



Bashimyzon cheni, a new genus and species of sucker loach (Teleostei, Gastromyzontidae) from South China

Xiong Gong^{1,2}, E Zhang¹

- 1 Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, China
- 2 University of Chinese Academy of Sciences, Beijing, China

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Corresponding author: E Zhang (zhange@ihb.ac.cn)

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Abstract

Bashimyzon, new genus, is here established for Erromyzon damingshanensis, and a new species of the genus is described from the You-Jiang of the Pearl River (=Zhu-Jiang in mandarin Chinese) basin in Guangxi Province, South China. This new genus has a small gill opening above the pectoral-fin base and short pectoral fins extending backwards short of pelvic-fin insertions, both characters combined to separate it from all currently-recognized gastromyzontid genera except Erromyzon and Protomyzon, but differs from the two genera in having a larger gap between the posterior edge of eye and the vertical through the pectoral-fin insertion and very small fleshy lobes posterior to the maxillary-barbel bases. It is further distinct from its most similar genus Erromyzon in having a relatively larger gill opening, fewer branched pectoral-fin rays folded against body, and more posteriorly placed pectoral fins with a shorter fin base. Bashimyzon cheni, new species, and B. damingshanensis, the single congeneric species, differ in number of lateral-line pored scales, body coloration, and cephalic contour, and also in substantial genetic divergence.

Key Words

Bashimyzon, new genus, new species, taxonomy, Zhu-Jiang basin

Introduction

Species of the family Gastromyzontidae (so-called sucker loaches) are small-sized bottom dwellers, mainly feeding on algae scraped off underwater rocks (Chen 1980a; Chen and Tang 2000; Chen and Zhang 2006). These species are adapted to dwell in rapid-running waters by evolving many morphological traits, such as a depressed head, an inferior mouth, and laterally-expanded pectoral and pelvic fins (Chen 1978; Chen and Zheng 1989; Kottelat 2004). This family is by far among the lesser-known taxonomic freshwater fish groups, and new species are described each year (Kottelat 2012; Zhang and Cao 2021). Numerous unidentified species are waiting for formal descriptions.

There are a total of 20 valid genera currently recognized in the Gastromyzontidae (Fricke et al. 2023). Kottelat (2004) proposed *Erromyzon* to accommodate

a Chinese species formerly misplaced in the genus Protomyzon Hora, 1932 (P. sinensis), and described a new species E. compactus from northeastern Vietnam. Subsequently, three new species were described from South China: E. yangi by Neely et al. (2007), E. kalotaenia by Yang et al. (2012) and E. damingshanensis by Xiu and Yang (2017). In Kottelat's (2012) inventory of the loaches (Teleostei, Cypriniformes, Cobitoidei) of the world, 17 valid genera were included in the Gastromyzontidae. Nevertheless, its generic classification remains far from satisfactory mainly due to a poor understanding of the phylogenetic relationships within the family. Following the erection of Erromyzon, two new genera were erected: Yaoshania by Yang et al. (2012) and Engkaria by Tan (2021). Labigastromyzon have been elevated to generic status (Chen et al. 2023). Erromyzon damingshanensis is reclassified in this study into a new genus of the Gastromyzontidae in China.

Currently, E. damingshanensis is found in a stream tributary to the Qingshui-He draining the northeastern slope of the Damingshan Mountain (Xiu and Yang 2017). This stream empties into the Hongshui-He of the middle Xi-Jiang basin. Our recent survey of freshwater fishes, conducted in the Wuming-He (a stream flowing into the You-Jiang) with its source in the southwestern slope of the Damingshan Mountain, yielded many specimens superficially most similar to E. damingshanensis but not conspecific with it or all other congeneric species, therefore representing an unrecognized species. Further morphological examination and comparisons demonstrated that these two species have remarkable differences in the length of the gap between the posterior edge of eye and the vertical through the pectoral-fin insertion, gill opening size, number of last branched pectoral-fin rays folded against the flank, and mouth-part structures with the rest of congeneric species, which are sufficient to place both in their own genus. The generic status of *Bashimyzon* gen. nov. is also justified by its substantial genetic divergence (p-distances:10-12%) with phylogenetically allied genera Erromyzon and Yaoshania. The present study aims to propose a new genus Bashimyzon, with E. damingshanensis Xiu & Yang, 2017 used as the type species, and then provide a formal description of the unidentified species, here named as Bashimyzon cheni gen. et sp. nov.

Materials and methods

Specimen sampling and preservation

All specimens were collected in our field surveys conducted during 2020–2023, in accordance with the Chinese Laboratory Animal Welfare and Ethics animal welfare laws (GB/T35892–2018). Specimens were caught using electrofishing and/or trap nets. After being anaesthetized, all caught individuals were killed by immersion in ethanol or formalin. Some of them were stored in 10% formalin preservative for morphological examination or permanent curation, and the others in 95% ethanol for DNA extraction. The specimens examined in this study are housed in the collection of Kunming Institute of Zoology (KIZ), Chinese Academy of Sciences, Kunming City, Yunnan Province, and the Institute of Hydrobiology (IHB), Chinese Academy of Sciences, Wuhan City, Hubei Province, P. R. China.

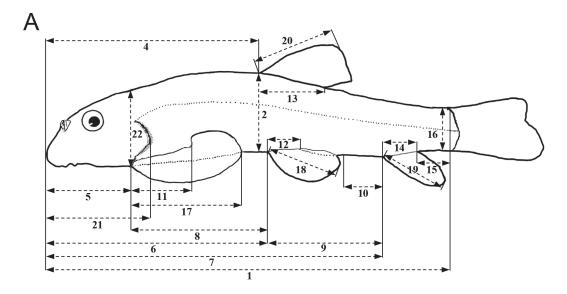
Morphological and geometric morphometric analysis

Measurements, as depicted in Fig. 1, were made point-topoint with digital calipers connected to a data recording computer and recorded to the nearest 0.1mm. Pre-dorsal, pre-pectoral, pre-pelvic and pre-anal lengths were taken from the snout tip to the dorsal-, pectoral-, pelvic- and anal-fin origin or insertion, respectively. Meristic counts were taken with a binocular Zeiss Stereo Discovery V6, following the methods utilized by Kottelat (1990); the last two branched rays of dorsal and anal fins were counted as one when borne by the same pterygiophore. All morphometric measurements and meristic counts were made on the left side of specimens whenever possible. Vertebral count was taken from X-ray photographs. The Weberian apparatus is considered as including four vertebrae. GraphPad Prism 8 (GraphPad Prism Inc.) was used for the basic statistical analysis on morphometric data. Abbreviations utilized here include: GL — gill opening length, G-P — distance from the lowest extremity of the gill opening to the pectoral-fin insertion, HD — head depth, PPL — pre-pectoral length, PBL — pectoral-fin base length, and SL — standard length.

Geometric morphometric (GM) analyses were utilized, targeted at cephalic contour and mouthpart structures to further distinguish B. cheni and B. damingshanensis. A total of 20 specimens were examined in GM analyses for each of these two species. Individual specimen was photographed for the head in ventral and lateral views using Capture 2.3 connected to the micro-imaging cameras. The software tpsUtil 1.83 (Rohlf 2015) was used to ordinate the digitalized images in the same file under the TPS format. The tpsDig2 software (Rohlf 2015) was used to record landmarks and semi-landmarks. The different sets and descriptions of landmarks and semi-landmarks (curve points) on the digitized image were illustrated in Fig. 2. The tps curve files were converted to landmarks data in tpsUtil 1.83 (Rohlf 2015). Subsequent data analyses were run using MorphoJ 1.07a software (Klingenberg 2011). A generalized Procrustes analysis (GPA) was conducted to scale landmarks of each specimen to a common body size, rotate each specimen to a common alignment and generate a consensus shape. Principal component analysis (PCA) was performed after checking for outliers and constructing a covariate matrix to better assess and visualize shape variation across all individuals.

DNA extraction, amplification and sequencing

Total genomic DNA was extracted from 95% ethanol-stored fin or muscle tissue samples using TIANamp Genomic DNA Kit (Tiangen Biotech Co., Ltd, Beijing, China) following manufactures' instructions. The mitochondrial cytochrome c oxidase subunit I (COI) gene was chosen for phylogenetic analysis. Two new pairs of specific primers were designed for amplification (COI F: ATCCTACCTGTGGCAATCAC / COI_R: AATAGG-GGRAATCAGTGGAC) and sequencing (COI F1: **GCATCWGTAGACCTRACYATYTT** COI R1: GCATARTATGCYACGACGTGRG) of the COI region in Bashimyzon and its close relatives. This gene was amplified by the polymerase chain reaction (PCR) in 25 µl reactions containing 12.5 µl Master mix Taq (Genesand Biotech Co. Ltd., Beijing, China), 1µl of each primer, 1µl template DNA and 9.5µl double distilled water (dd H₂O).



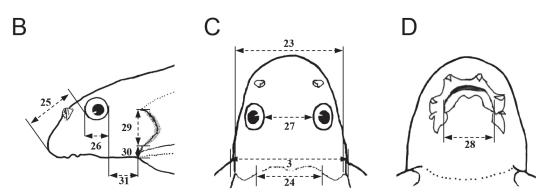


Figure 1. Measurements taken on species of *Bashimyzon* and its relatives. Lateral view of body (**A**), and lateral (**B**), dorsal (**C**) and ventral (**D**) view of head. 1, standard length; 2, body depth; 3, body width crossing pectoral-fin insertions; 4, pre-dorsal length; 5, pre-pectoral length; 6, pre-pelvic length; 7, pre-anal length; 8, distance from pectoral- to pelvic-fin insertion; 9, distance from pelvic-fin insertion to anal-fin origin; 10, distance from anus to anal-fin origin; 11, pectoral-fin base length; 12, pelvic-fin base length; 13, dorsal-fin base length; 14, anal-fin base length; 15, caudal-peduncle length; 16, caudal-peduncle depth; 17, pectoral-fin length; 18, pelvic-fin length; 19, anal-fin length; 20, dorsal-fin length; 21, head length; 22, head depth; 23, head width; 24, width between upper extremities of gill openings; 25, snout length; 26, eye diameter; 27, interorbital width; 28, mouth width; 29, gill opening length; 30, distance from lowest extremity of gill opening to pectoral-fin insertion; 31, distance between posterior edge of eye and vertical through pectoral-fin insertion.

The PCR conditions were given as follows: initial denaturation at 95 °C for 3min followed by 35 cycles of 94 °C for 25s, 54 °C for 25s, and elongation at 72 °C for 45s; and final extension at 72 °C for 5min. Amplified products were stored at 4 °C. The sequencing was done through Aokedingsheng Biotechnology Company (Wuhan, China). All sequences amplified in this study were submitted to GenBank.

Phylogenetic analyses

The amplified 158 gene sequences from six species, namely *E. sinensis* (26), *E. compactus* (two), *E. yangi* (32), *E. kalotaenia* (20), *B. damingshanensis* (43), and *B. cheni* (35), were utilized for molecular phylogenetic analysis along with other seven GenBank-retrieved

sequences of the same gene from three outgroups: Sinogastromyzon tonkinensis (KY352773), Vanmanenia pingchowensis (KP005457) and Yaoshania pachychilus (AP012132, KT031050, KX588239, KY352775 and NC030634). Detailed information on specimens used for molecular analysis in this study are given in Table 1. Raw sequences were edited using Segman in DNAstar (DNAStar Inc., Madison, WI, USA), aligned with Seaview v4.2.5 (Gouy et al. 2010), and checked by eye for some ambiguous alignments. Nucleotide sequences were initially aligned utilizing Clustal X v2.0 (Larkin et al. 2007) with default parameters, and then re-checked visually. The sequence data were translated into amino acids in MEGA v7.0 (Kumar et al. 2016) to confirm the absence of premature stop codons. Then DnaSP v6 (Rozas et al. 2017) was used for genetic diversity analyses and filter the haplotype.

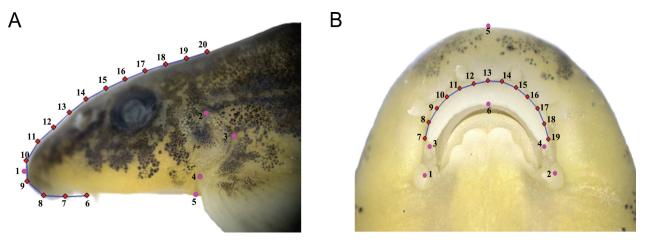


Figure 2. Landmarks (pink dot) and semi-landmarks (red diamond) used in geometric morphometrics. Photographs of *B. cheni*, IHB 202303064716, 42.7 mm SL. **A.** Lateral view of head; 1–5 landmark points (anterior-most tip of snout, topmost, hindmost and lowermost point of gill opening, and pectoral-fin insertion), and 6–20 semi-landmark points to which the curve from the maxillary-barbel root to the posteromedial tip of the supraoccipital was resampled by length; **B.** Ventral view of head; 1–6 landmark points (roots of left and right maxillary barbels, left and right lateral end of upper lip, anterior-most tip of snout, and median point of distal margin of upper lip), and 7–19 semi-landmark points to which the curve between the left and right end of the rostral groove was resampled by length.

Table 1. Detailed information of specimens used for molecular phylogenetic analyses in this study.

Species	N	Specimen voucher	Sampling localities	Haplotypes	GenBank no.
E. sinensis	21	IHB 202203177109, 20, 27-	Liuding-He, Gui-Jiang, tributary of Zhu-	Hap1-6	OR744909-29
		34, 36, 42–46, 51–53, 55–56	Jiang, Changle Village, Jinxiu County, China		
	5	IHB 202010049920-24	Lu-Jiang, Gui-Jiang, tributary of Zhu-Jiang,	Hap5, 7–9	OR744930-34
			Lujiang Village, Lingchuan County, China		
E. compactus	2	IHB 01-QN-2023, 02-QN-2023	Ba Che River, Ba Che city, Ba Che District, Quang Ninh Province, Vietnam	Hap10-11	OR744935–36
E. yangi	27	IHB 202203177157-59,	Meicun-He, Liu-Jiang, tributary of Zhu-	Hap12-15	OR744937-63
		62–85	Jiang, Meicun Village, Jinxiu County, China		
	5	IHB 202104053762-66	Shuijing-He, Liu-Jiang, tributary of Zhu-	Hap13, 15–16	OR744964-68
-			Jiang, Heping Village, Jinxiu County, China		
E. kalotaenia	18	IHB 202203177107–08,	Liuding-He, Gui-Jiang, tributary of Zhu-	Hap17–18	OR744969-86
		10–19, 21–26	Jiang, Changle Village, Jinxiu County, China		
	2	IHB 202203177160-61	Dishui-He, Gui-Jiang, tributary of Zhu-Jiang,	Hap17	OR744987–88
			Shibajia Village, Jinxiu County, China		
B. damingshanensis	3	IHB 202109136061-63	Qingshui-He, Hongshui-He, tributary of Zhu- Jiang, Shanglin County, China	Hap19	OR744989-91
	40	IHB 202109136064-103	Qingshui-He, Hongshui-He, tributary of	Hap19–20	OR744992-5031
			Zhu-Jiang, Naxue Village, Dafeng Town,		
			Shanglin County, China		
B. cheni	20	IHB 202109136104-23	Wuming-He, You-Jiang, tributary of Zhu-	Hap21-22	OR745032-51
			Jiang basin, Xinyang Village, Gulin Town,		
		UID 000000054575 55	Mashan County, China		00745050 00
	15	IHB 202303064575-89	Wuming-He, You-Jiang, tributary of Zhu-	Hap22	OR745052-66
			Jiang basin, Jiaobei Village, Liangjiang		
			Town, Wuming District, China		

The haplotype sequence matrix was used for subsequent phylogenetic analyses. MrBayes 3.2.2 (Ronquist et al. 2012) was utilized for Bayesian inference (BI) analysis. PartitionFinder v2.1.1 (Lanfear et al. 2017) was used to select the best partitioning strategy and the optimal nucleotide substitution model for the dataset using the Bayesian information criterion (BIC). Three codon partitions and their corresponding substitution model for COI gene sequences were proposed: 1st codon with SYM+I, 2nd with F81+I, and 3rd with GTR+G.

Two independent runs were carried out with four Monte Carlo Markov chains (three hot chains and one cold chain) for 20 million generations to calculate posterior probability. Trees were sampled for every 1000 generations. The initial 25% of sampled trees were discarded as burn-in. Convergence of the runs was assessed by the average standard deviation of split frequencies (< 0.01). Partitioned maximum likelihood (ML) analyses were conducted in RAxMLHPC v7.0 (Stamatakis 2006) with the same partitioning strategy as for

Bayesian inference. The more complex model (GTR + I + G) was used for all subsets, and 100 replicates ML inferences were performed with a complete random starting tree. Nodal support for the clades was estimated with 1000 bootstrap pseudoreplicates (Stamatakis et al. 2008). Phylogenetic tree was edited in FigTree v1.4.2 (Rambaut 2009). Additionally, the uncorrected genetic distances (p-distances) between lineages were calculated with MEGA v7.0 (Kumar et al. 2016).

Results

Bashimyzon gen. nov.

https://zoobank.org/1AA6CE02-CC93-4512-9EEC-F9298FF6A3C1Figs 3D, 5-7

Type species. *Erromyzon damingshanensis* Xiu & Yang, 2017: 893 (type locality: Qingshui-He, tributary to Hongshui-He of Zhu-Jiang basin, in Shanglin County, Guangxi Province)

Diagnosis. Bashimyzon is distinct from all currently identified gastromyzontid genera except Erromyzon and Protomyzon in the presence of a gill opening restricted above pectoral-fin base (vs. gill opening elongate, extending downwards to or beyond the pectoral-fin insertion to the ventral surface of head), and pectoral fins backwards extending away from (vs. close to or beyond) pelvic-fin insertions. This new genus is separated from the two genera in having a larger (vs. small) gap between the posterior edge of eye and the vertical through the pectoral-fin insertion, with the gap length being nearly equal to (vs. less than) eye diameter (Fig. 3; Table 2) and a very small fleshy lobe (vs. a relatively large fleshy lobe or enlarged papillae; see Kottelat 2004: Page 306, figs 4-6, and Yang et al. 2012: Page 176, fig. 3; Table 2) posterior to the maxillary-barbel base.

Other characters useful for distinguishing *Bashimyzon* and *Protomyzon* include: upper and lower lips continuous around the corners of mouth (vs. discontinuous or interrupted by an arched blade-like structure; see Kottelat 2004: Page 306, figs 4–6), lower lip smooth (vs. papillated), and no externally distinct opercle (vs. present) (Table 2).

Bashimyzon is morphologically most similar to Erromyzon, but further differs from it in having a relatively larger (vs. smaller) gill opening (length 46.5–60.7% of HD vs. 25.8–44.9, and the distance from its lower extremity to the pectoral-fin insertion 3.6–16.1% of HD vs. 27.2–43.5; see Figs 3, 4A, B); fewer last branched pectoral-fin rays folded against the flank (3–4 vs. 7–8); and more posteriorly located pectoral fins (pre-pectoral length 17.0–22.3% of SL vs. 12.5–15.8; see Fig. 4C), with a shorter (vs. longer) base (length 8.8–10.8% of SL vs. 13.9–17.4; see Fig. 4D).

Etymology. The generic name is derived from *Bashi* (岜是), the local name of the Damingshan Mountain in Zhuang nationality language, and *myzon*, a common suffix used in the Balitoridae. Gender masculine. The Chinese common generic name is here suggested as "岜是鳅属".

Bashimyzon cheni sp. nov.

https://zoobank.org/8E5656C2-1F1A-4085-962F-45D1594B1D58 Figs 5–7

Type material. *Holotype.* IHB 202109064273, 28.3 mm SL; Guangxi Province: Nanning City: Mashan County: Wuming-He, a stream tributary to You-Jiang of Zhu-Jiang basin, at Xinyang Village (23°35'05"N, 108°15'38"E, roughly 237m above sea level) of Gulin Town; collected by X. Gong, D. M. Guo and Y. Liu; 17 September 2021.

Paratypes. IHB 202109064274–78, 5 specimens, 28.3–39.9 mm SL; other data same as holotype. IHB 202303064712–36, 25 specimens, 35.0–42.7 mm SL; Guangxi Province: Nanning City: Wuming District: Wuming-He, a stream tributary to You-Jiang of Zhu-Jiang basin, at Jiaobei Village (23°31'27"N, 108°18'24"E, about 176 m above sea level) of Liangjiang Town; collected by X. Gong, D. M. Guo and P. Shan; 21 March 2023.

Diagnosis. Bashimyzon cheni is clearly distinguished from the single congeneric species B. damingshanensis in having 8–9 teardrop-shaped black blotches wider than interspaces, with the majority portion of each blotch located above the lateral line on the flank (vs. 12 irregular black bars narrower than interspaces, and located along the lateral line on the flank; see Figs 5, 6); fewer rows of blackish spots

Table 2. Comparison of diagnostic characters among Bashimyzon gen. nov. and other related genera.

	Protomyzon auct.	Erromyzon s.str.	Bashimyzon gen. nov.	
Gap between posterior edge of eye and vertical through pectoral-fin insertion	small	small	large	
Arched blade-like structure between upper and lower lip	present; upper and lower lip interrupted	absent; upper and lower lip continuous	absent; upper and lower lip continuous	
Lower lip	papillated (except for P. aphelocheilus)	smooth	smooth	
Externally opercle	present	absent	absent	
Gill opening	restricted to a small slit above pectoral-fin base (except for P. whiteheadi and P. griswoldi)	restricted to a small slit above pectoral-fin base	larger with its lower extremity stopping short of the pectoral-fin base	
Structure posterior to each maxillary barbel	enlarged papillae	a large fleshy lobe	a very small fleshy lobe	

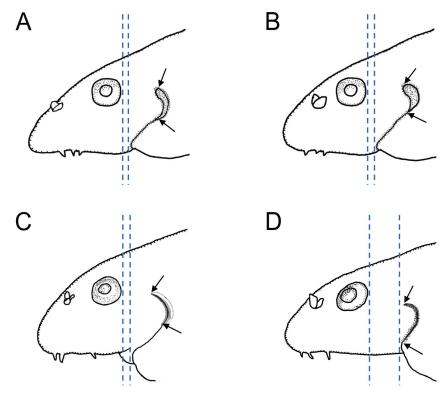


Figure 3. Lateral views of heads for: *Protomyzon aphelocheilus* (**A**), *Protomyzon borneensis* (**B**), *Erromyzon sinensis* (**C**), and *Bashimyzon damingshanensis* (**D**). Arrows indicate extremities of gill openings, and blue dotted lines pass vertically through the pectoral-fin insertion and the posterior margin of eye, respectively.

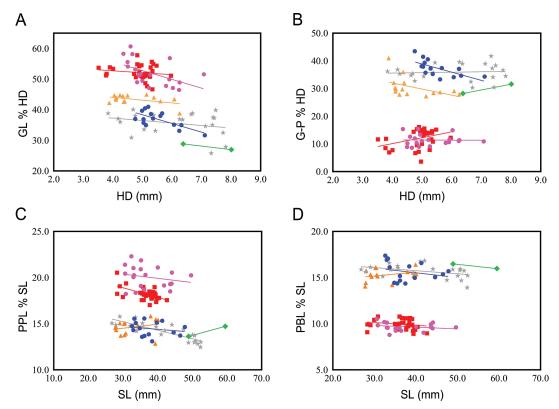


Figure 4. Comparisons (linear regression) of some morphometric measurements between sampled species of *Erromyzon [E. sinensis* (blue dot), *E. compactus* (green diamond), *E. yangi* (grey star), and *E. kalotaenia* (yellow triangle)] and *Bashimyzon [B. damingshanensis* (ponk dot) and *B. cheni* (red square)]. **A.** Between GL% of HD and HD; **B.** Between G-P% of HD and HD; **C.** Between PPL% of SL and SL; and **D.** Between PBL% of SL and SL. **GL**—gill opening length; **G-P**—distance from the lowest extremity of gill opening to the pectoral-fin insertion; **HD**—head depth; **PPL**—pre-pectoral length; **PBL**—pectoral-fin base length; **SL**—standard length.



Figure 5. Dorsal (top), lateral (middle) and ventral (bottom) views of body in *B. cheni*, IHB 202109064273, holotype, 28.3 mm SL; China: Guangxi Province: Nanning City: Mashan County: Wuming-He, a stream tributary to You-Jiang of Zhu-Jiang basin, at Xinyang Village of Gulin Town.

across dorsal-fin rays (3 vs. 4); fewer lateral-line pored scales (84–86 vs. 88–95); a gradual (vs. abrupt) upward dorsal profile of head in front of nostrils (Fig. 8A); a deeply (vs. slightly) curved rostral groove, or the greater (vs. less) distance from the median point of the distal margin of the upper lip to the anterior-most tip of the snout than half of the mouth width (Fig. 8B), a distinct (vs. indistinct) incision on both side of the fleshy pad of the lower lip (Figs 2B, 7).

Description. General body shape and appearance illustrated in Figs 5, 6 and lateral view of head in Fig. 7. Morphometric measurements and meristic counts provided in Table 3. Body moderately elongate, anteriorly nearly cylindrical, slightly deeper than wide or both equal, and posteriorly compressed laterally, with greatest depth at dorsal-fin origin, and greatest width at middle of pectoral-fin base. Caudal peduncle stout, deeper than long, with minimum caudal-peduncle depth closer to caudal-fin base. Dorsal profile of head gradually rising from

anterior-most tip of snout to posterior end of supraoccipital and predorsal body slightly convex or straight; dorsal profile of dorsal-fin base and post-dorsal body slightly concave. Ventral profile of head straight, then slightly convex from pectoral-fin insertion to anal-fin origin, and evenly rising towards caudal-fin base.

Head longer than deep, and shallower than wide. Snout broadly rounded in dorsal view and obtuse in lateral view, nearly equal to postorbital head. Eyes small, situated dorsolaterally in half of head, with broad and slightly flat interorbital space. Anterior and posterior nostrils close together, and short tubular flap on anterior nostril. Mouth small, inferior and arched in ventral view. Rostral fold modified into three rounded, fleshy lobes; median one wider than or equal to two lateral ones, separated from upper lip by distinct shallow groove. Lips fleshy and smooth; upper lip broad and curved, reflected on base of upper jaw; lower lip restricted to corners of mouth.

Table 3. Morphometric data for Bashimyzon cheni gen. et sp. nov.

	Holotype		Daratuna	s (n = 30)	
	поютуре	Min	Paratype Max	SD	
Morphometric measurements		IVIIII	IVIAX	Mean	30
Standard length (mm)	28.3	28.3	42.7	36.8	3.4
% Standard length	20.5	20.5	72.7	30.0	J. T
Pre-dorsal length	48.9	45.7	50.9	48.6	1.4
Pre-pectoral length	18.2	17.0	20.5	18.23	0.69
Pre-pelvic length	50.0	50.3	55.3	52.3	1.2
Pre-anal length	81.3	79.5	85.4	81.6	1.4
Distance between pectoral- and pelvic-fin insertion	33.2	32.0	46.5	34.7	2.7
Distance from pelvic-fin insertion to anal-fin origin	29.7	26.5	31.2	28.9	1.1
Distance from anus to anal-fin origin	8.5	7.3	11.4	9.8	1.0
Body depth	16.5	15.5	21.2	18.3	1.5
Body width	17.9	17.2	19.3	18.2	0.6
Caudal-peduncle length	9.7	8.4	11.7	10.2	0.8
Caudal-peduncle depth	11.4	9.9	12.2	10.9	0.6
lead length	21.3	19.4	23.2	21.3	0.7
lead depth	13.8	12.3	23.2 14.9	13.4	0.7
lead depth lead width	17.2	14.6	14.9	16.6	0.6
Snout length	9.5	9.4	17.9	10.7	0.7
6 Head length	9.5	9.4	11.9	10.7	0.5
	44.5	44.1	56.5	50.3	2.5
Snout length	44.5 25.7	21.6		24.7	
ye diameter	49.0		29.6		2.0
nterorbital width		36.9	49.3	42.8	3.0
Porsal-fin base length	48.8	47.6	62.6	54.4	3.8
Pectoral-fin base length	46.5	41.7	50.8	46.8	2.7
Pelvic-fin base length	23.2	20.3	26.5	24.1	1.6
mal-fin base length	19.5	22.7	31.4	28.2	2.3
Porsal-fin length	84.8	80.7	100.8	93.7	4.2
Pectoral-fin length	123.2	109.8	134.0	123.6	5.5
Pelvic-fin length	78.6	79.4	97.4	87.8	4.4
Anal-fin length	60.6	59.3	77.8	70.3	3.9
Distance between the posterior margin of eye and the	18.4	16.0	22.3	18.6	1.6
ertical through the pectoral-fin insertion					
6 Body width crossing pectoral-fin insertions	22.6	27.6	20.0	21.4	0.0
Mouth width	33.6	27.6	38.9	31.4	2.3
Vidth between upper extremities	88.7	79.9	92.8	86.4	3.0
f gill openings 6 Caudal-peduncle length					
	1175	100 E	1007	100 E	6.0
Caudal-peduncle depth	117.5	100.5	123.7	109.5	6.9
6 Head depth Distance from lowest extremity of	6.9	2.6	1.6.1	10.0	2.0
istance from lowest extremity of ill opening to pectoral-fin insertion	6.9	3.6	16.1	12.2	3.2
ill opening to pectoral-ill insertion ill opening length	53.9	46.6	57.8	52.1	2.4
6 Eye diameter	55.9	40.0	37.0	JZ.1	۷.4
-	135.5	109.9	160.3	133.5	11.3
hill opening length					
Distance from lowest extremity of gill opening to pectoral-fin insertion	17.4	9.5	43.2	31.4	9.0
Meristic counts					
Porsal-fin rays	iii, 7	iii, 7			
nal-fin rays	iii, 7 ii, 5				
		ii, 5 i 15			
Pectoral-fin rays	i, 15	i, 15			
Pelvic-fin rays	i, 7	i, 7–8			
Lateral line scales	85	84–86			
'ertebrae	29	29–30			

Upper and lower lips continuous around corner of mouth, with small notch on confluence between both anterior to corners of mouth. A small papilla present on this notch, but indistinct in some individuals. Postlabial groove

widely interrupted, or short and restricted only to corners of mouth, so leaving an anteriorly bilobed median fleshy pad on chin. A distinct incision also on both side of the fleshy pad. Upper and lower jaws bearing thick,





Figure 6. Lateral view of freshly captured individual of **A.** *B. damingshanensis*, IHB 202109064259, topotype, 30.8 mm SL; China: Guangxi Province: Shanglin County: Qingshui-He at Naxue Village of Dafeng Town; and **B.** *B. cheni*, IHB 202109064273, holotype, 28.3 mm SL; China: Guangxi Province: Mashan County: Wuming-He at Xinyang Village of Gulin Town.

flexible horny sheaths on cutting edges. Lower jaw largely exposed. Two pairs of rostral barbels; outer pair larger than inner pair. Maxillary-barbel pair rooted at corners of mouth, longer than rostral-barbel pair. A very small fleshy lobe posterior to maxillary barbel present on lower lip. Gill opening relatively large above pectoral-fin base, with its lower extremity extending away from the pectoral-fin insertion.

Scales minute, cycloid. Lateral line complete, with 84–86 pored scales, slightly curved upwards about half of pectoral-fin length, then downwards to posterior end of anal-fin base, extending along middle of caudal-peduncle to caudal-fin base. No scales on head, abdomen adjacent to ventral midline extending for three-fourths of distance between pectoral- to pelvic-fin insertions, and post-pelvic ventral region in front of anus. Numerous small tubercles densely distributed in suborbital portions of head and sides of snout; not obvious in other areas of body. Vertebrae 4+29–30.

Dorsal fin with three unbranched and seven branched rays; distal margin truncate or straight; origin anterior to pelvic-fin insertion, and closer to caudal-fin base than to snout tip. Pectoral fins with three unbranched and seven branched rays, enlarged and expanded laterally, longer

than head; inserted slightly in front of lowest extremity of gill opening; tip of adpressed fin extending away from pelvic-fin insertion; last three or four branched rays folded dorsally against flank, shorter than others. Gap between posterior margin of eye and vertical through pectoral-fin insertion nearly equal to eye diameter. Pelvic fins short and not fused, with one unbranched and seven or eight branched rays; inserted slightly closer to caudal-fin base than to snout tip; tip of adpressed fin reaching anus, but far away from anal-fin origin. Anal fin with two unbranched and five branched rays; distal margin truncate; origin closer to caudal-fin base than to pelvic-fin insertion; and tip of adpressed fin reaching caudal-fin base. Anus located midway from posterior end of pelvic-fin base to anal-fin origin or slightly behind. Caudal-fin emarginated, lower lobe slightly longer than upper lobe.

Coloration. In freshly-captured specimens, background of body yellowish or whitish yellow, with 8–9 vertical brown blotches of variable size on flank, oblong prodorsally and teardrop-shaped below dorsal-fin base and postdorsally (Fig. 6B). In formalin-stored individuals, body faded to lighter brown. Blotches on flank only 2/3 of body depth, wider than interspaces, and interrupted on dorsum. Dorsal surface of body covered

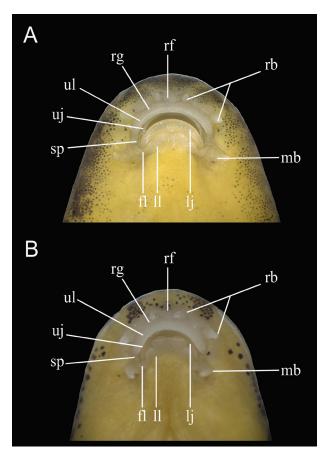


Figure 7. Ventral view of mouth in A. B. damingshanensis, IHB 202109064259, topotype, 30.8 mm SL; B. B. cheni, IHB 202109064273, holotype, 28.3 mm SL. fl— flesh lobe; lj— lower jaw; ll—lower lip; mb—maxillary barbel; rg—rostral groove; rf—rostral fold; rb—rostral barbels; sp—small papilla; uj—upper jaw; ul—upper lip.

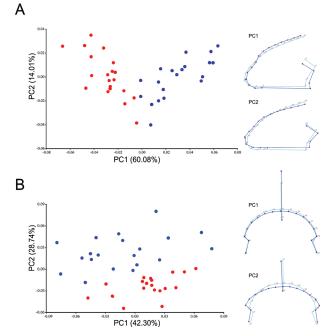


Figure 8. Scatterplots for the first two principal components (PC1 and PC2) of *B. cheni* (red dot) and *B. damingshanensis* (blue dot) principal component analyses for contours of the lateral head (**A**) and mouth-part structures (**B**).

with 7–8 dark elliptical patches along dorsal midline; predorsal patches usually linked to each other. Body pale yellowish-cream on ventral and ventrolateral surfaces, with some small blackish blotches located above pelvic-fin base. Pectoral-fin base lightly speckled with three to four irregular dark brown spots; pelvic fins and anal fin hyaline. Three rows of blackish spots on dorsal-fin rays, and two or three rows on caudal-fin rays; a relatively large black bar at caudal-fin base. Sides of head yellowish brown to black, with dark brown vermiculations visible.

Distribution and habitat. Bashimyzon cheni is so far known from the Wuming-He, a stream tributary to the You-Jiang discharging into the Xi-Jiang of the Zhu-Jiang basin, in Guangxi Province, China (Fig. 9). It was caught in shallow fast-flowing waters, at depths of 0.2–0.8 m, with a mixed bottom substrate of boulders, cobbles, and sands (Fig. 10). Coexisting species included Opsariichthys bidens, Osteochilus salsburyi, Schistura fasciolata, Traccatichthys pulcher, Mystus guttatus, Glyptothorax fokiensis, and Mastacembelus armatus.

Etymology. The specific epithet is named after Prof. Chen Yi-Yu, a Chinese Academician, in honor for his great contribution to Chinese freshwater fish research. In particular, he discovered and named the type species of *Erromyzon* and *Yaoshania*, which are two close relatives of the genus under description. The Chinese common specific name "陈氏岜是鳅" is here suggested.

Sequence variation and molecular phylogeny

A total of 158 COI gene sequences of 1167 bp (base pair) in length from all four species of *Erromyzon*, and two species of *Bashimyzon* were amplified in this study (Table 1). These sequences were used for molecular phylogenetic analysis together with three outgroups: *Sinogastromyzon tonkinensis* (one), *Vanmanenia pingchowensis* (one), and *Yaoshania pachychilus* (five). Twenty-seven haplotypes (22 and five, respectively for ingroups and outgroups) were detected from the sequences (Table 1). The haplotype sequences matrix consisted of 833 conserved sites, 334 variable sites, 259 parsimony informative sites and 75 singleton sites. The nucleotide frequencies were 25.2% (A), 29.4% (T), 25.8% (C), and 19.5% (G).

Given that BI and ML analyses produced overall identical topologies, only the BI tree with Bayesian posterior probabilities (PP) and bootstrap support (BS) value were presented in Fig. 11. From the tree topologies, samples of *B. cheni* were strongly supported (PP = 1.0 / BS = 100) to group into a lineage further forming a well-supported (PP = 1.0 / BS = 100) clade together with those of *B. damingshanensis. Bashimyzon* was weakly supported (PP = 0.57 / BS = 51) to stand as the sister group consisting of sampled species of *Erromyzon* (*E. sinensis*, *E. compactus*, *E. yangi* and *E. kalotaenia*). The two genera together were highly supported (PP = 1.0 / BS = 94) to be sister of *Yaoshania*.

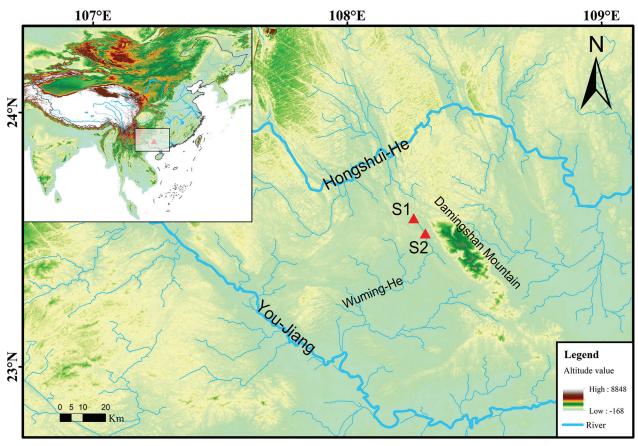


Figure 9. Map showing the two sampling sites of the type specimens of *B. cheni*. S1, Xinyang Village, Gulin Town, Mashan County, and S2, Jiaobei Village, Liangjiang Town, Wuming District, Nanning City, Guangxi Province, P. R. China.



Figure 10. Sampling locality of the holotype (IHB 202109064273) of *B. cheni* in the Wuming-He, tributary to the You-Jiang in Xinyang Village, Gulin Town, Mashan County, Nanning City, Guangxi Province, P. R. China; 17 September 2021, photographed by Yi Liu.

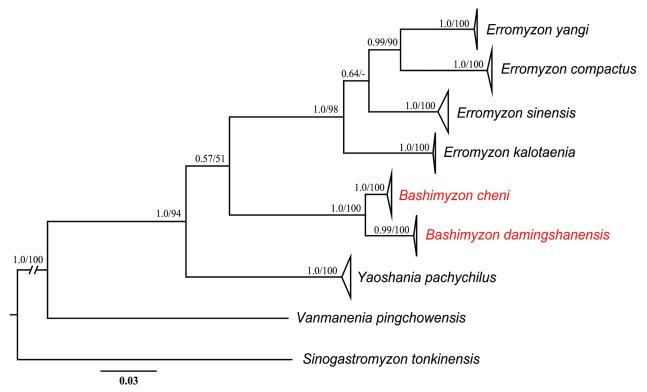


Figure 11. Bayesian Inference tree derived from the mitochondrial COI gene for seven analyzed species of three genera (*Erromyzon*, *Bashimyzon*, and *Yaoshania*). Bayesian posterior probabilities (> 0.5), and maximum likelihood bootstrap values (> 50%) are shown, respectively. Dash represents a node with bootstrap support lower than 50%.

The genetic distances (p-distances) within and between genera were provided in Table 4. Intraspecific genetic distances were 0.03–0.26%, 0.0–0.01%, and 0.19% for sampled species of *Erromyzon*, *Bashimyzon* and *Yaoshania*, respectively. Interspecific genetic distances were 6–8% for *Erromyzon*, 3% for *Bashimyzon* and NA for *Yaoshania* (not available owing to the fact that it is a monotypic genus). The intergeneric genetic distances here calculated were 10–12% (*Bashimyzon / Erromyzon*), 10% (*Bashimyzon / Yaoshania*), and 11–12% (*Yaoshania / Erromyzon*), respectively.

Discussion

It has been widely recognized that gill-opening size and pectoral-fin extension are of taxonomic importance at the generic level for the Gastromyzontidae (Pellegrin and Fang 1935; Chen 1980b; Kottelat 2004). On the basis of the two characters, the family is subdivided into four groups. The first group is composed of *Annamia* Hora, 1932, *Formosania* Oshima, 1919, *Glaniopsis* Boulenger, 1899, *Katibasia* Kottelat, 2004, *Liniparhomaloptera* Fang, 1935, *Parhomaloptera* Vaillant, 1902, *Plesiomyzon* Zheng & Chen, 1980 and *Vanmanenia* Hora, 1932. These eight genera possess a large gill opening extending downwards beyond the pectoral-fin insertion to the ventral surface of head. The second group, comprising *Paraprotomyzon* s. str. and *Yaoshania*, have an intermediate gill opening extending downwards to but not beyond the

Table 4. Genetic distances (uncorrected p-distance) of COI gene computed by MEGA v7.0 amongst 7 species of *Erromyzon*, *Bashimyzon* and *Yaoshania*.

Species	Within	1	2	3	4	5	6
	Group						
1. E. sinensis	0.0021						
2. E. compactus	0.0026	0.07					
3. E. yangi	0.0008	0.07	0.06				
4. E. kalotaenia	0.0003	0.07	0.08	0.07			
5. B. damingshanensis	0.0000	0.11	0.12	0.11	0.11		
6. B. cheni	0.0001	0.10	0.12	0.10	0.10	0.03	
7. Y. pachychilus	0.0019	0.11	0.12	0.12	0.11	0.10	0.10

pectoral-fin insertion to the ventral surface of head. The third group consists of *Beaufortia* Hora, 1932, *Engkaria* Tan, 2021, *Gastromyzon* Günther, 1874, *Hypergastromyzon* Roberts, 1989, *Labigastromyzon* Tang & Chen, 1996, *Neogastromyzon* Popta, 1905, *Pseudogastromyzon* Nichols, 1925, and *Sewellia* Hora, 1932, all of them having a gill opening restricted only to a very small slit above the pectoral-fin base, and pectoral fins extending backwards beyond or close to pelvic-fin insertions. The fourth group has a relatively small gill opening above the pectoral-fin base and pectoral fins extending backwards away from pelvic-fin insertions, two characters shared with other two genera (*Erromyzon* and *Protomyzon* auct.) and also with *Bashimyzon* herein described.

Only two species are referred to *Bashimyzon: B. daming-shanensis* (Xiu & Yang, 2017) and *B. cheni* here described. The former was previously placed in *Erromyzon*, a genus

erected to include a Chinese species formerly misidentified in Protomyzon and a new species of northeastern Vietnam (Kottelat 2004). Also misplaced in Protomyzon was the type species of Yaoshania in Chinese literature. From Kottelat's (2004) point of view, the species was unlikely to be the member of either Protomyzon or Erromyzon. It was later utilized as the type species to erect Yaoshania (Yang et al. 2012). Its generic status was confirmed in Shi et al.'s (2018) molecular phylogenetic analysis based on complete mitochondrial genomes for the superfamily Cobitoidea. Our study demonstrated that Y. pachychilus had a minimum genetic distance of 11% with E. kalotaenia and E. sinensis for the COI gene. And the maximum genetic distances of two species of Bashimyzon were 12% with E. compactus. The congeneric recognition of these species would make the maximum interspecific genetic distance (12%) of Erromyzon s.l. slightly greater than the minimum one between Yaoshania and Erromyzon (11%) (Table 4). Apparently, the generic status of *Bashimyzon* is justified, particularly given its monophyletic nature (see Fig. 11) and the current recognition of Erromyzon and Yaoshania, two closely allied monophyletic groups, as two distinct genera (Shi et al. 2018).

The erection of *Bashimyzon* as a new genus highlights the need to scrutinize the classification of currently identified species of *Paraprotomyzon* s.l., which was initially erected by Pellegrin and Fang (1935) to accommodate a new species P. multifasciatus. The original description of this species stated that it possesses a large gill opening extending downwards close to, but not beyond, the pectoral-fin insertion. However, all other currently identified species of Paraprotomyzon, namely P. bamaensis Tang, 1997, P. lungkowensis Xie, Yang & Gong, 1984, P. niulanjiangensis Lu, Lu & Mao, 2005, and P. yunnanensis Li, Mao, Lu, Sun & Lu, 1998, have a gill opening only restricted to a small slit above the pectoral-fin base (see Xie et al. 1984; Lu et al. 2005). Moreover, marked differences in mouth-part structures are also found between P. multifasciatus and these congeneric species (Tang and Chen 2000). It is more likely that these four species represent their own genus, and *Paraprotomyzon* is actually a monotypic genus.

The constituent species of *Protomyzon* also needs strict scrutiny. The type species of this genus (*P. whiteheadi* Vaillant, 1893) shares with *P. griswoldi* (Hora & Jayaram, 1952) the presence of a relatively large gill opening extending downwards close to but not beyond the pectoral-fin insertion (see Vaillant 1893; Hora and Jayaram 1950, 1952). However, a gill opening restricted to a small slit above the pectoral-fin base is exhibited by the other two congeners: *P. aphelocheilus* Inger & Chin, 1962 and *P. borneensis* Hora & Jayaram, 1952. Given the generic-level taxonomic importance of gill-opening size revealed by Yang et al. (2012) and also in this study, *P. whiteheadi* and *P. griswoldi* likely belong to a distinct genus, and the taxonomy of *Protomyzon* needs further study in the future.

The genetic distance between *B. damingshanensis* and *B. cheni* (3%) is greater than 2%, a threshold used

for vertebrates' species delimitation (Avise and Walker 1999; Hebert et al. 2003), thus supporting the recognition of B. cheni as a valid species. The discovery of this new species has also conservation implications. As pointed by Tan (2006), the torrent loaches of Borneo usually have a restricted distribution; a single species is often restricted only to a single stream. Their restricted distribution makes these rheophilic fishes susceptible to anthropogenic interferences. This is the case for B. cheni. In terms of our observation in the fieldworks, the new species has a large number of individuals currently confined only to a stream tributary to the Wuming-He in Guangxi Province of southern China. The stream drains through two scenic spots—Damingshan Mountain and Jinlun Cave. The rapid development of local tourism industry is predicted to have adverse impacts on species diversity of freshwater fishes and aquatic ecosystem. Due to the construction of many weirs across the Wuming-He for agricultural irrigation, habitats of this new species are fragmented. Its survival is thus put under threat from touristic industry and agriculture. In addition, the new species exhibited an extremely low genetic diversity. Only two haplotypes were detected from a total of 35 COI gene sequences of 1167 bp in length; the haplotype diversity (Hd) was 0.0571 and nucleotide diversity (Pi) was 0.00010 within the Wuming-He population (unpublished data). The presence of this new species, with fragmented populations and low genetic diversity, in a single stream can reduce its evolutionary potential and increase the risk of extinction in the wild. Therefore, conservation efforts should be taken to preserve B. cheni.

So far, *B. cheni* occurs in a stream with its source in the southwestern slope of the Damingshan Mountain, and so does *B. damingshanensis* in its northeastern slope. The allopatric distribution of this sister pair suggests that the uplift of the Damingshan Mountain has been acting as the driving force of speciation between both. In such a small scale, mountain build-up plays a crucial role in speciation of these two species. Given that the species of *Erromyzon* also have a montane distribution, it is speculated that the species diversity of the genus in China is underestimated. More in-depth taxonomic research is urgently required for widespread species such as *E. sinensis*.

Comparative material

Erromyzon sinensis: IHB 75-IV-2572–2574, 75-IV-2579, 75-IV-2581, 5 specimens, syntypes, 33.9–53.3 mm SL, Xiuren Town, Lipu County, Guilin City, Guangxi Province, China; IHB 75-V-2804, 1 specimen, syntype, 30.3 mm SL, Lipu County, Guilin City, Guangxi Province, China; IHB 202203064279–88, 10 specimens, 32.63–38.40 mm SL, Liuding-He, Gui-Jiang, tributary of Zhu-Jiang, Changle Village, Jinxiu County, Laibin City, Guangxi Province, China; IHB 202010051119–23, 5 specimens, 39.74–47.85 mm SL, Lu-Jiang, Gui-Jiang, tributary of Zhu-Jiang, Lujiang Village, Lingchuan County, Guilin City, Guangxi Province, China.

Erromyzon compactus: ZRC 49636, holotype, 30.8 mm SL, Ba Che River, a river that flows independently into the sea, Ba Che City, Ba Che District, Quang Ninh Province, Vietnam (photograph examined); IHB 01-QN-2023, 02-QN-2023, 2 specimens, 48.95–59.52 mm SL, other data same as the holotype.

Erromyzon yangi: KIZ 200304423, holotype, 42.8 mm SL, Meicun-He, Liu-Jiang, tributary of Zhu-Jiang, Meicun Village, Jinxiu County, Laibin City, Guangxi Province, China; KIZ 200304422, 2003004424, 2 specimens, paratypes, 36.7–37.1 mm SL, other data same as holotype; IHB 2017056658–61, 2017056678–83, 10 specimens, 26.83–47.47 mm SL, other data same as holotype; IHB 202104052295–99, 202203064413–18, 11 specimens, 41.02–52.45 mm SL, Shuijing-He, Liu-Jiang, tributary of Zhu-Jiang, Heping Village, Jinxiu Town, Jinxiu County, Laibin City, Guangxi Province, China.

Erromyzon kalotaenia: KIZ 200304310, holotype, 41.3 mm SL, Liuding-He, Gui-Jiang, tributary of Zhu-Jiang, Changle Village, Jinxiu County, Laibin City, Guangxi Province, China; KIZ 200304313–14, 200304317, 3 specimens, paratypes, 46.9–47.9 mm SL, other data same as holotype; KIZ 200304049–52, 200304071, 5 specimens, paratypes, 32.4–45.8 mm SL, Dishui-He, Gui-Jiang, tributary of Zhu-Jiang, Shibajia Village, Jinxiu County, Laibin City, Guangxi Province, China; IHB 202203064289–4303, 15 specimens, 27.78–40.13 mm SL, other data same as holotype.

Bashimyzon damingshanensis: KIZ 2014091301, holotype, 48.2 mm SL, Qingshui-He, Hongshui-He, tributary of Zhu-Jiang, Naxue Village, Dafeng Town, Shanglin County, Nanning City, Guangxi Province, China; KIZ2014091302, 2014091304–08, 5 specimens, paratypes, 43.8–47.3 mm SL, same data as the holotype (photograph examined); IHB 202109064253–4272, 20 specimens, 30.57–49.65 mm SL, other data same as the holotype.

Ethics approval and consent to participate

All procedures described in this paper were in accordance with Chinese laws and were licensed by the Ministry of Ecology and Environment of the People's Republic of China.

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Xiong Gong conceived the study and conducted the data analysis. Xiong Gong led the writing and E Zhang revised the manuscript. All authors contributed to the writing of the paper.

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