was run with the default settings (a relative gap width of 1.5, an intraspecific divergence of 0.001–0.1, 10 steps, and the Kimura method) in iTaxoTools 0.1 (Vences et al. 2021).

RESULTS

Hongshuia wangi Zeng & Zhang, sp. nov.

(Figs. 2A, 3A, 4A and 5A; Table 2)
urn:lsid:zoobank.org:act:E507BE8C-648F-4332-BB45-DD6031637055

Material examined: Holotype: IHB 201909260001, 43.1 mm SL; from the Zhangbu River flowing into the Caodu River in the Pearl River Basin at Zhangbu Village (26°03'39"N 107°03'55"E), Pingtang County, Guizhou Province, China; collected by Guo-Fu Wang in Sep 2019. Paratypes: IHB 201909260002–08, 7 specimens, 36.0–39.6 mm SL; other data same as holotype. IHB 20230819001–02, 2 specimens, 31.2–43.8 mm SL; collected in Aug 2023; other data same as holotype. IHB 202402146679–80, 202420140001–04, 6 specimens, 39.4–49.6 mm SL; collected from the Dagui River flowing into the Caodu River in the Pearl River Basin at Tongzhou Town (25°49'38"N 107°02'08"E), Pingtang County, Guizhou Province, China; collected by Zhi-Xuan Zeng in Feb 2024. The common Chinese name proposed for the new species is '王氏红水鲮'.

Etymology: The specific epithet is derived from the family name of the collector (Mr. Guo-Fu Wang), in honor of his contribution in protecting the Zhangbu River and discovering the new species.

Diagnosis: *Hongshuia wangi*, new species, shares with *H. brevibarba* and *H. boulobos*, new species, the presence of a lower lip with a sector-shaped median lobe (the maximum width positioned at anterior potion of median lobe), by which they are separated from *H. microstomata* and *H. megalophthalmus*, which have a roughly rounded median lobe of lower lip. It is distinct from *H. brevibarba* and *H. boulobos* through a wider median lobe of lower lip (width 47.8–57.6% of corresponding head width vs. 40.5–43.4% in *H. brevibarba* and 38.5–44.0% in *H. boulobos*). It further differs from *H. brevibarba* in peripheral part of median lobe of lower lip (greatly protruded vs. slightly protruded); and from *H. boulobos* in having a longer caudal peduncle (18.2–25.7% SL vs. 16.0–18.6%), and the width of lateral lobe of lower lip less (vs. more) than one-third of the median lobe width. The major diagnostic characters are summarized in table 3.

Description: Morphometric measurements are given in table 2 (original measurements are provided in figshare), and the general body appearance and oromandibular morphology are shown in figures 2A and 3A, respectively. Body elongate, cylindrical anteriorly and slightly compressed posteriorly. Dorsal profile from snout tip to dorsal-fin origin slightly convex, and from dorsal-fin origin to dorsal origin of caudal-fin base slightly declining. Maximum body depth positioned at

Table 1. GenBank accession numbers of specimens involved in molecular analysis. *, newly generated in present study

Species Sampling site/ river basin	Voucher specimen	Accession No.	
		Cyt *b*	*COI*
Hongshuia wangi sp. nov.			
Zhangbu, Pingtang, Guizhou/ Pearl River	IHB 201909260011	OR231095*	PQ361887*
	IHB 201909260012	OR231096*	PQ361891*
	IHB 201909260013	OR231097*	PQ361890*
	IHB 201909260014	OR231098*	PQ361889*
	IHB 201909260015	OR231099*	PQ361888*
Tongzhou, Pingtang, Guizhou/ Pearl River	IHB 202402146679	PQ365728*	PQ361892*
	IHB 202402146680	PQ365727*	PQ361893*
	IHB 202402146688	PQ365726*	PQ361894*
	IHB 202402146689	PQ365725*	PQ361895*
	IHB 202402146690	PQ365724*	PQ361896*
Hongshuia boulobos sp. nov.			
Yaosuo, Libo, Guizhou/ Pearl River	IHB 202405016696	PQ365723*	PQ361898*
	IHB 202405016697	PQ365722*	PQ361899*
	IHB 202405016698	PQ365721*	PQ361900*
	IHB 202405026699	PQ365719*	PQ361901*
	IHB 202405026700	PQ365718*	PQ361902*
	IHB 202405020001	PQ365720*	PQ361903*
Qiaocun, Nandan, Guangxi/ Pearl River	IHB 202211063671	OR231090*	PQ361897*
Hongshuia brevibarba			
Baishuihe, Wudang, Guizhou/ Yangtze River	IHB 202307010001	OR231089*	PQ361273*
Hongshuia paoli			
Paoli, Fengshan, Guangxi/ Pearl River	IHB 202307066653	PQ365732*	PQ361274*
	IHB 202307066654	PQ365733*	PQ361275*
	IHB 202307066655	PQ365734*	PQ361276*
	IHB 202307066656	PQ365735*	PQ361277*
	IHB 202307066657	PQ365736*	PQ361278*
Hongshuia aff. *paoli*			
Lingyun, Guangxi/ Pearl River	KIZDLN20080003	GU086560	GU086598
Hongshuia megalophthalmus			
Banmo, Tian'e, Guangxi/ Pearl River	KIZMR20080234	GU086555	GU086593
Hongshuia microstomata			
Xiaoqikong, Libo, Guizhou/ Pearl River	KIZMR200800576	GU086548	GU086586
	IHB 202311064923	PQ365729*	PQ361279*
Malai, Nandan, Guangxi/ Pearl River	IHB 202311064932	PQ365730*	PQ361280*
	IHB 202311064933	PQ365731*	PQ361281*
Pseudocrossocheilus bamaensis			
Congli, Luodian, Guizhou/ Pearl River	IHB 202307010002	OR231100*	PQ361282*
Discogobio tetrabarbatus			
Libo, Guizhou/ Pearl River	KIZMR20080058	GU086556	GU086594
Linichthys laticeps			
Maling, Huaxi, Guizhou/ Pearl River	N/A	OR343919	OR343919
Ageneiogarra imberba			
Wenshan, Yunnan/ Unknown	KIZWWY20080051	GU086568	GU086606
Pseudogyrinocheilus prochilus			
Yiliang, Yunnan/ Unknown	KIZ200405021	GU086534	GU086572

insertion of dorsal-fin origin. Ventral profile from snout tip to anal-fin origin almost straight, and from anal-fin origin to ventral origin of caudal-fin base slightly concave.

Head slightly depressed, length 23.6–26.5% (mean = 24.8%) of SL, greater than width and width greater than depth. Eye medium-sized, positioned at dorsal-lateral part of head. Interorbital space wide and slightly convex. Snout blunt in dorsal view and slightly pointed in lateral view. A shallow groove extending along anteroventral margin of lachrymal from rostral-barbel base to lateral extremity of rostral cap on each side of snout. Tubercles small, conical with blunt tip, irregularly arranged in two rows on snout tip and anterior part of lachrymal. Two pairs of barbels, short but strong. Rostral barbels rooted at front end of shallow grooves on side of snout, length 49.6–75.0% (mean = 63.4%) of eye diameter. Maxillary barbels rooted at corners of mouth, length 29.9–55.0% (mean = 41.1%) of eye diameter.

Table 2. Morphometric characters of *Hongshuia wangi* and *Hongshuia boulobos*

	Hongshuia wangi (*n* = 16)		*Hongshuia boulobos* (*n* = 15)	
	Holotype	Range (Mean, SD)	Holotype	Range (Mean, SD)
As percentage of SL (%)				
Head length	25.2	23.6–26.5 (24.8, 0.9)	22.3	21.0–25.9 (23.3, 1.2)
Body depth	20.8	18.2–24.3 (20.5, 1.4)	19.3	13.7–22.5 (17.6, 2.7)
Caudal peduncle length	25.7	18.2–25.7 (20.6, 2.3)	18.6	16.0–18.6 (17.3, 0.7)
Caudal peduncle depth	11.5	9.8–11.7 (10.8, 0.5)	10.5	8.3–12.5 (10.0, 1.0)
Dorsal-fin length	25.4	22.6–26.4 (24.7, 1.1)	23	19.9–26.0 (23.9, 1.7)
Pectoral-fin length	23.3	20.3–23.3 (22.2, 1.0)	19.8	18.0–24.4 (21.5, 1.6)
Pelvic-fin length	19.4	15.8–20.8 (18.4, 1.4)	17.8	15.6–22.3 (18.9, 1.9)
Anal-fin length	17.5	16.1–20.2 (18.0, 1.2)	18.6	15.2–19.3 (18.0, 1.1)
Caudal-fin length	27.3	25.8–30.1 (28.0, 1.2)	26.6	23.1–32.0 (28.0, 2.2)
Predorsal length	49.8	48.9–54.2 (51.4, 1.3)	51	49.1–52.6 (50.7, 0.9)
Prepectoral length	23.4	21.9–25.4 (23.6, 1.0)	21.5	20.0–24.0 (22.0, 1.0)
Prepelvic length	49.9	49.9–56.1 (52.9, 2.1)	52.8	50.4–55.0 (52.7, 1.2)
Preanal length	70.3	69.5–76.0 (73.7, 1.9)	76.9	72.4–76.9 (74.7, 1.3)
Preanus length	68.8	67.5–72.2 (69.9, 1.6)	71.8	68.7–73.2 (70.4, 1.2)
As percentage of HL (%)				
Snout length	42.3	37.1–44.9 (40.5, 2.6)	40.0	36.0–45.7 (41.3, 2.1)
Eye diameter	27.1	21.8–28.8 (26.0, 2.0)	22.0	22.0–26.1 (24.2, 1.4)
Interorbital width	50.3	45.3–58.6 (50.3, 3.1)	52.8	47.6–55.4 (50.3, 2.1)
As percentage of head width (%)				
Median lobe of lower lip width	48.6	47.8–57.6 (50.9, 2.7)	38.5	38.5–44.0 (41.6, 1.4)
Lateral lobe of lower lip width	11.9	11.6-15.9 (13.4, 1.3)	16.6	13.9–17.0 (15.6, 0.9)
As percentage of eye diameter (%)				
Rostral barbel	69	49.6-75.0 (63.4, 7.2)	83.9	50.0–86.1 (63.9, 11.9)
Maxillary barbel	40.1	29.9–55.0 (41.1, 5.8)	61.1	37.7–71.5 (54.7, 8.8)

SD, standard deviation; SL, standard length; HL, head length.

Table 3. Summary of major diagnostic characters for five valid species of *Hongshuias*

Characters	*H. wangi* (*n* = 16)	*H. boulobos* (*n* = 15)	*H. brevibarba* (*n* = 11)	*H. microstomata* (*n* = 9)	*H. megalophthalmus*	
					Types of *H. paoli* (*n* = 15)	Topotypes (*n* = 10)
Width of median lobe of low lip (% head width)	47.8–57.6 (50.9)	38.5–44.0 (41.6)	40.5–43.4 (41.5)	36.0–47.8 (42.0)	44.3–58.5 (49.8)	43.4–57.1 (50.2)
Median lobe of lower lip	Sector-shaped	Sector-shaped	Sector-shaped	Roughly Rounded	Roughly rounded	Roughly rounded
Maxillary-barbel length (% eye diameter)	29.9–55.0 (41.1)	37.7–71.5 (54.7)	30.0–45.5 (39.6)	48.7–96.3 (74.0)	51.0–67.9 (59.1)	45.6–86.9 (61.7)
Caudal-peduncle length (% SL)	18.2–25.7 (20.6)	16.0–18.6 (17.3)	16.9–22.1 (19.9)	17.0–20.7 (18.3)	17.5–22.7 (19.8)	18.6–22.3 (21.1)
Peripheral part of median lobe of lower lip	Greatly protruded	Greatly protruded	Slightly protruded	Greatly protruded	Greatly protruded	Greatly protruded
lateral lobe greater than 1/3 of median lobe width	No	Yes	No	No	No	No

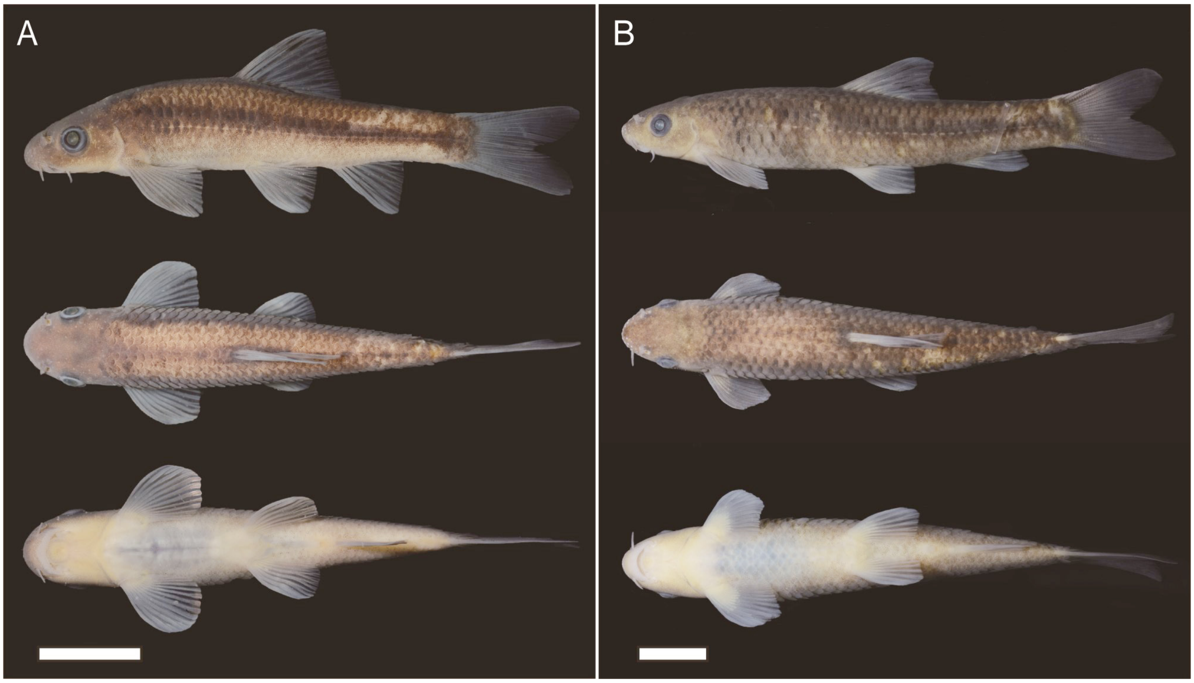

Fig. 2. (A) *Hongshuia wangi*, IHB 201909260001, holotype, 43.1 mm SL, from the Zhangbu River in the Pearl River Basin at Zhangbu Village, Pingtang County, Guizhou Province, China; (B) *Hongshuia boulobos*, IHB 202211062541, holotype, 80.5 mm SL, from the major outlet of the Dongsai Subterranean River in the Pearl River Basin at Yaosuo Village, Libo County, Guizhou Province, China. White bar = 1 cm.

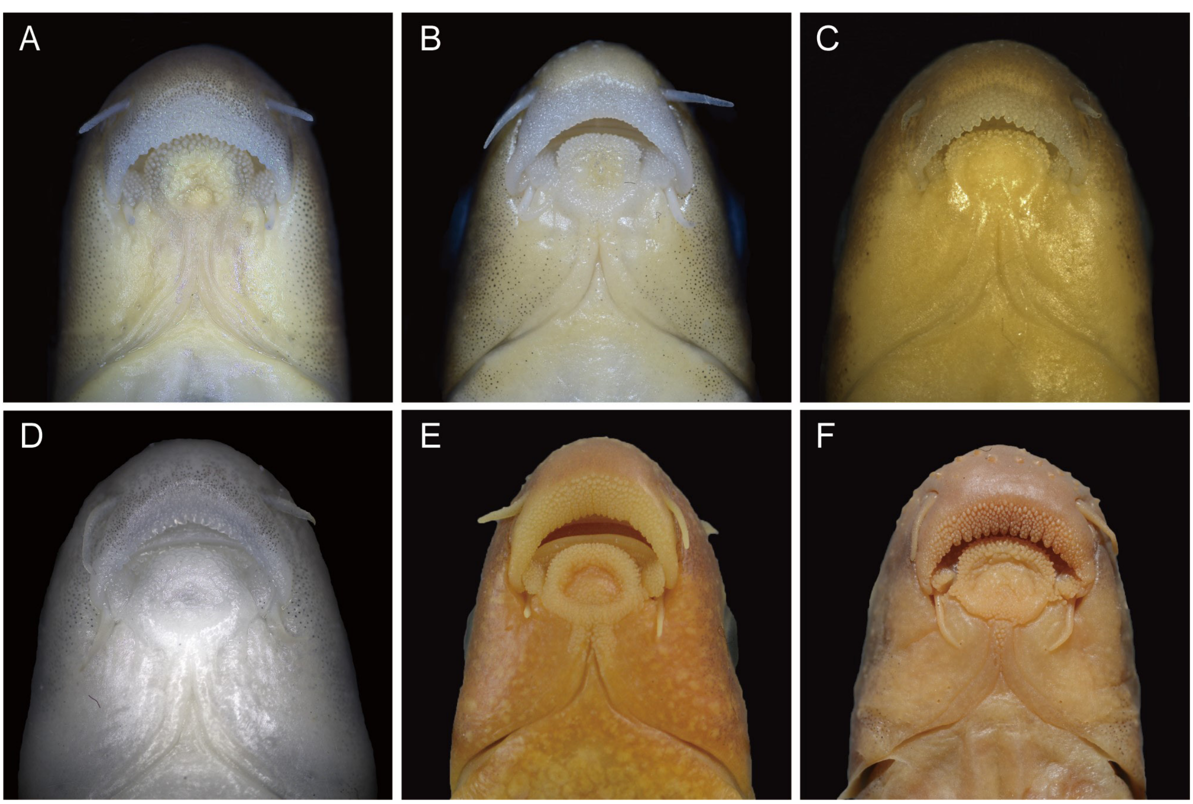

Fig. 3. Oromandibular structures in: (A) *Hongshuia wangi* IHB 201909260001, holotype, 43.1 mm SL; (B) *H. boulobos*, IHB 202211062541, holotype, 63.4 mm SL; (C) *H. brevibarba*, IHB 202006048859, holotype, 56.4 mm SL; (D) *H. microstomata*, IHB 202311064943, 65.2 mm SL; (E) *H. megalophthalmus*, IHB 20214105501, topotype, 63.5 mm SL; (F) *H. paoli*, IHB 200210911, holotype, 54.6 mm SL.

Mouth inferior and slightly arched. Rostral cap with slightly indented distal margin, densely covered with weakly-developed papillae, terminating next to base of maxillary barbel, and disconnected with lower lip at corners of mouth. Upper lip absent. Upper jaw with a thick flexible horny sheath on cutting edge, covered by rostral cap, and disconnected with lower lip at corners of mouth. Low lip divided into a relatively large median lobe, width 47.8–57.6% (mean = 50.9%) of head width, and two relatively small lateral lobes, width 11.6–15.9% (mean = 13.4%) of head width, less than one-third of median lobe width. Median lobe of lower lip modified into a fleshy plate anteriorly wider than posteriorly, peripheral part greatly protruded, forming a ring-like fold, and central part sunken, forming a flat, fleshy pad. Ring-like fold densely covered with well-developed papillae anteriorly and weakly-developed papillae posteriorly. A shallow groove extending along margin of the ring-like fold, separating it anteriorly from lower jaw, laterally from two lateral lobes, and posterolaterally from mental region. Posteromedian margin of ring-like fold reduced, forming a relatively wide connection between flat fleshy pad and mental region, width 17.1–29.3% (mean = 21.9%) of head width.

Dorsal fin with 3 simple and 7 (in 16 specimens) branched rays, last one split to base; distal margin slightly concave; origin at midpoint between snout tip and caudal-fin base. Pectoral fin with 1 simple and 10 (in 1 specimen), 11 (in 5 specimens) or 12 (in 10 specimens) branched rays; extending beyond halfway to pelvic-fin insertion, but not reaching to dorsal-fin origin. Pelvic fin with 1 simple and 7 (in 16 specimens) branched rays; inserted slightly posterior to dorsal-fin origin; reaching to anus. Anal fin with 3 unbranched and 5 (in 16 specimens) branched rays, last one split to base; distal margin slightly concave or straight; inserted closer to pelvic-fin origin than to caudal-fin base. Caudal fin deeply forked; tip of upper and lower lobes pointed, both lobes equal in length and shape.

Body covered with moderately sized scales. Scales on chest and belly slightly smaller than those on flank. Lateral line complete, horizontal, with 39 (in 2 specimens), 40 (in 9 specimens) or 41 (in 5 specimens) lateral line scales. Scale rows above lateral line 4+1/2 (in 3 specimens) or 5+1/2 (in 13 specimens), and below 3+1/2 (in 3 specimens) or 4+1/2 (in 13 specimens). Circumpeduncular scales 12 (in 10 specimens), 13 (in 2 specimens) or 14 (in 4 specimens). Predosoral scales on midline 13 (in 2 specimens), 14 (in 13 specimens) or 15 (in 1 specimen), slightly smaller than those on flank, not embedded subcutaneously.

Vertebrae: 18+16 = 34 (in 1 specimen). Two rows of pharyngeal teeth; tooth pattern 2,5–5,3 (in 1 specimen); tooth tips compressed and pointed (Fig. 4A). Air bladder with 2 chambers; anterior chamber oval; posterior chamber elongate, ~2 times length of anterior one. Intestine long, ~4 times of standard length, forming many coils. All original morphometric measurements are available in table S2.

Coloration: In freshly collected individuals (Fig. 5A), head and body yellowish dorsally and laterally, slightly lighter in ventral view. A black stripe extending along lateral line from nape to caudal-fin base, ~2 scales wide anteriorly, slightly narrower posteriorly. A light yellowish dot intermediately posterior to nape. A narrow light yellowish stripe running along dorsal midline from nape to dorsal-fin origin. A narrow interrupted black stripe from posterior end of dorsal-fin base to dorsal origin of caudal-fin base. Dorsal fin with a black base, and in some individuals with black interradial membrane; all other fins transparent. In formalin-

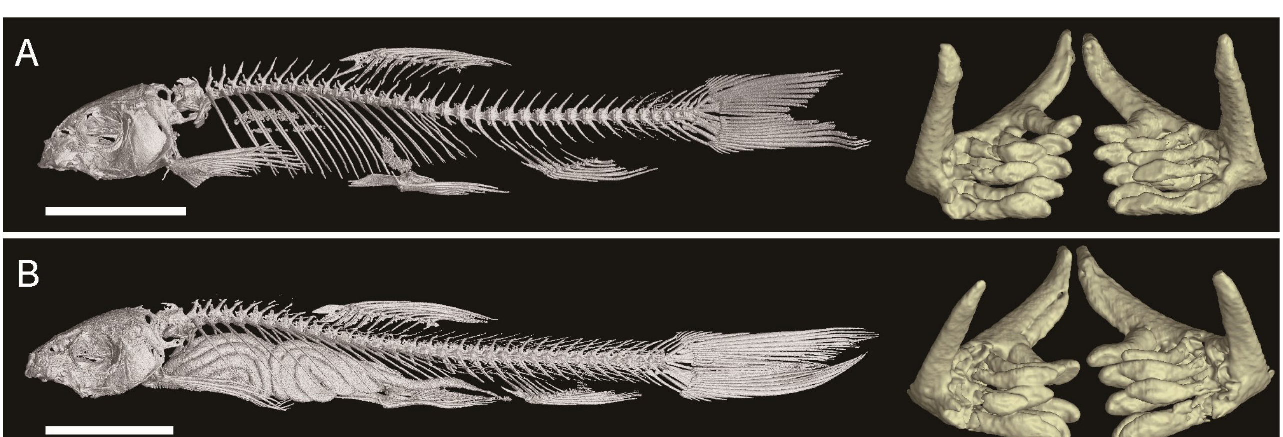

Fig. 4. Reconstructed Micro-CT graph of whole specimen in lateral view (with a white bar = 1 cm) and amplified pharyngeal dentition. (A) *Hongshuia wangi*, IHB 202402146680, paratype, 49.6 mm SL; (B) *H. boulobos*, IHB 202405026702, paratype, 55.17 mm SL.

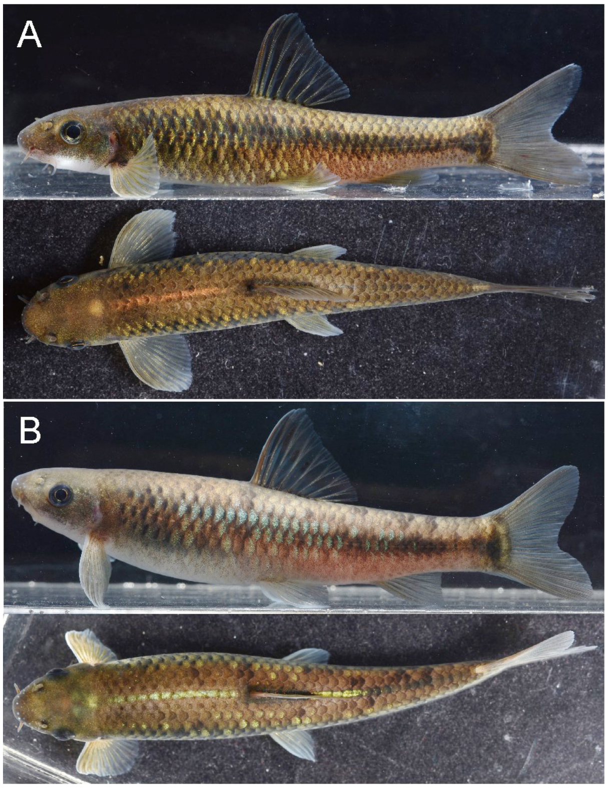

Fig. 5. Freshly collected specimen of (A) *Hongshuia wangi*, IHB 202402146680 and (B) *H. boulobos*, IHB 202405026699.

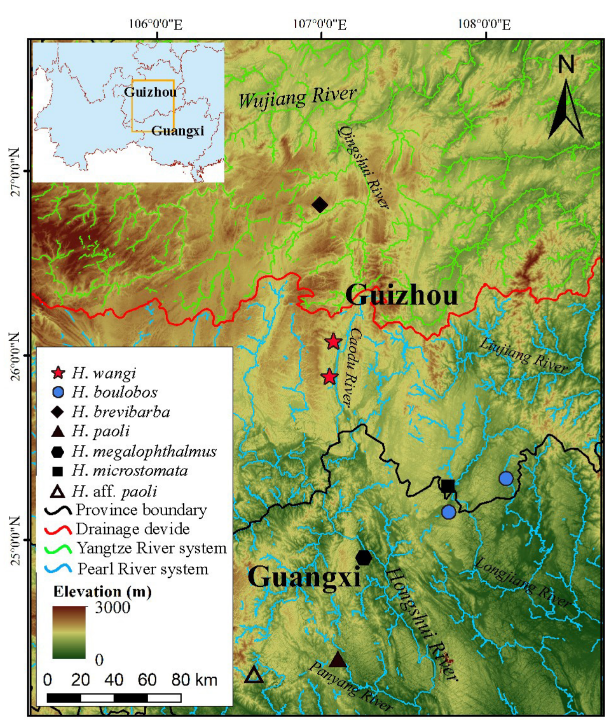

Fig. 6. Distribution of species of *Hongshuia*.

preserved specimens (Fig. 2A), dorsal and lateral head blackish, and ventral head whitish gray. Ground color of body dusty yellowish dorsally and laterally, and pale yellow ventrally. A dark black longitudinal band extending along lateral line, anteriorly deep, and slightly declining in depth posteriorly. An inconspicuous blackish dorsal midline stripe starting from nape to caudal-fin base. No light dot intermediately posterior to nape. All fins whitish gray.

Distribution and habitat: Currently known from the Zhangbu and Dagui Rivers, two tributaries flowing into the Caodu River System of the Pearl River Basin in Pingtang County, Guizhou Province, China (Fig. 6). *Hongshuia wangi* prefers hill streams with clear, fast-flowing water over gravel substrates (Fig. 7A) and feeds mainly on periphyton. Other sympatric labeonin species include *Pseudocrossocheilus bamaensis*, *Discogobio laticeps* Chu, Cui & Zhou, 1993, and *Pseudogyrincheilus prochilus*.

Hongshuia boulobos **Zeng & Zhang, sp. nov.**

(Figs. 2B, 3B, 4B and 5B; Table 2)
urn:lsid:zoobank.org:act:B55F7844-7CCD-442B-B1DC-3E9B78577306

Hongshuia microstomatus: Xiao & Lan, 2023: 255 (Nandan County, Guangxi Province).

Material examined: Holotype: IHB 202211062541, 63.4 mm SL; from the major outlet of the Dongsai Subterranean River flowing into the Longjiang River, a main tributary of the Liujiang River

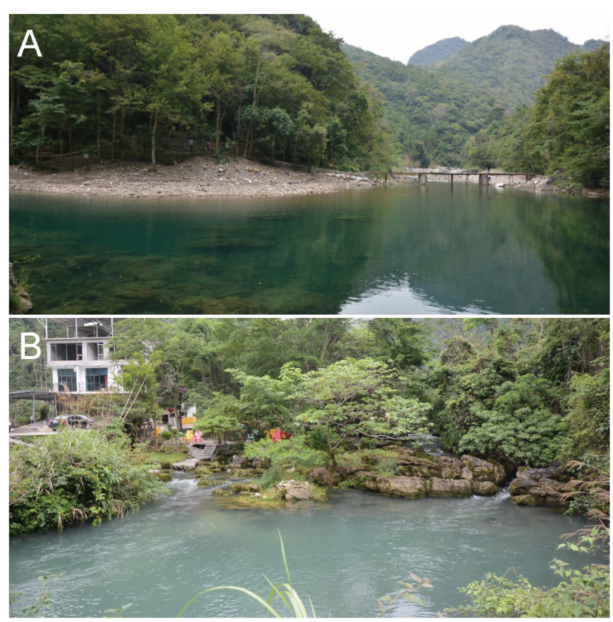

Fig. 7. Habitat of (A) *Hongshuia wangi*: the Zhangbu River (the surface section) and (B) *H. boulobos*: the Dongsai Subterranean River (the outlet).

in the Pearl River Basin at Yaosuo Village (25°17'25"N 108°04'00"E), Libo County, Guizhou Province, China; collected by Zhi-Xuan Zeng in Sep 2022**.** Paratypes: IHB 202211063671, 1 specimen, 50.7 mm SL; collected from a branch outlet of the Lihu Subterranean River flowing into the Longjiang River in the Pearl River Basin at Qiao Village (25°03'34"N 107°36'27"E), Nandan County, Guangxi Province, China; collected by Zhi-Xuan Zeng in Sep 2022. IHB 202405020001–08, 202405026699–703, 13 specimens, 34.1–61.5 mm SL in May 2024, other data same as holotype.

Etymology: The epithet, used as a noun, is derived from the Greek words 'bou-' (large) and 'lobos'(lobe), in allusion to the two large lateral lobes of the lower lip. The common Chinese name proposed for the new species is '大叶红水鲮'.

Diagnosis: *Hongshuia boulobos*, new species, is distinct from all congeneric species in having a lower lip with larger lateral lobes (width more than vs. less than one-third of the median lobe width). It is similar to *H. wangi* and *H. brevibarba* in having a sector-shaped median lobe of lower lip, a character used to separate them from *H. microstomata* and *H. megalophthalmus* where the median lobe of lower lip is roughly rounded. *Hongshuia boulobos* is further distinguishable from *H. wangi* in having a shorter caudal peduncle (16.0–18.6% SL vs. 18.2–25.7%); and further from *H. brevibarba* in having longer maxillary barbels (37.7–71.5% eye diameter vs. 30.0–45.5%), and greatly (vs. slightly) protruded peripheral part of median lobe of lower lip (Table 3).

Description: Morphometric measurements are given in table 2 (original measurements are provided in figshare), and general body appearance and oromandibular morphology are shown in figures 2B and 3B, respectively. Body elongate, cylindrical anteriorly and slightly compressed posteriorly. Dorsal profile from snout tip to dorsal-fin origin slightly convex, and from dorsal-fin origin to dorsal origin of caudal-fin base slightly declining. Maximum body depth positioned at insertion of dorsal-fin origin. Ventral profile from snout tip to anal-fin origin almost straight or slightly convex, and from anal-fin origin to ventral origin of caudal-fin base slightly concave.

Head slightly depressed, length 21.0–25.9% (mean = 23.3%) of SL, greater than width and width greater than depth. Eye medium-sized, positioned at dorsal-lateral part of head. Interorbital space wide and slightly convex. Snout blunt in dorsal view and roughly pointed in lateral view. A shallow groove extending along anteroventral margin of lachrymal from rostral-barbel base to lateral extremity of rostral cap on each side of snout. Tubercles small, conical with blunt tip, irregularly arranged in two rows on snout tip and anterior part of lachrymal. Two pairs of strong barbels. Rostral pair rooted at front end of shallow grooves on side of snout, length 50.0–86.1% (mean = 63.9%) of eye diameter. Maxillary barbels rooted at corners of mouth, length 37.7–71.5% (mean = 54.7%) of eye diameter.

Mouth inferior and slightly arched. Rostral cap with slightly indented distal margin, densely covered with weakly-developed papillae, terminating next to base of maxillary barbel, and disconnected with lower lip at corners of mouth. Upper lip absent. Upper jaw with a thick flexible horny sheath on cutting edge, covered by rostral cap, and disconnected with lower lip at corners of mouth. Lower lip divided into a median lobe and two lateral lobes. Median lobe relatively small, width 38.5–44.0% (mean = 41.6%) of head width. Lateral lobes relatively large, width 13.4–17.0% (mean = 15.5%) of head width, greater than one-third of median lobe width. Median lobe of lower lip modified into a plate anteriorly wider than posteriorly, peripheral part greatly protruded, forming a ring-like fold, and central part sunken, forming a flat, fleshy pad. Ring-like fold densely covered with well-developed papillae. A shallow groove extending along anterior and lateral margin of the ring-like fold, separating it from lower jaw and two lateral lobes, and posterolaterally from mental region. Posteromedian margin of the ring-like fold reduced to form a relatively narrow connection between the flat, fleshy pad and mental region, width 5.8–10.8% (mean = 8.3%) of head width.

Dorsal fin with 3 simple and 7 (in 15 specimens) branched rays, last one split to base; distal margin slightly concave; origin at midpoint between snout tip and caudal-fin base. Pectoral fin with 1 simple and 11 (in 7 specimens), 12 (in 7 specimens) or 13 (in 1 specimen) branched rays; extending beyond halfway to pelvic-fin insertion, but not reaching to dorsal-fin origin. Pelvic fin with 1 simple and 7 (in 15 specimens) branched rays; inserted slightly posterior to dorsal-fin origin; reaching to anus. Anal fin with 3 unbranched and 5 (in 15 specimens) branched rays, last one split to base; distal margin slightly concave or straight; inserted closer to pelvic-fin origin than to caudal-fin base. Caudal fin deeply forked; tip of upper and lower lobes pointed, both lobes equal in length and shape.

Body covered with moderately sized scales. Scales on chest and belly slightly smaller than those on flank. Lateral line complete, horizontal, with 37 (in 4 specimens), 38 (in 3 specimens), 39 (in 6 specimens) or 40 (in 2 specimens) lateral line scales. Scale rows above lateral line 4+1/2 (in 13 specimens) or 5+1/2 (in 2), and below 3+1/2 (in 13 specimens) or 4+1/2 (in 2 specimens). Circumpeduncular scales 11 (in 1 specimen), 12 (in 10 specimens) or 13 (in 4 specimens). Predosoral scales on midline 13 (in

3 specimens) or 14 (in 12 specimens). slightly smaller than those on flank, not embedded subcutaneously.

Vertebrae: 18+16 = 34 (in 1 specimen). Two rows of pharyngeal teeth; tooth pattern 3,5–5,3 (in 1 specimen); tooth tips compressed and pointed (Fig. 4B). Air bladder with 2 chambers; anterior chamber oval; posterior chamber elongate, ~2 times length of anterior one. Intestine long, ~4 times of standard length, forming many coils.

All original morphometric measurements are available in table S2.

Coloration: In fresh specimens (Fig. 5B), head and body yellowish in dorsal and lateral views, slightly lighter in ventral view. A black stripe extending along lateral line from nape to caudal-fin base, ~3 scales wide anteriorly, and ~2 scales wide posteriorly. A light yellowish dot intermediately posterior to nape. A narrow light yellowish stripe running along dorsal midline from nape to dorsal-fin origin. A narrow interrupted light yellow or black stripe from posterior end of dorsal-fin base to dorsal origin of caudal-fin base. Dorsal fin with a black base, in some individuals with interradial membrane black; all other fins transparent. In formalin-preserved specimens (Fig. 2B), dorsal and lateral head blackish, and ventral head whitish gray. Ground color of body dusty yellowish dorsally and laterally, and pale yellow ventrally. A dark black longitudinal band extending along lateral line, anteriorly deep, and slightly declining in depth backwards. An inconspicuous blackish dorsal midline stripe starting from nape to caudal-fin base. No light dot intermediately posterior to nape. All fins whitish gray.

Distribution and habitat: The new species is currently known from the outlets of subterranean tributaries of the Longjiang River, a western tributary of the upper Liujiang River flowing into the Pearl River in Libo County, Guizhou Province and Nandan County, Guangxi Province (Fig. 6). *Hongshuia boulobos* inhabits hill streams with clean, fast-flowing water over gravel substrates (Fig. 7B) or adjacent karst windows with water connected with the subterranean river, feeding mainly on periphyton. Other coexisting labeonin species include *Pseudocrossocheilus nigrovittatus* (Su, Yang & Cui 2003), *Discogobio tetrabarbatus* and *Parasinilabeo assimilis* Wu & Yao 1977.

Morphometric comparisons

In the PCA for the species with large median lobe, with width about half of the corresponding head width (including *H. wangi*, *H. paoli* and *H. megalophthalmus*), the first three components explained 88.92% of the total variance, including 80.54% by PC1, 5.47% by PC2, and 2.91% by PC3. The specimens of *H. wangi* constitute a distinct cluster in PC1 vs. PC2 (Fig. 8A). On the PC1 axis, *H. wangi* is separated from *H. paoli* with the main loadings comprising preanal length, preanus length, caudal peduncle depth, pectoral-fin length and standard length (Table S3). On the PC2 axis, *H. wangi* is distinct from *H. megalophthalmus* with the main loadings on this axis comprising connection width, maxillary barbel length, eye diameter, snout length and prepectoral length (Table S3). In the PCA for the rest three species with small median lobe, with width ~40% of the head width (including *H. boulobos*, *H. microstomata* and *H. brevibarba*), the first three components explained 96.22% of the total variance, including 85.75% by PC1, 8.66% by PC2, and 1.81% by PC3. *Hongshuia boulobos* is distinct from the other two species through PC2 vs. PC3 (Fig. 8B). On the PC2 axis, *H. boulobos* differs from *H. brevibarba* and *H. microstomata*, and the first five loadings are connection width, body depth, caudal peduncle depth, caudal-fin length and caudal peduncle length (Table S3). In the PCA for the species from the Pearl River Basin of Guizhou Province (including *H. wangi*, *H. boulobos* and *H. microstomata*), all species form distinguishable clusters in PC1 vs. PC2 (Fig. 8C). *Hongshuia microstomata* differs from the other two species on the PC1 axis, with standard length, predorsal length, preanus length, prepectoral length and preanal length as the major loadings, while *H. wangi* is separated from *H. boulobos* on the PC2 axis, with connection width, body depth, median lobe of lower lip width, maxillary barbel length and head length as the major loadings (Table S3). In the PCA for *H. paoli* and *H. megalophthalmus*, the first three components explain 87.92% of the total variance, including 78.93% by PC1, 5.44% by PC2, and 3.55% by PC3. The two species, however, were indistinguishable (Fig. 8D–F). A linear regression further supported the distinguishing features of *H. wangi*, *H. boulobos*, and *H. microstomata*: *H. microstomata* is distinguishable from *H. wangi* and *H. boulobos* in the relationship between eye diameter and maxillary barbel length, *H. wangi* differs from *H. microstomata* and *H. boulobos* in head width to median lobe width and standard length to Caudal-peduncle length relations, whereas *H. boulobos* from the other two species in the relationship between median and lateral lobe widths (Fig. 9).

Genetic comparisons

A total of 1100 bps of the Cyt *b* dataset and 821 bps of the *COI* dataset were included in the molecular analysis. The sequences of the Cyt *b* dataset contained 809 conserved sites, 291 variable sites, 174 parsimony informative sites, and 117 singleton sites, and that of the *COI* dataset contained 638 conserved sites, 183 variable sites, 118 parsimony informative sites, and 65 singleton sites.

The BI and ML trees based on the combined Cyt *b* and *COI* datasets shared identical topology (Fig. 10). Sequences of *H. wangi* formed a monophyletic lineage with 100% posterior probability and 99% bootstrap support, as sister to *H. microstomata*. The monophyly of *H. boulobos* was favored by 100% posterior probability and 100% bootstrap support, and this species was separated at the base of the clade formed by all species from the Pearl River Basin. In addition, sequences of *H. paoli* from its type locality—Paoli Village in Fengshan County—were closely associated with *H. megalophthalmus*. However, sequences from material identified as *H.* aff. *paoli* from Lingyun did not cluster with those of *H. paoli* from the type locality. The ABGD analysis returned seven partitions, of which partition 6 (maximum prior distance, $p = 0.0129$) was the most congruent with the data and supported *H. wangi* and *H. boulobos* as two new species, and also favored the synonymization of *H. paoli* and *H. megalophthalmus* (Fig. 10).

Average genetic distances derived from the *COI* sequences showed that the average intraspecific distance of *H. wangi* was 0%, with interspecific distances ranging from 1.5% (vs. *H. megalophthalmus* and *H. microstomata*) to 5.1% (vs. *H. boulobos*). The intraspecific genetic distance of *H. boulobos* was 0.3%, with interspecific distances ranging from 4.6% (vs. *H. megalophthalmus*) to 5.1% (vs. *H. wangi* and *H. microstomata*) (Table 4).

DISCUSSION

Hongshuia paoli was described and designated as the type species of the genus by Zhang et al. (2008). However, the PCA results from the specimens caught from the type localities of both *H. paoli* and *H. megalophthalmus* showed that both species are

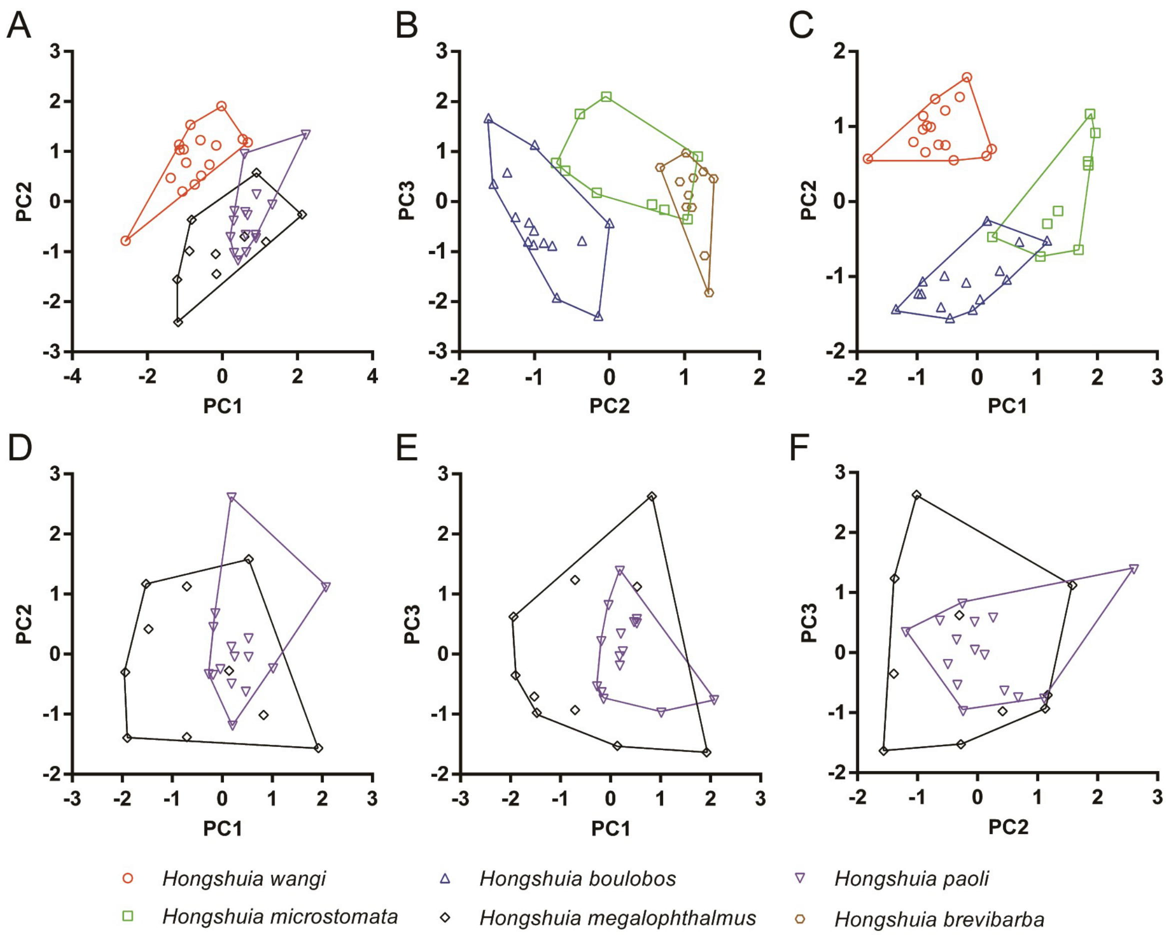

Fig. 8. Scatter plot of (A) PC1 vs. PC2 for *Hongshuia wangi*, *H. paoli* and *H. megalophthalmus*; (B) PC2 vs. PC3 for *H. boulobos*, *H. microstomata* and *H. brevibarba*; (C) PC1 vs. PC2 for *H. wangi*, *H. boulobos* and *H. microstomata*; and (D–F) PC1 vs. PC2, PC1 vs. PC3 and PC2 vs. PC3 for *H. paoli* and *H. megalophthalmus*.

indistinguishable (Fig. 8D–F), and the main diagnostic characters substantially overlap (Table 3). The characters previously used to distinguish between the two species, such as differences in the papillae on the rostral cap, and presence or absence of black blotches on the interradial membranes of dorsal fin, were found to be variable. Although the type locality of *H. megalophthalmus* is 58 km away from that of *H. paoli* (Chen et al. 2006; Zhang et al. 2008), their genetic distance is merely 0.4% (Table 4). The topotypes of both species also clustered together in the molecular phylogenetic analysis, and ABGD species delimitation supported the synonymization of the two species (Fig. 10). These findings strongly suggest that *H. paoli* is a junior synonym of *H. megalophthalmus* and indicate that *H. megalophthalmus* is widely distributed in the Hongshui River Basin. However, this assignment will not alter the designation of *Hongshuia paoli* as the type species of the genus, according to Art. 67 of the International Code of Zoological Nomenclature (ICZN 1999). In addition, the voucher specimen of the sequence KIZDLN20080003 from Lingyun County constituted a phylogenetically distinct lineage, which we identify as *H.* aff. *paoli* (see preceding section) (Fig. 10). Although previously identified as *H. paoli* (Zheng et al. 2010; Xiao and Lan 2023), this population in fact represents an undescribed species.

All freshly collected specimens of the two new species possessed a light yellowish round spot immediately posterior to the nape and a narrow light yellowish stripe along the dorsal midline of the pre-dorsal body (Fig. 5). This color pattern is also present in other *Hongshuia* species (Fig. 3a of Zeng et al. 2022) and has not been reported in any other labeonin genera. Thus, this color pattern can be considered autapomorphic for the genus *Hongshuia*. Our experience in the field also confirm that the two new species have different habitat requirements: *H. wangi* is a typical epigean species usually found in hill streams with clear, rapid-running water (Fig. 7A), whereas *H. boulobos* occurs in the outlets of subterranean rivers with clean, fast-flowing water over gravel substrates (Fig. 7B) or

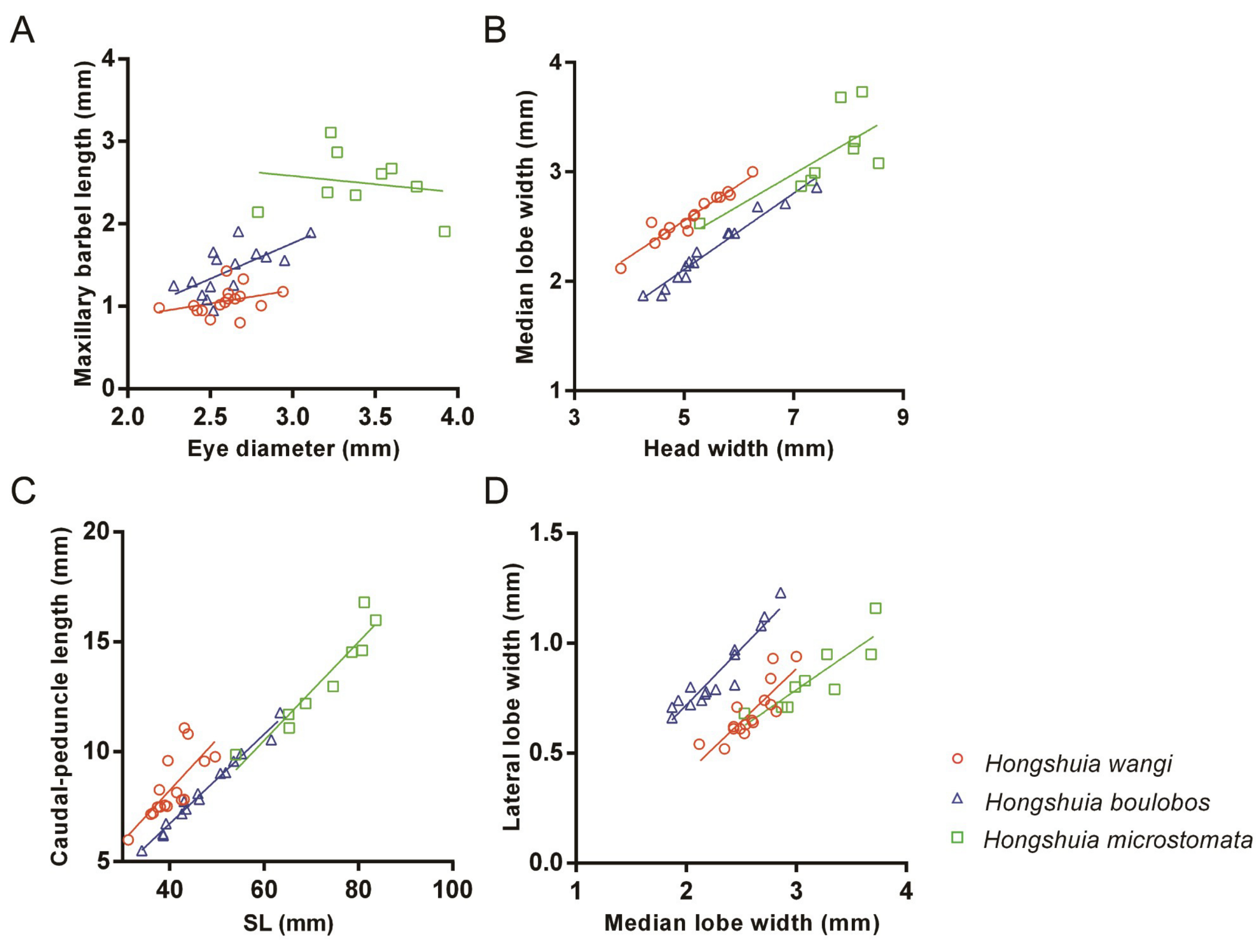

Fig. 9. Linear regression for morphometric measurements of *Hongshuia* species: (A) Eye diameter against maxillary barbel length; (B) Head width against width of median lobe of lower lip; (C) Standard length (SL) against caudal-peduncle length; (D) Median lobe width against lateral lobe width.

in adjacent karst windows with water connected to the subterranean river. All five *Hongshuia* spp. can be classified into two ecotypes based on our field data: the epigean type, including *H. brevibarba* and *H. wangi*, and the hypogean type, comprising *H. microstomata*, *H. megalophthalmus*, *H.* aff. *paoli* (from Lingyun) and *H. boulobos*. All currently recognized hypogean *Hongshuia* spp. can be defined as troglophilic fishes (or atypical cavefishes) defined by Zhao et al. (2011).

Species diversification of *Hongshuia* is closely associated with landscape evolution and ecological disparity. *Hongshuia brevibarba*, the species currently known from the upper Yangtze River Basin, diverged from the remaining Pearl River Basin congeners (Fig. 10), possibly the result of the formation of the watershed between the two basins, owing to the uplift

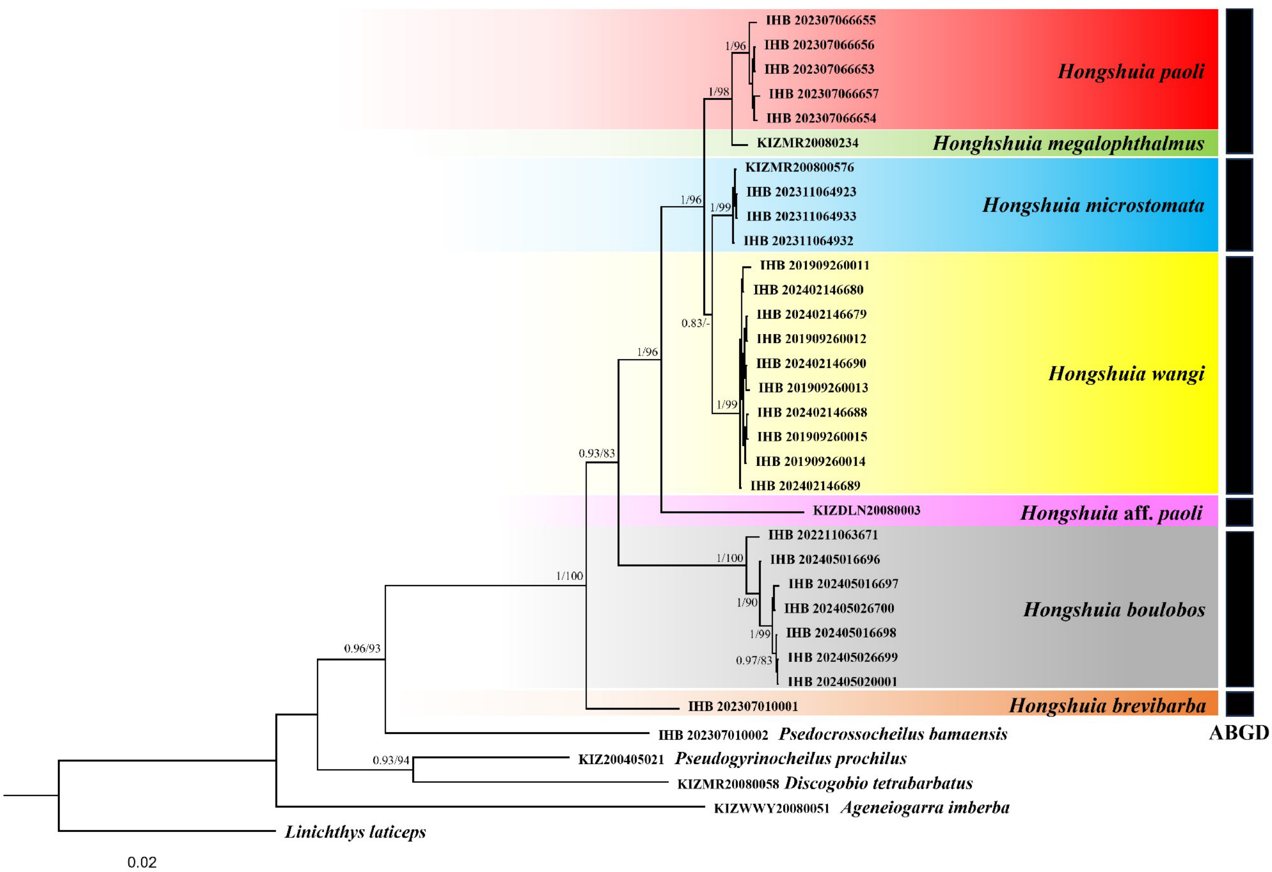

Fig. 10. Bayesian inference and maximum likelihood trees of *Hongshuia* species inferred from the combined Cyt *b* and *COI* datasets. Posterior probability/ bootstrap support was shown if greater than 50%. ABGD species delimitation results were also shown.

Table 4. Average intraspecific and interspecific genetic distances (%) derived from the *COI* gene in species of *Hongshuia*. n/a, not available

Species	Intraspecific	Interspecific					
		1.	2.	3.	4.	5.	6.
1. *H. wangi*	0						
2. *H. boulobos*	0.3	5.1					
3. *H. brevibarba*	n/a	4.2	5.0				
4. *H. microstomata*	0	1.5	5.1	4.4			
5. *H. megalophthalmus*	n/a	1.5	4.6	4.7	1.5		
6. *H. paoli*	0.2	1.7	4.8	4.9	1.7	0.4	
7. *H.* aff. *paoli*	n/a	3.8	5.0	5.2	4.3	3.8	4.2

of the Miaoling Mountains during the Early Pleistocene (Zhou and Chen 1993). The next divergence involved *H. boulobos*, known from hypogean streams flowing into the Longjiang River of the Liujiang River, and subsequently *H.* aff. *paoli*, known from hypogean streams flowing into the Youjiang River of the Yujiang River (Zhang et al. 2022). Their marginal distribution compared to all other congeners and highly specialized habitat preferences suggest that peripheral isolation and ecological disparity may have led to the speciation of the Longjiang River population into *H. boulobos* and the Lingyun County population into *H.* aff. *paoli*.

The low genetic distances between species pair among *H. megalophthalmus*, *H. microstomata*, and *H. wangi* (Table 4) indicate recent divergences of the three species. *Hongshuia microstomata* is restricted to the Huanghou Underground River of the upper Longjiang River (Wang and Chen 1989; Li et al. 1999), while *H. wangi* is found in the Caodu River close to the northern boundary of the Hongshui River Basin (Jing 2013). *Hongshuia megalophthalmus* is also present in the Hongshui River, however, further downstream than *H. wangi*. Herein, we hypothesize that the upper Longjiang River was a part of the Hongshui River system and was separated from the lower reaches of Longjiang River. The common ancestor of the three species may have been troglophilic and widespread in the Hongshui and upper Longjiang Rivers. The changes in drainage patterns of these two rivers, in other words the capture of the upper Longjiang River (including the Huanghou Underground River) by its lower reaches likely facilitated speciation of *H. microstomata*. Notably, although *H. microstomata* and *H. boulobos* are both troglophlic and co-occur in the Longjiang River system, they also have an allopatric distribution—*H. microstomata* is known only from the upper reaches of the Longjiang River and *H. boulobos* only from its middle reaches. Furthermore, ecological disparity likely played a crucial role in species diversification between *H. megalophthalmus* and *H. wangi*, as the former is a hypogean species widespread in the Hongshui River, and the latter is an epigean species occurring in its higher elevation reaches.

Comparative materials

Hongshuia brevibarba: IHB 201909300001–10, 202006048859 (holotype), 11 specimens, 38.8–56.4 mm SL; from a stream tributary flowing into the Qingshui River of the Wujiang River System in the Yangtze River Basin at Baishuihe Village, Wudang District, Guiyang City, Guizhou Province.

Hongshuia megalophthalmus: IHB 20214105501–5, 202197556–60, 10 specimens, 38.4–63.5 mm SL; from an outlet of small subterranean tributary flowing into the Pearl River at Banmo Village, Tian'e County, Guangxi Province.

Hongshuia microstomata: IHB 87IV506–7, 87IV509 (holotype), 87IV510, 4 specimens, 78.6–83.7 mm SL; from a major outlet (Wolong-Tan) of the Huanghou Subterranean River flowing into the Longjiang River of the Liujiang River System in the Pearl River Basin at Wangmeng Village in Libo County, Guizhou Province. IHB 202311064940–44, 5 specimens, 65.3–74.7 mm SL; from the Huanghou Subterranean River flowing into the Longjiang River of the Liujiang River System in the Pearl River Basin at Malai Village, Nandan County, Guangxi Province.

Hongshuia paoli: IHB 200210900–10, 202210911 (holotype), 200210912–14, 15 specimens, 47.2–65.4 mm SL; from an outlet of the Panyang River discharging into the Pearl River at Paoli Village, Fengshan County, Guangxi Province.

CONCLUSIONS

This work identifies two new species of *Hongshuia* based on morphological and molecular evidence, and provides strong evidence to consider *H. paoli* as a junior synonym of *H. megalophthalmus*. However, this study is still limited as it was based on merely two mitochondrial genes. A genomics-based study is warranted to further access the evolutionary history of the genus *Hongshuia* and explore the geographic changes within Southwest China.

Acknowledgments: We are also appreciative of the agencies (the management departments of Maolan National Natural Reserve and Zhangbu Scenic Area, as well as Guiyang Qianren Ecological Conservation Center) for their support in the field surveys, and to Wei-Han Shao, Man Wang, Xiong Gong and Juan-Juan Chen (IHB) for their kind assistance in DNA extraction and photo taking. This work was supported by the Young Scientists Fund of the National Natural Science Foundation of China (Grant No. 82300894). We would like to thank MogoEdit (https://www.mogoedit.com) for its English editing during the preparation of this manuscript.

Authors' contributions: EZ designed the study and revised the manuscript. ZXZ examined the specimens, performed the molecular analysis and prepared the manuscript. CJT and BLZ launched the surveys.

Competing interests: The authors declare that they have no conflict of interests.

Availability of data and materials: The sequences used in this study are available in GenBank with accession numbers provided in table 1.

Consent for publication: Not applicable.

Ethics approval consent to participate: Not applicable.

REFERENCES

Chen XY, Yang JX, Cui GH. 2006. A new fish species of genus *Sinocrossocheilus* (Cyprinidae) from Guangxi, China. Zool Res **27:**81–85. (in Chinese)

Guo F, Yuan D, Qin Z. 2010. Groundwater contamination in karst areas of southwestern China and recommended countermeasures. Acta Carsologica **39:**389–399. doi:10.3986/ac.v39i2.107.

Hubbs CL, Lagler KF. 1947. Fishes of the great lakes region. Bulletin of the Cranbrook Institute of Sciences **26:**1–186.

ICZN (International Commission on Zoological Nomenclature). 1999. International Code of Zoological Nomenclature, 4th ed. International Trust for Zoological Nomenclature. Available at: https://www.iczn.org/iczn/index.jsp.

Jing ZS. 2013. Encyclopedia of rivers and lakes in China - Section of Zhujiang Rivers Basin. Beijing: China Water and Power Press, Beijing. (in Chinese)

Kimura M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J Mol Evol **16:**111–120. doi:10.1007/BF01731581.

Kottelat M. 2001. Fishes of Laos. Wildlife Heritage Trust Publications, Colombo.

Kumar S, Stecher G, Tamura K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Mol Biol Evol **33:**1870–1874. doi:10.1093/molbev/msw054.

Lanfear R, Frandsen PB, Wright AM et al. 2017. PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. Mol Biol Evol **34:**772–773. doi:10.1093/molbev/msw260.

Li JY, Ji CM, Yan GQ et al. 1999. Characteristics and assessment on water resources of Huanghou Karst Underground Drainage at Dushan in South Guizhou Province. Carsologica Sinica **18:**234–244.

Liu SQ, Mayden RL, Zhang JB et al. 2012. Phylogenetic relationships of the Cobitoidea (Teleostei: Cypriniformes) inferred from mitochondrial and nuclear genes with analyses of gene evolution. Gene **508:**60–72. doi:10.1016/j.gene.2012.07.040.

Nguyen LT, Schmidt HA, von Haeseler A et al. 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol **32:**268–274. doi:10.1093/molbev/msu300.

Puillandre N, Lambert A, Brouillet S et al. 2012. ABGD, Automatic Barcode Gap Discovery for primary species delimitation. Mol Ecol **21:**1864–1877. doi:10.1111/j.1365-294X.2011.05239.x.

Roberts TR. 1989. The freshwater Fishes of Western Borneo (Kalimantan Barat, Indonesia). Memoirs of the California Academy of Science **14:**1–210.

Ronquist F, Teslenko M, van der Mark P et al. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol **61:**539–542. doi:10.1093/sysbio/sys029.

Vences M, Miralles A, Brouillet S et al. 2021. iTaxoTools 0.1: Kickstarting a specimen-based software toolkit for taxonomists. Megataxa **6:**77–92. doi:10.1101/2021.03.26.435825.

Wang DZ, Chen YY. 1989. Descriptions of three new species of Cyprinidae from Guizhou Province, China (Cypriniformes: Cyprinidae). Acta Academiae Medicinae Zunyi **12:**29–34. (in Chinese)

Xiao S, Lan JH. 2023. Classification atlas of freshwater fishes of Guangxi. Henan Science and Technology Press, Zhengzhou. (in Chinese)

Xiao WH, Zhang YP, Liu HZ. 2001. Molecular systematics of Xenocyprinae (Teleostei: Cyprinidae): taxonomy, biogeography, and coevolution of a special group restricted in East Asia. Mol Phylogenet Evol **18:**163–173. doi:10.1006/mpev.2000.0879.

Zeng ZX, Shao WH, Jin ZJ et al. 2022. *Hongshuia brevibarba*, a new species of labeonin fishes (Pisces: Cyprinidae) from the upper ChangJiang basin in Guizhou Province, South China. Ichthyol Explor Freshw **1184:**1–11. doi:10.23788/IEF-1184.

Zhang CG, Shao GZ, Wu HL et al. 2020. Species catalogue of China, vol. 2. Animals, vertebrates(V), fishes(i). Science Press, Beijing. (in Chinese)

Zhang E, Qiang X, Lan JH. 2008. Description of a new genus and two new species of labeonine fishes from South China (Teleostei: Cyprinidae). Zootaxa **1682:**33–44. doi:10.11646/zootaxa.1682.1.3.

Zhang QJ, Bei WC, Deng Y et al. 2022. Structural characteristics of Shuiyuandong underground river system in Lingyun County of Guangxi. Mineral Resources and Geology **36:**640–645+674.

Zhao YH, Gozlan RE, Zhang CG. 2011. Out of sight out of mind: current knowledge of Chinese cave fishes. J Fish Biol **79:**1545–1562. doi:10.1111/j.1095-8649.2011.03066.x.

Zheng LP, Yang JX, Chen XY et al. 2010. Phylogenetic relationships of the Chinese Labeoninae (Teleostei, Cypriniformes) derived from two nuclear and three mitochondrial genes. Zool Scr **39:**559–571. doi:10.1111/j.1463-6409.2010.00441.x.

Zheng LP, Chen XY, Yang JX. 2016. Molecular systematics of the Labeonini inhabiting the karst regions in southwest China (Teleostei, Cypriniformes). ZooKeys **612:**133–148. doi:10.3897/zookeys.612.9085.

Zhou QY, Chen PY. 1993. Lithofacies change and palaeogeographical evolution during Late Cenozoic in Guizhou and its vicinity. Geology of Guizhou **10:**201–207. (in Chinese)

Supplementary materials

Table S1. Selected best models for 6 pre-defined partitions used in IQ-tree and MrBayes (download)

Table S2. Original morphometric measurements of the type specimens of the two new species (download)

Table S3. PCA loadings of the first three principal components extracted from 23 morphometric data for species of Hongshuia (download)