

Letter to the editor

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A new species of the gudgeon genus *Huigobio* Fang, 1938 (Cypriniformes: Cyprinidae) from the Yangtze River Basin, southern China

During revision of the genus *Microphysogobio* Mori, 1934, we discovered a new species of *Huigobio* from the Yangtze River Basin in China. The new species, named *Huigobio heterocheilus* sp. nov., can be distinguished from its congeners by a combination of the following characters: medial pad on lower lip not visible; ventral region scaleless before pelvic-fin insertion; lateral-line scales 39–40; caudal peduncle depth 49.2%–59.1% of length; barbel length 6.6%–10.7% of head length; black blotch between anterior margin of eye and upper lip. Using the K2P model and cytochrome *b* (cyt *b*) sequences, interspecific genetic distances between the new species and *Huigobio exilicauda* Jiang & Zhang, 2013 and *Huigobio chenhsienensis* Fang, 1938 were 13.2% and 15.1%, respectively. The new species is known from the upper reaches of the Xiangjiang River in the middle Yangtze River Basin. A diagnostic key for species of *Huigobio* Fang, 1938 is provided.

Huigobio is a small benthic gudgeon genus, which was established in 1938 based on *Huigobio chenhsienensis* (Fang, 1938). At present, the genus contains only two valid species, i.e., *H. chenhsienensis* and *H. exilicauda*. The genus is mainly distributed in the coastal river basins of Zhejiang Province, tributaries of Poyang Lake in the middle Yangtze River Basin in Jiangxi Province, and northern tributaries of the Pearl River Basin in Guangdong Province and the Guangxi Zhuang Autonomous Region (Fang, 1938; Huang & Wu, 2010; Jiang & Zhang, 2013; Luo et al., 1977; Yue, 1998; Zhang et al., 1996).

The validity of this genus has been discussed in previous studies. Bănărescu & Nalbant (1966) placed *H. chenhsienensis* in the genus *Microphysogobio* due to its similarity to *Microphysogobio chinssuensis* in general shape and regarded *Huigobio* as a synonym of *Microphysogobio*, though without any further explanation. Several recent studies

have also suggested that *Huigobio* is invalid based on molecular phylogeny, with *H. chenhsienensis* nested within *Microphysogobio* (Huang et al., 2016, 2018; Li et al., 2018).

However, other studies support *Huigobio* as a valid genus based on morphological differences with *Microphysogobio* (i.e., unique papillae pattern on lower lip) (Jiang & Zhang, 2013; Luo et al., 1977; Yue, 1998) and on molecular evidence (Jiang & Zhang, 2013). The latest Chinese freshwater fish catalogs (Zhang & Zhao, 2016; Zhang et al., 2020) also recognize *Huigobio* as a valid genus. In the present study, after examining and comparing the two known species of *Huigobio* (*H. exilicauda* and *H. chenhsienensis*) with *Microphysogobio* species, we agree that they are unique, especially in regard to lip papillae. Thus, we treated *Huigobio* as a valid genus in this study.

A new species, morphologically similar to *H. exilicauda*, was discovered through morphological comparison and molecular phylogenetic analyses. The discovery of this species should improve our understanding of the diversity and distribution of this genus.

As some of the specimens were bought from a local market in Guanyang County, locality data were not precise. The latitude and longitude coordinates were inferred by the authors based on information provided by a local fisherman. Other comparative materials were collected by hand net or bought from local markets. Detailed information on the specimens is listed in the Comparative Materials (Appendix I). Specimens used for morphological study were fixed in 10% formalin solution for three days, followed by 70% ethanol for long-term preservation. Examined materials, including the holotype, were deposited at the National Zoological Museum, Institute of Zoology, Chinese Academy of Sciences, Beijing, China

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(ASIZB, institutional abbreviation follows [Leviton et al. 1985](#)) and the Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, China (IHB).

All measurements were taken point-to-point with digital calipers to 0.01 mm on the left side of the specimens. Counts were also made on the left side of specimens. Pictures of the fish lip papillae were taken using a Nikon D600 camera with a 60 mm fixed focus lens on a light background with a ruler for scale. Lip papillae images were analyzed with ImageJ software. Once the scale was settled, the distance between two points was measured with a straight line ([Lunghi et al., 2019](#)). Counts and measurements followed [Sun et al. \(2021\)](#).

Molecular studies were based on the *cyt b* sequence. DNA was extracted from the pectoral fin on the right side of the fish. *cyt b* was amplified using the primers *cytbF1* (5'-TGACT TGAAGAACCACCGTTGTA-3') and *cytbR1* (5'-CGATCTTCG GATTACAAGACCGATG-3') following [Huang et al. \(2016\)](#). Sequencing reactions were performed according to the operating instructions of BigDye Terminator v3.1 (BDT), with 1 μ L of primer (3.2 pmol/ μ L), 1 μ L of template DNA, 2 μ L of BigDye[®] Terminator v3.1, and 6 μ L of double distilled water (dd H₂O) for a total reaction volume of 10 μ L. The thermocycling conditions were: initial denaturation for 2 min at 96 °C, denaturation for 10 s at 96 °C, annealing for 10 s at 50 °C, and extension for 1 min at 60 °C. After 30 cycles, a final extension was performed at 60 °C for 3 min and the polymerase chain reaction (PCR) products were preserved at 4 °C. Sequencing was carried out by Beijing TsingKe Biotech Co., Ltd. (China).

The sequencing results were assembled using SeqMan II, with other sequences acquired from the NCBI database. The code of each individual and GenBank accession Nos. are given in Supplementary Table S1. Nineteen sequences from *Huigobio* species, four sequences from *Microphysogobio* species, and four sequences from *Platysmacheilus* species were added. *Pseudogobio guilinensis* and *Squalidus argentatus* were used as outgroups. Nucleotide sequence alignment was verified using MEGA v6.0., and ModelFinder ([Kalyaanamoorthy et al., 2017](#)) was used to select the best-fit model using Bayesian information criterion (BIC). Bayesian inference (BI) phylogenies were inferred using MrBayes v3.2.6 ([Ronquist et al., 2012](#)) under a HKY+F+G4 model (two parallel runs, 1 000 000 generations), in which the initial 25% of sampled data were discarded as burn-in. Maximum-likelihood (ML) phylogenetic analysis was performed using RaxML-HPC2.

Taxonomy

Huigobio heterocheilus sp. nov.

[Figure 1A–D](#); [Table 1](#).

Holotype: ASIZB 218733, 57.3 mm standard length (SL); China: Guangxi Zhuang Autonomous Region: Guanyang County: stream of the Guanjiang River, a tributary of the Xiangjiang River, Yangtze River Basin; N25°33'50", E111°13'41"; collected by Zhi-Xian Sun; 26 November 2020.

Paratypes: ASIZB 218729–32, ASIZB 218734–43, 14 specimens, 52.4–60.6 mm SL; same data as holotype; ASIZB 218728, one specimen, 52.7 mm SL; China: Hunan Province:

Yanling County: Caoping Village: upper drainage of the Mishui River, a tributary of the Xiangjiang River, Yangtze River Basin; N26°28'13", E113°48'0"; September 2018.

Diagnosis: *Huigobio heterocheilus* sp. nov. can be distinguished from its congeners by a combination of the following characteristics: medial pad not visible; midventral region before pelvic-fin insertion scaleless; lateral-line scales 39–40; caudal peduncle depth 49.2%–59.1% of length; barbel length 6.6%–10.7% of head length; black blotch between anterior margin of eye and upper lip.

Description: Body elongated, lower surface of head and breast flattened, abdomen rounded, caudal peduncle short, slightly compressed. Dorsal body profile rising smoothly from nostrils to back of head, then rising gradually to dorsal-fin origin, sloping substantially along dorsal-fin base, then sloping gradually to caudal-fin base. Maximum body depth at dorsal-fin origin, body depth 17.5%–20.1% of standard length. Head short, head length longer than body depth (104.8%–122.2% of body depth); snout bluntly pointed, with shallow concavity on top of snout before nostrils; eyes large, 24.6%–31.7% of head length, placed in dorsal half of head; nostrils positioned closely, in front of eyes; interorbital region flattened, width smaller than eye diameter (67.9%–92.3% of eye diameter). Dorsal-fin length shorter than pectoral-fin length (63.6%–82.7% of pectoral-fin length). Anus positioned at anterior, one-third distance from pelvic-fin insertion to anal-fin origin. Caudal peduncle slender, length 13.6%–16.2% of standard length, depth 7.7%–8.6% of standard length.

Mouth horseshoe shaped and inferior, with pair of short barbels at extremity of mouth, barbel length shorter than half eye diameter (17.9%–39.3% of eye diameter); upper and lower jaws with horny sheaths on cutting margins, upper jaw cutting-edge width greater than half mouth width (59.0%–71.2% of mouth width), lower jaw exposed obviously. Lips thick, well developed, with pearl-liked papillae; central portion of anterior papillae arranged in one or two rows (papillae on second row small, hidden under papillae on first row) and usually larger than all other papillae on upper lip, lateral portions of anterior papillae in several rows; medial pad on lower lip not visible (medial pad fragmented into several small papillae, equal size to inside papillae on lateral lobes) ([Figure 1E](#)); lateral lobes on lower lip covered with around 150 well-developed confluent papillae, laterally connected with upper lip anterior papillae at corners of mouth.

Body covered with moderately small cycloid scales. Lateral line complete, almost straight at center, slightly bent under dorsal origin. Lateral-line scales 39 (5 specimens), 40 (10); scales above lateral line 3.5 (12), 4 (1), 4.5 (2); scales below lateral line 2 (15); predorsal scales 9 (2), 10 (10), 11 (3); circumpeduncular scales 12 (15). Midventral region of body scaleless before pelvic-fin insertion ([Figure 1B, H](#)).

Dorsal fin with three unbranched and seven (15 specimens) branched rays; upper margin slightly concave or straight, origin nearer to snout than caudal-fin base. Pectoral fin with one unbranched and 12 (15) branched rays; tip of adpressed fin extending beyond vertical through dorsal-fin origin but not approaching pelvic-fin origin. Pelvic fin with one unbranched and seven (15) branched rays, inserted below fourth branched dorsal-fin ray; tip of adpressed fin reaching over midway to

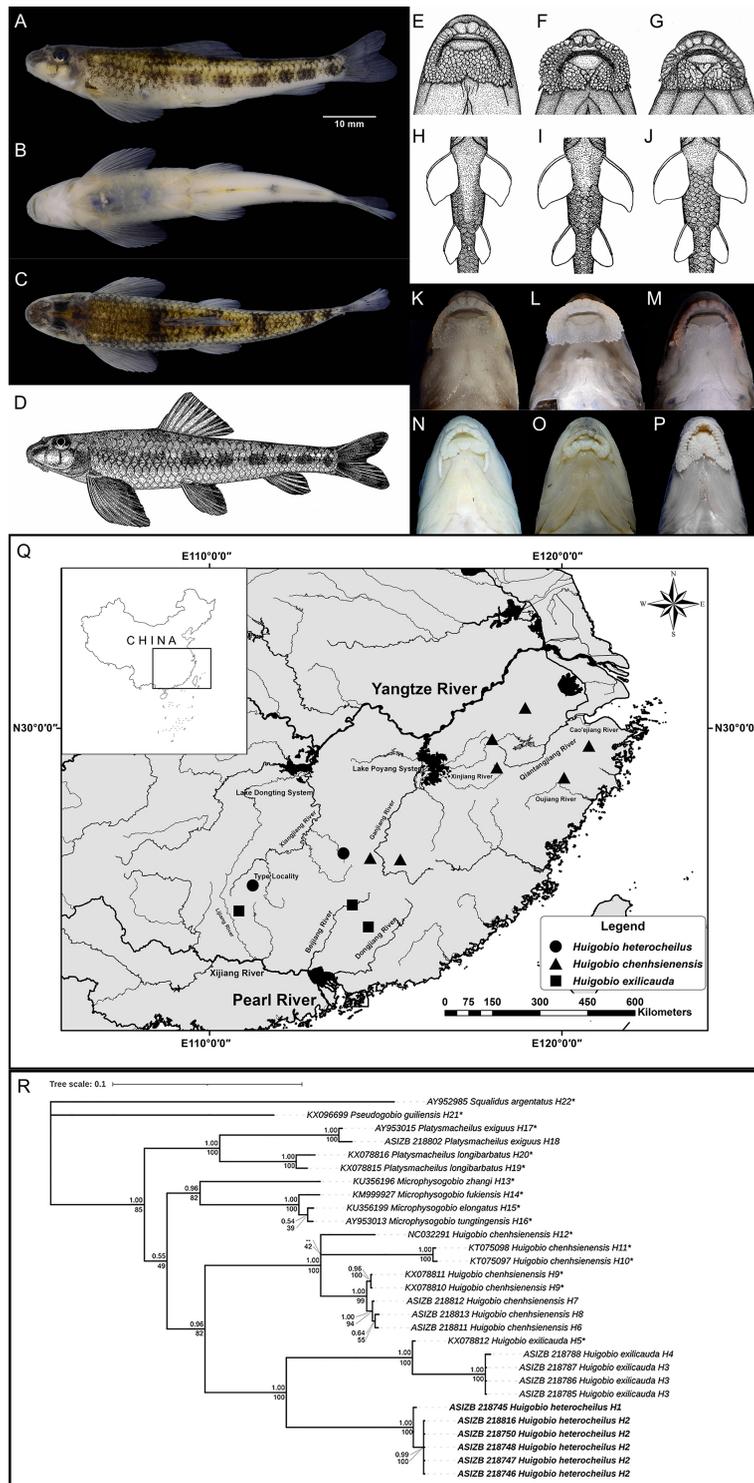


Figure 1 Type photos, morphological comparison, distribution, and phylogenetic position of *Huigobio heterocheilus* sp. nov.

A–C: Picture shows lateral view (A), ventral view (B), and dorsal view (C) of holotype (ASIZB 218733). D: Original drawing. E–G: Original drawing of lip papillae system of *Huigobio* species: *Huigobio heterocheilus* sp. nov. (E), *H. exilicauda* (F), *H. chenhsienensis* (G). H–J: Original drawing of midventral region of *Huigobio* species: *Huigobio heterocheilus* sp. nov. (H), *H. exilicauda* (I), *H. chenhsienensis* (J). K–P: Lip papillae system: *Huigobio heterocheilus* sp. nov. (K, holotype, ASIZB 218733), *H. exilicauda* (L, ASIZB 218787), *H. chenhsienensis* (M, ASIZB 218774, Sun et al., 2021), *Microphysogobio chinssuensis* (N, ASIZB 168301, Sun et al., 2021), *Microphysogobio hsinglungshanensis* (O, ASIZB 215100 Sun et al., 2021), *Platysmacheilus exiguus* (P, ASIZB 220879). Q: Distribution of *Huigobio heterocheilus* sp. nov. and its congeners. R: Molecular phylogenetic tree of *Huigobio heterocheilus* sp. nov. (bold) and its congeners based on *cyt b* sequence reconstructed by Bayesian inference (values above branch) and maximum-likelihood (values below branch). Photos by Zhi-Xian Sun.

Table 1 Morphometric measurements of *Huigobio heterocheilus* sp. nov. and its congeners

| Characters | <i>H. heterocheilus</i> sp. nov. (n=15) | | | | <i>H. exilicauda</i> (n=15) | | | <i>H. chenhsienensis</i> (n=9) | | |
|---------------------------------|---|------------------------|------|-----|-----------------------------|------|-----|--------------------------------|------|-----|
| | Holotype | Holotype and Paratypes | | | Range | Mean | SD | Range | Mean | SD |
| | | Range | Mean | SD | | | | | | |
| Dorsal-fin rays | 7 | 7 | 7 | – | 7 | 7 | – | 7 | 7 | – |
| Anal-fin rays | 5 | 5 | 5 | – | 5 | 5 | – | 5 | 5 | – |
| Pectoral-fin rays | 12 | 12 | 12 | – | 11–12 | 11 | – | 11–12 | 11 | – |
| Pelvic-fin rays | 7 | 7 | 7 | – | 7 | 7 | – | 7 | 7 | – |
| Lateral line scales | 39 | 39–40 | 40 | – | 37–39 | 38 | – | 37–39 | 38 | – |
| Scales above lateral line | 3.5 | 3.5–4.5 | 4 | – | 3.5 | 3.5 | – | 3.5–4 | 3.5 | – |
| Scales below lateral line | 2 | 2 | 2 | – | 2 | 2 | – | 2 | 2 | – |
| Pre-dorsal scales | 10 | 9–11 | 10 | – | 9–10 | 9 | – | 9–10 | 10 | – |
| Circumpeduncular scales | 12 | 12 | 12 | – | 12 | 12 | – | 12 | 12 | – |
| Standard length (SL, mm) | 57.3 | 52.4–60.6 | 56.9 | – | 41.3–52.3 | 46.2 | – | 44.6–60.8 | 53.3 | – |
| In percentage of SL (%) | | | | | | | | | | |
| Body depth | 18.6 | 17.5–20.1 | 18.7 | 0.7 | 16.4–19.1 | 17.8 | 0.7 | 18.3–20.9 | 19.4 | 0.9 |
| Head length | 21.0 | 20.3–22.7 | 21.4 | 0.6 | 21.4–23.9 | 22.9 | 0.6 | 20.1–23.1 | 21.5 | 0.9 |
| Dorsal-fin length | 18.9 | 18.2–21.0 | 19.5 | 0.8 | 19.9–23.9 | 21.4 | 1.0 | 17.6–21.9 | 19.5 | 1.3 |
| Dorsal-fin base length | 14.2 | 12.0–14.4 | 13.7 | 0.7 | 13.9–16.4 | 15.0 | 0.6 | 13.4–15.3 | 14.5 | 0.6 |
| Pectoral-fin length | 24.9 | 24.9–28.7 | 26.3 | 1.1 | 23.8–28.3 | 26.4 | 1.2 | 23.0–25.5 | 24.1 | 0.8 |
| Pectoral-fin base length | 5.6 | 5.2–6.4 | 5.8 | 0.3 | 5.4–6.6 | 5.8 | 0.4 | 5.2–6.2 | 5.7 | 0.3 |
| Pelvic-fin length | 19.1 | 19.0–22.1 | 20.2 | 0.9 | 19.2–23.1 | 21.5 | 1.1 | 17.7–18.8 | 18.2 | 0.4 |
| Pelvic-fin base length | 3.8 | 3.7–4.8 | 4.1 | 0.3 | 4.4–5.0 | 4.7 | 0.2 | 4.0–5.7 | 4.8 | 0.5 |
| Anal-fin length | 16.4 | 15.0–17.7 | 16.4 | 0.6 | 15.5–18.4 | 16.9 | 1.0 | 13.7–16.0 | 15.1 | 0.7 |
| Anal-fin base length | 6.1 | 6.1–7.1 | 6.8 | 0.3 | 5.4–6.5 | 6.1 | 0.4 | 5.8–6.9 | 6.4 | 0.5 |
| Caudal peduncle length | 16.1 | 13.6–16.2 | 15.2 | 0.8 | 14.3–16.9 | 15.6 | 0.9 | 14.2–17.0 | 16.1 | 0.9 |
| Caudal peduncle depth | 8.0 | 7.7–8.6 | 8.1 | 0.2 | 7.5–8.7 | 8.2 | 0.3 | 9.5–10.4 | 10.0 | 0.3 |
| Head length (HL, mm) | 12.1 | 10.8–13.5 | 12.2 | – | 9.4–12.1 | 10.6 | – | 9.0–13.0 | 11.5 | – |
| In percentage of HL (%) | | | | | | | | | | |
| Head depth | 62.7 | 60.4–68.7 | 63.8 | 2.4 | 60.4–72.9 | 64.9 | 2.8 | 61.2–73.4 | 67.3 | 3.8 |
| Head width | 68.5 | 66.1–74.6 | 69.5 | 2.5 | 66.2–76.7 | 71.9 | 3.1 | 71.7–84.0 | 76.4 | 4.1 |
| Eye diameter | 25.6 | 24.6–31.7 | 28.0 | 2.3 | 28.6–33.8 | 31.7 | 2.0 | 25.7–34.5 | 29.7 | 3.0 |
| Interorbital width | 23.2 | 21.0–25.6 | 23.5 | 1.4 | 20.1–25.5 | 23.0 | 1.7 | 24.9–31.3 | 28.1 | 2.3 |
| Snout length | 47.2 | 42.2–48.8 | 46.0 | 1.9 | 38.9–50.2 | 43.0 | 2.8 | 42.0–48.1 | 44.4 | 2.0 |
| Anterior papillae length | 45.4 | 40.4–49.7 | 45.7 | 2.5 | 38.4–58.1 | 46.1 | 5.5 | 39.1–51.2 | 44.5 | 4.3 |
| Anterior papillae width | 46.9 | 41.3–57.7 | 48.2 | 4.4 | 47.3–68.5 | 56.6 | 6.3 | 46.7–56.3 | 50.7 | 3.3 |
| Central anterior papillae width | 17.3 | 12.6–17.5 | 15.4 | 1.5 | 11.5–18.9 | 15.5 | 1.8 | 14.7–20.7 | 17.4 | 1.9 |
| Upper jaw cutting edge width | 23.7 | 19.2–28.9 | 24.4 | 2.6 | 22.8–31.7 | 25.6 | 2.8 | 25.0–30.7 | 27.8 | 1.8 |
| Medial Pad width | – | – | – | – | 8.4–14.9 | 11.6 | 1.8 | 10.3–13.9 | 12.5 | 1.4 |
| Mouth depth | 27.4 | 21.6–28.7 | 25.9 | 1.9 | 21.3–31.9 | 25.1 | 3.6 | 23.7–31.6 | 27.1 | 2.6 |
| Mouth width | 34.9 | 32.0–39.7 | 36.5 | 2.8 | 32.3–47.3 | 36.8 | 4.5 | 32.4–47.0 | 38.1 | 4.0 |
| Barbel length | 9.2 | 6.6–10.7 | 8.7 | 1.5 | 13.7–22.0 | 16.5 | 2.3 | 10.6–18.3 | 13.9 | 2.9 |

–: Not available.

anal-fin origin. Anal fin with three unbranched and five (15) branched rays; origin almost equidistant between pelvic-fin insertion and caudal-fin base. Caudal fin emarginated, with nine branched rays on upper lobes and eight branched rays on lower lobes, lobes pointed.

Coloration of fresh specimen: Dorsal side of head and body deep yellowish brown, midlateral side light yellowish brown, ventral side grayish white. Dorsal side of body with four distinct black crossbars (first and second at dorsal-fin base origin and ending, respectively, third at vertical position above anal-fin base origin, fourth on caudal peduncle). Interorbital

region with black crossbar. Operculum and suborbital region with three distinct black blotches (first between anterior margin of eye and upper lip, second on suborbital plate, third on cheek and lower opercle, [Figure 1D](#)). Two horizontally aligned black dashes above and below each lateral-line scale; six or seven black blotches positioned along lateral line (first blotch at vertical position below dorsal-fin base origin); scales above lateral line black margined. One black blotch above pectoral-fin base. All fins pale and translucent; caudal-fin membrane with one row of tiny black spots.

Coloration in preservative: Dorsal side of head and body

yellowish brown, midlateral side light yellowish brown, ventral side grayish white. Interorbital region with black crossbar. Dorsal side of body with four distinct black crossbars, same position as living specimen (Figure 1C). Operculum and suborbital region with three distinct black blotches, same position as living specimen. Two horizontally aligned black dashes above and below each lateral-line scale. Scales above lateral line black margined. All fins pale and caudle-fin membrane with black spots, same as living specimen (Figure 1A).

Distribution: The new species is known from the upper reaches of the Xiangjiang River, a main tributary of the Yangtze River, located in the Guangxi Zhuang Autonomous Region and Hunan Province, China (Figure 1Q).

Etymology: The specific name *heterocheilus*, from the Greek *hetero* (meaning different) and *cheilus* (meaning lips), refers to the different lip papillae system of this species, with the medial pad on the lower lip not visible. We propose the common name “异唇胡鲃” in Chinese.

Genetic comparisons: A total of 22 haplotypes from 29 individuals for the *cyt b* gene were included in analysis. The molecular phylogenetic results based on the mitochondrial *cyt b* sequence showed that *Huigobio heterocheilus sp. nov.* is closely related to *H. exilicauda*, with these two species forming a sister clade to the *H. chenhsienensis* clade. Based on the K2P model, the interspecific genetic distances between *Huigobio heterocheilus sp. nov.* and the two congeners (*H. exilicauda* and *H. chenhsienensis*) were 13.2% and 15.1%, respectively, for *cyt b* (Supplementary Table S2). The intraspecific genetic distances within *H. exilicauda* and *H. chenhsienensis* were 1.5% and 4.5%, much lower than the interspecific genetic distances between *Huigobio heterocheilus sp. nov.*, *H. exilicauda*, and *H. chenhsienensis*. Thus, the genetic evidence strongly supported *Huigobio heterocheilus sp. nov.* as a distinct species. The results also showed that *Huigobio* is a monophyletic lineage and sister to *Microphysogobio*. The BI and ML phylogenetic trees showed the same tree topology with relatively strong support (Figure 1R).

Remarks: *Huigobio* is similar to *Microphysogobio* and *Platysmacheilus*, with all possessing sharp horny sheaths on the cutting edges of their upper and lower jaws (Jiang & Zhang, 2013). However, the three genera differ in lip papillae pattern and width of the upper jaw cutting edge. The lateral lobes on the lower lip were posteriorly in contact with each other behind the medial pad but not confluent in *Huigobio* (Figure 1F, G). In addition, the cutting-edge width on the upper jaw of *Huigobio* is greater than half the mouth width (63.8%–73.8% of mouth width in *H. exilicauda*, Figure 1L; 65.2%–77.6% of mouth width in *H. chenhsienensis*, Figure 1M). However, the lip papillae system of the new species is quite unique. The new species does not exhibit the typical characteristics of *Microphysogobio*, i.e., lateral lobes posteriorly disconnected from each other behind medial pad (Figure 1N, O) (Sun et al., 2021). The cutting-edge width on the upper jaw of the new species is also greater than the half mouth width (59.0%–71.2% of mouth width, Figure 1K), rather than the narrow cutting edge in the genus *Platysmacheilus* (Figure 1P).

Based on the two valid species in the genus, the new species can be distinguished from *H. chenhsienensis* by having greater ventral scaleless region (midventral region before pelvic-fin insertion scaleless (Figure 1H) vs. scaleless before pectoral-fin insertion (Figure 1J)), slender caudal peduncle (caudal peduncle depth 49.2%–59.1% of length vs. caudal peduncle depth 56.3%–68.9% of length), and different lip papillae pattern (medial pad not visible vs. medial pad visible).

Huigobio heterocheilus sp. nov. is most closely related to *H. exilicauda* based on morphological and molecular results. Both species have a slender caudal peduncle compared to *H. chenhsienensis* (depth 49.2%–59.1% of length in *Huigobio heterocheilus sp. nov.*, depth 45.9%–58.3% of length in *H. exilicauda*, depth 56.3%–68.9% of length in *H. chenhsienensis*). However, *Huigobio heterocheilus sp. nov.* can be distinguished from *H. exilicauda* based on the following morphological differences: lip papillae medial pad not visible (vs. medial pad visible), lateral-line scales 39–40 (vs. 37–39), ventral scaleless region before pelvic-fin insertion (vs. scaleless before half distance from pectoral-fin insertion to pelvic-fin insertion, Figure 1I), barbels 6.6%–10.7% of head length (vs. 13.7%–22.0% of head length), and operculum black blotch between anterior margin of eye and upper lip present (vs. absent).

We found that the squamation pattern on the midventral region of *Huigobio* is an important diagnostic key for specimen comparison. Luo et al. (1977) described the midventral region of *H. chenhsienensis* as scaleless before the pelvic-fin insertion. Studies on *Microphysogobio* and *Huigobio* have followed this description (Jiang et al., 2012; Yue, 1998). However, according to the original description and hand drawings, the scaleless region of *H. chenhsienensis* is only restricted before the pectoral-fin insertion (thorax before pectoral fins naked, Figure 1J) (Fang, 1938). We collected several specimens of *H. chenhsienensis* from its type locality, Chen-hsien, Chekiang Province (now Shengzhou City, Zhejiang Province, located at the upper reaches of the Cao'ejiang River Basin) and found the characteristics to be consistent with the original description.

Huigobio heterocheilus sp. nov. is distributed in the upper drainage of the Xiangjiang River, a tributary that flows into the Lake Dongting system of the middle Yangtze River Basin (Figure 1Q). The distribution of *Huigobio heterocheilus sp. nov.* does not overlap with the other *Huigobio* species. In addition, the distribution patterns of the *Huigobio* species seem to be well explained by barriers. The Luoxiao Mountains are the main barrier between the Xiangjiang and Ganjiang rivers (Liu et al., 2020; Wang, 1998), and represent the main boundary between the Lake Dongting and Lake Poyang systems. *Huigobio heterocheilus sp. nov.* is distributed on the west side of the Luoxiao Mountains, while the *H. chenhsienensis* clade is distributed on the east side. The Nanling Mountains are the main barrier between the Yangtze and Pearl river basins, and a key boundary of ichthyofauna in eastern Asia (Huang et al., 2020; Li, 1981; Mori, 1936). *Huigobio exilicauda* is distributed south of the Nanling Mountains, while *Huigobio heterocheilus sp. nov.* and *H. chenhsienensis* are distributed north of the mountains.

Interestingly, the phylogenetic relationships within *Huigobio* indicated that the new species is more closely related to *H. exilicauda*, which is distributed south of the Nanling Mountains, rather than to *H. chenhsienensis*. This suggests that some of the northern tributaries of the Pearl River may have a close relationship to the Yangtze River Basin. Huang et al. (2017) stated that the artificial Lingqu Canal, which was built in the third century BC, may have formed a direct connection between the Yangtze and Pearl rivers, thus allowing the distribution of *Microphysogobio zhangii* in both. This may explain recent dispersal events (less than 2 500 years ago), but not the longer-term speciation of *Huigobio*. In fact, some of the northern tributaries of the Pearl River were previously Yangtze River tributaries. Notably, the upper reaches of the Lijiang River System, such as the Darongjiang, Xiaorongjiang, Liudonghe, and Chuanjiang rivers, originally belonged to the Xiangjiang River System, and were captured by the Lijiang River (Ru, 2011). Based on our current knowledge regarding the distribution of this genus, the main distribution area suggests that *Huigobio* is a “Yangtze River genus”. River capture or mountain building events in the Nanling Mountains may have caused species dispersal. This may explain why some Yangtze endemic fish species appear in the Pearl River.

A key to known species of *Huigobio*

- 1a)** Medial pad on lower lip visible **2**
1b) Medial pad on lower lip not visible **3**
2a) Midventral region of body scaleless before pectoral-fin origin, caudal peduncle robust, depth 56.3%–68.9% of length, interorbital width 24.9%–31.3% of head length (coastal rivers in Zhejiang Province, Poyang Lake rivers, and lower Yangtze River Basin) *Huigobio chenhsienensis* Fang, 1938
2b) Midventral region of body scales before half distance from pectoral-fin insertion to pelvic-fin insertion, caudal peduncle slender, depth 48.9%–59.7% of length, interorbital width 20.1%–25.5% of head length (northern tributaries of Pearl River Basin) *Huigobio exilicauda* Jiang & Zhang, 2013
3) Lateral-line scales 39–40, midventral region of body scaleless before pelvic-fin insertion. (upper reaches of Xiangjiang River, Yangtze River Basin)
..... *Huigobio heterocheilus* sp. nov.

NOMENCLATURE ACTS REGISTRATION

The electronic version of this article in portable document format will represent a published work according to the International Commission on Zoological Nomenclature (ICZN), and hence the new names contained in the electronic version are effectively published under that Code from the electronic edition alone (see Articles 8.5–8.6 of the Code). This published work and the nomenclature acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information can be viewed through any standard web browser by appending the LSID to the prefix <http://zoobank.org/>.

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SCIENTIFIC FIELD SURVEY PERMISSION INFORMATION

The collection of fish used in this study complied with the Wildlife Protection Act of China.

SUPPLEMENTARY DATA

Supplementary data to this article can be found online.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

Z.X.S. collected the fish. Z.X.S. and Y.H.Z. designed the study. Z.X.S. and X.J.L. examined the specimens. Z.X.S. and X.J.L. performed morphological comparisons. Z.X.S. conducted molecular analysis. Z.X.S. and Y.H.Z. wrote the draft. W.Q.T. and Y.H.Z. revised the draft. All authors read and approved the final version of the manuscript.

Zhi-Xian Sun^{1,#}, Xue-Jian Li^{2,#}, Wen-Qiao Tang^{2,*},
Ya-Hui Zhao^{1,*}

¹ Key Laboratory of Zoological Systematics and Evolution, Institute of Zoology, Chinese Academy of Sciences, Beijing, 100101, China

² Shanghai Universities Key Laboratory of Marine Animal Taxonomy and Evolution, Shanghai Ocean University, Shanghai, 201306, China

[#]Authors contributed equally to this work

*Corresponding authors, E-mail: wqtang@shou.edu.cn;
zhaoyh@ioz.ac.cn

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Appendix I

Comparative materials

Huigobio chenhsienensis

ASIZB 218774-82, nine specimens, 44.6–60.8 mm SL, China: Zhejiang Province: Shengzhou City (type locality): Cangyan Village: Xiaowuxi River, stream of the Chengtanjiang River, Cao'ejiang River Basin; N29°30'19", E120°45'58"; collected by Zhi-Xian Sun; 13 December 2020; IHB 2016105388, one specimen, 47.6 mm SL; China: Jiangxi Province: Ganzhou City: Xingguo County: tributary of the Ganjiang River, Yangtze River Basin; IHB 201703010621, one specimen, 55.6 mm SL; China: Jiangxi Province: Ji'an City: Suichuan County: tributary of the Ganjiang River, Yangtze River Basin.

Huigobio exilicauda

ASIZB 218783-95, ASIZB 218799-800, 15 specimens, 41.3–52.3 mm SL; Guangdong Province: Shaoguan City: Shixing County: stream of the Zhenjiang River, tributary of the Beiji River, Pearl River Basin; N25°1'13", E114°3'11"; collected by Zhi-Xian Sun; 23 December 2020; ASIZB 196530, one specimen, 54.9 mm SL; China: Guangxi Zhuang Autonomous Region: market in Gongcheng Yao Autonomous County, Pearl River Basin; 29 March 2011.

Microphysogobio chinssuensis

ASIZB 168301, one specimen, 44.0 mm SL; China: Henan Province: Wulongkou County: Qinhe River, Yellow River Basin; 4 September 2007 (for photograph).

Microphysogobio hsinglungshanensis

ASIZB 215100, one specimen, 56.1 mm SL; China: Hebei Province: Xinglong County: Lijaying Township: Zhaojiadian Village: Liuhe River, Luanhe River Basin; N40°37'59", E117°46'20"; collected by Zhi-Xian Sun and Rui Zhang; 9 June 2020 (for photograph).

Platysmacheilus exiguus

ASIZB 220879, one specimen; China: Guangxi Zhuang Autonomous Region: Guilin City: Pingle County: Lijiang River, tributary of the Xijiang River, Pearl River Basin; collected by Zhi-Xian Sun; 8 July 2019 (for photograph).