

Letter to the editor

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A new species of the gudgeon genus *Microphysogobio* Mori, 1934 (Cypriniformes: Cyprinidae) from Zhejiang Province, China

DEAR EDITOR,

A new gobionine species, *Microphysogobio oujiangensis* sp. nov., was collected from the Oujiang River Basin during field work in Lishui City, Zhejiang Province in 2021. The new species can be well distinguished from its congeners by the combination of the following characteristics: mouth shallow arc-shaped, width of cutting edge on upper jaw equal to half mouth width, central portion of anterior papillae arranged in one row with 6–10 well-developed papillae of equal size, medial pad on lower lip bisected into two squircle-shaped pads, and grooved; midventral region completely scaled and thorax scaleless, lateral line scales 36–38, pre-dorsal scales 8–10; barbel length 26.7%–31.4% of head length; eye diameter 30.9%–36.9% of head length, interorbital width 21.3%–27.6% of head length; posterior chamber of air-bladder weak, length smaller than half eye diameter. Bayesian inference and maximum-likelihood phylogenetic analyses based on the *cyt b* gene sequence and species delineation also supported the specimens as a distinct species, sister to *M. brevirostris* (Günther, 1868).

East Asia has the most diverse gobionine fish in the world, and *Microphysogobio* is one of the most species-rich genera in the subfamily Gobioninae. There are 30 valid species in this genus, 23 of which are distributed in China (Sun et al., 2021). Among the Chinese species, nine are found in independent coastal rivers in southeast China (e.g., Zhejiang, Fujian, Guangdong, and Taiwan provinces), including *M. brevirostris*, *M. fukiensis* (Nichols, 1926), *M. bicolor* (Nichols, 1930), *M. tafangensis* (Wang, 1835), *M. alticorpus* (Bănărescu & Nalbant, 1968), *M. microstomus* Yue, 1995, *M. xianyouensis* Huang, Chen & Shao, 2016, *M. zhangji* Huang, Zhao, Chen & Shao, 2017, and *M. luhensis* Huang, Chen, Zhao & Shao,

2018 (Huang et al., 2016, 2018). Thus, southeast China is a hotspot of species diversity of the genus *Microphysogobio*.

Microphysogobio species usually inhabit the upper and middle streams of a river system. Unlike gobionine genera from lower reaches, such as *Hemibarbus* and *Saurogobio*, *Microphysogobio* species are diverse, with well-developed lip papillae and a small-sized air-bladder, indicating strong adaptability to benthic life (Yue, 1998). The horny sheaths of the cutting margins on their upper and lower jaws enable them to scrape algae from pebbles and stones in slow-flowing shallow waters (Sun et al., 2021). Given their microhabitat characteristics and benthic habitat preferences, *Microphysogobio* species may be easily separated by common barriers, such as mountains and lower reaches of streams. Thus, the coastal rivers in southeast China provide ideal habitats and speciation environments for *Microphysogobio*.

In 2021, during field collection in Zhejiang Province, southeast China, we discovered a population of *Microphysogobio* that was morphologically and genetically distinct from all known congeners, which we describe herein as a new species within the genus.

Specimens were collected by hand nets, fish traps, or from local markets. Detailed information on the compared specimens is provided in the Comparative Materials (Supplementary Materials). Specimens used for morphological study were fixed in 10% formalin solution for three days, followed by 70% ethanol for long-term preservation at the Institute of Zoology, Chinese Academy of Sciences, Beijing (ASIZB). Specimens used for molecular studies were preserved in 95% ethanol. Other examined materials were deposited at ASIZB, Biodiversity Research Museum, Biodiversity Research Center, Academia Sinica, Taipei

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Received: 02 February 2022; Accepted: 30 March 2022; Online: 31 March 2022

Foundation items: This work was supported by the National Science & Technology Fundamental Resources Investigation Program of China (2019FY101800), Project of Biodiversity Survey and Conservation in Lishui, Zhejiang Province, and Sino BON-Inland Water Fish Diversity Observation Network

(ASIZP), and Taiwan Ocean University, Keelung (NTOUP).

Morphological comparisons and measurements followed Sun et al. (2021) and Zhao & Sun (2021). The meristic values in parentheses after counts indicate frequency and asterisks indicate holotype count. Molecular study was 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 in Huang et al. (2016). Sequencing results were assembled using SeqMan II, and other sequences were acquired from the NCBI database. Individual codes, locality information, haplotype details, and GenBank accession numbers are given in Supplementary Table S1. In total, 37 *cyt b* sequences from *Microphysogobio* species were used in this study, with *Pseudogobio guilinesis* used as an outgroup. Nucleotide sequence alignment was verified using MEGA v6.0 (Tamura et al., 2013) with ClustalW. ModelFinder (Kalyanamoorthy et al., 2017) was used to select the best-fit model using Bayesian information criterion (BIC). The BI phylogenies were inferred using MrBayes v3.2.6 (Ronquist et al., 2012) under the GTR+F+G4 model (two parallel runs, 1 000 000 generations), with the initial 25% of sampled data discarded as burn-in. ML phylogenetic analysis was performed using MEGA 6.0 under the TN93+G model (1 000 bootstrap replications). In addition, two independent methods, i.e., assemble species by automatic partitioning (ASAP) and Poisson tree process (PTP), which rely on different operational criteria, were applied to infer molecular species delineation for *Microphysogobio* (Kapli et al., 2017; Puillandre et al., 2021). Aligned sequences were uploaded to the ASAP online server using the Jukes-Cantor (JC69) model (<https://bioinfo.mnhn.fr/abi/public/asap/>) and the rooted phylogenetic trees (BI and ML), without an outgroup, were uploaded to the PTP online server (<http://species.h-its.org/ptp/>). The evolutionary divergence of sequence pairs between and within groups was estimated using the Kimura 2-parameter model (Kimura, 1980).

Taxonomy

Microphysogobio oujiangensis Sun & Zhao, sp. nov.

Figure 1A–D; Supplementary Figure S1

Holotype: ASIZB 220814, 64.8 mm SL; from confluence of the Panxi and Haoxi rivers (Oujiang River Basin) in Yazhai Village (ca. N28°36'24.11", E120°3'39.91"), Jinyun County, Lishui City, Zhejiang Province, China; 19 April 2021; Zhi-Xian Sun, Rui Zhang, Bin-Qing Zhu, Qiu-Ju Chen, and Rui Xi col.

Paratype: ASIZB 220815–34, 20 specimens, 56.4–70.1 mm SL; same data as holotype.

Diagnosis: The new species can be distinguished from its congeners by a combination of the following characteristics: mouth shallow arc-shaped and inferior, cutting edge width of upper jaw equal to half mouth width, central portion of anterior papillae arranged in one row with 6–10 well-developed and basically equally sized papillae, medial pad on lower lip usually bisected into two round square pads, and grooved; midventral region before pectoral-fin insertion scaleless, lateral line scales 36–38 (modally 37), pre-dorsal scales 8–10 (modally 10); barbel length 26.7%–31.4% of head length; eye diameter 30.9%–36.9% of head length, interorbital width 21.3%–27.6% of head length; posterior chamber of air-bladder

weak, rice shaped, length smaller than half eye diameter.

Description: Body elongated, thoracic region dorsiventrally flattened, abdomen rounded, caudal peduncle short, slightly compressed laterally. Dorsal body profile rising from nostrils to dorsal-fin origin, then gradually sloping to caudal-fin base. Maximum body depth at dorsal-fin origin, body depth 19.8%–24.3% of standard length (Supplementary Table S2). Head short, length almost equal to body depth; snout blunt, with apparent concavity on top of snout before nostrils; eye diameter 30.9%–36.9% of head length, positioned at dorsal half of head; interorbital region flattened, width smaller than eye diameter (62.5%–80.8% of eye diameter). Anus positioned at anterior one-third of distance from pelvic-fin insertion to anal-fin origin.

Mouth shallow arc-shaped and inferior, with one pair of maxillary barbels rooted at extremity of upper lip, barbel length slightly shorter than eye diameter (81.3%–90.4% of eye diameter); upper and lower jaws with thin horny sheaths on cutting margins, cutting edge width of upper jaw equal to half mouth width (50.2% of mouth width, on average). Lips thick, well developed, with pearl-like papillae; central portion of anterior papillae arranged in one row with 6–10 well-developed and approximately equal-sized papillae; lateral portions of anterior papillae in several rows; medial pad on lower lip usually bisected into two squircle-shaped pads, and grooved; lateral lobes covered with 30–40 well-developed papillae, posteriorly disconnected from each other behind medial pad and laterally connected with upper lip anterior papillae around mouth corner (Figure 1E; Supplementary Figure S1A).

Body covered with moderately small cycloid scales. Lateral line complete, almost straight in center, slightly bent down under dorsal origin. Lateral line scales 36 (3), 37* (12), 38 (6); scales above lateral line 3.5* (9), 4 (2), 4.5 (10); scales below lateral line 2* (21); pre-dorsal scales 8 (1), 9 (12), 10* (8); circumpeduncular scales 12* (21). Midventral region completely scaled and thorax scaleless (Figure 1B).

Dorsal fin with three unbranched and seven* (21) branched rays; distal margin slightly concave, origin nearer snout than caudal-fin base. Pectoral fin with one unbranched and 10 (5) or 11* (16) branched rays; adpressed pectoral fin tip extending beyond vertical direction of the dorsal-fin origin but not reaching pelvic-fin origin. Pelvic fin with one unbranched and seven* (21) branched rays, inserted below fourth or fifth branched dorsal-fin ray; adpressed pelvic fin more than half distance between pelvic-fin origin and anal-fin origin. Anal fin with three unbranched and six* (21) branched rays; origin almost equidistant between pelvic-fin insertion and caudal-fin base. Caudal fin forked, with one* unbranched principal ray and nine* branched principal rays on upper lobes and eight* branched principal rays and one* unbranched principal ray on lower lobes (21), lobes pointed.

Gill rakers rudimentary. Pharyngeal teeth “5–5” (in one row). Air-bladder small, anterior chamber enveloped in thick fibrous capsule; posterior chamber weak, rice shaped, length smaller than half eye diameter.

Coloration in life: Dorsal side of head and body pinkish-red, mid-lateral side pinkish-red, and ventral side grayish white.

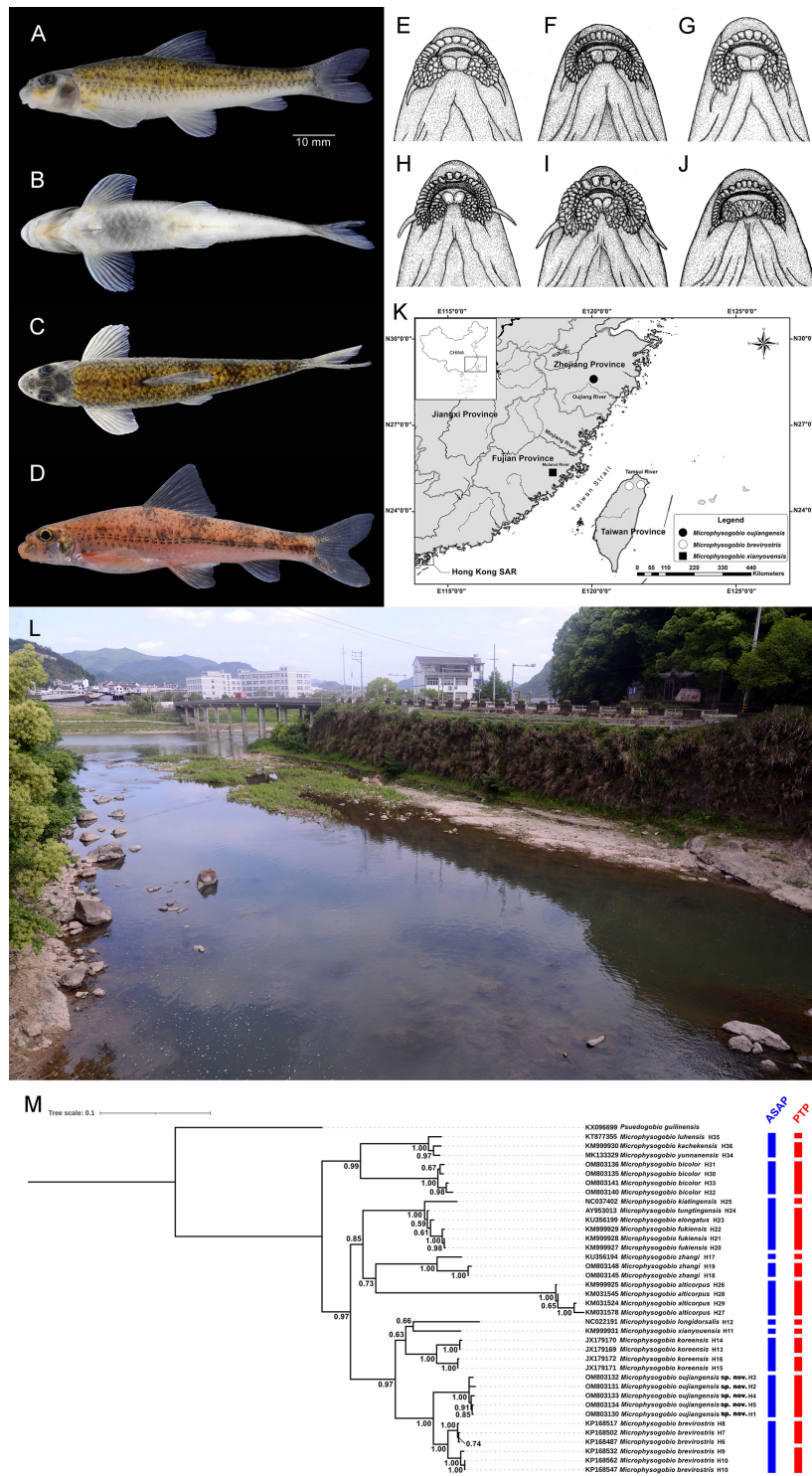


Figure 1 Type photos, live photos, morphological comparisons, distribution, habitat, and phylogenetic position of *Microphysogobio oujiangensis* sp. nov.

A–C: Picture shows lateral view (A), ventral view (B), and dorsal view (C) of holotype (ASIZB 220814). D: Live individual, collected from same locality as type specimens. E–J: Original drawing of lip papillae system of *Microphysogobio* species: *Microphysogobio oujiangensis* sp. nov. (E), *M. brevisrostris* (F), *M. xianyouensis* (G), *M. fukiensis* (H), *M. zhangii* (I), and *M. alticorpus* (J). K: Distribution of *Microphysogobio oujiangensis* sp. nov. and congeners. L: Habitat in type locality. M: Molecular phylogenetic tree of *Microphysogobio oujiangensis* sp. nov. and congeners based on *cyt b* sequence reconstructed by Bayesian inference; Bayesian posterior probabilities are shown on nodes and species delimitation results are shown on right. Photos by Zhi-Xian Sun.

Dorsal side of body with four distinct black saddles (first at dorsal-fin base origin, vague and small, second at dorsal-fin base ending, third at vertical position above anal-fin base origin, fourth on caudal peduncle). Lateral line scales with 7–8 vague grayish-brown blotches; some scales above lateral line with grayish-brown spots, two horizontally aligned black dashes above and below each lateral line scale (Figure 1D). Interorbital region without 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). One black mini-blotch above pectoral-fin base; caudal-fin base with small “<”-shaped black mark. Fins half translucent, with black pigments on some dorsal-fin rays and caudal-fin rays.

Coloration in preservation: Dorsal side yellowish-brown, mid-lateral side shallow yellowish-brown, ventral side grayish-white. Dorsal side of body with four distinct black saddles, same position as living specimen. Lateral line scales with 7–8 vague grayish-brown blotches; some scales above lateral line with grayish-brown spots, two horizontally aligned black dashes above and below each lateral line scale. Interorbital region without black crossbar. Operculum and suborbital region with three faded black blotches, same position as living specimen (Figure 1C). One faded black mini-blotch above pectoral-fin base; caudal-fin base with faded small “<”-shaped black mark. Fins pale, with black pigments in same place as living specimen.

Distribution: *Microphysogobio oujiangensis* sp. nov. is currently known only from the Oujiang River, a coastal river located in Zhejiang Province, China (Figure 1K).

Habitat and biology: *Microphysogobio oujiangensis* sp. nov. inhabits the clear water of rivers with sandy riverbeds containing gravel and pebbles (Figure 1L). Based on our observations and collection, *Microphysogobio oujiangensis* sp. nov. usually inhabits deeper water areas during the day, then moves to shallower areas at nightfall. Based on the horny sheaths on its upper and lower jaws, *Microphysogobio oujiangensis* sp. nov. usually feeds by scraping algae from pebbles. Coexisting species include *Acrossocheilus wenchowensis* Wang, 1935, *Distocheodon tumirostris* Peters, 1881, *Sarcocheilichthys parvus* Nichols, 1930, *Leptobotia brachycephala* Guo & Zhang, 2021, *Cobitis* spp., *Vanmanenia stenosoma* (Boulenger, 1901), and *Tachysurus ondon* (Shaw, 1930).

Etymology: The name of the new species, *oujiangensis*, is derived from the name of the locality river basin, Oujiang (瓯江). Its common name in Chinese is “瓯江小鱧鮡”.

Genetic comparisons: A total of 36 mitochondrial *cyt b* haplotypes from 37 *Microphysogobio* individuals were included in this analysis. Molecular phylogenetic analysis indicated that the new species was sister to *Microphysogobio brevirostris* (Figure 1M). Based on the K2P model, the interspecific genetic distance between *Microphysogobio oujiangensis* sp. nov. and its closest congener *M. brevirostris* for *cyt b* was 5.2%, while the intraspecific genetic distances of *Microphysogobio oujiangensis* sp. nov. and *M. brevirostris* were much lower (0.7% and 1.5%, respectively). ASAP analysis recognized 11 molecular operational taxonomic units (MOTUs) among the 15 species, while the PTP method for the

BI tree supported 15 MOTUs, with posterior probabilities of 0.57, 0.36, 0.74, 0.97, 0.90, 1.00, 0.96, 0.93, 1.00, 1.00, 0.96, 0.97, 0.82*, 0.71, and 0.68, respectively (* refers to node of *Microphysogobio oujiangensis* sp. nov.). The mPTP analysis for the ML tree supported 14 MOTUs, with posterior probabilities of 0.95, 0.92, 1.00, 0.81, 0.89, 0.50, 0.88, 1.00, 1.00, 0.84, 0.88, 0.81*, 0.64, and 0.59 respectively (Supplementary Figure S2). Both methods supported *Microphysogobio oujiangensis* sp. nov. as a distinct taxon. The genetic evidence also supported *Microphysogobio oujiangensis* sp. nov. as a possible distinct species based on currently available sequences. The BI and ML phylogenetic trees showed different tree topologies, with Bayesian posterior probabilities and bootstrap values. The genetic distances based on *cyt b* among the 15 species of *Microphysogobio* are given in Supplementary Table S3. The ML tree is provided in Supplementary Figure S2.

Remarks: Among the 30 valid *Microphysogobio* species, *Microphysogobio oujiangensis* sp. nov. can be easily distinguished from *M. chinssuensis* (Nichols, 1926), *M. yaluensis* (Mori, 1928), *M. kiatingensis* (Wu, 1930), *M. hsinglungshanensis* Mori, 1934, *M. amurensis* (Taranez, 1937), *M. anudarini* Holcık & Pivnicka, 1969, *M. linghensis* Xie, 1986, *M. liaohensis* (Qin, 1987), *M. rapidus* Chae & Yang, 1999, *M. wulonghensis* Xing, Zhao, Tang & Zhang, 2011, and *M. nudiventris* Jiang, Gao & Zhang, 2012 by having completely scaled mid-ventral region (vs. incompletely scaled mid-ventral region (Xing et al., 2011)).

Compared to those species with completely scaled mid-ventral regions, the new species can also be distinguished from *M. tungtingensis* (Nichols, 1926), *M. bicolor*, *M. tafangensis*, and *M. zhangji* by having six branched anal-fin rays (vs. five) and wider cutting edge on upper jaw (equal or larger than half mouth width vs. smaller than half mouth width).

Microphysogobio oujiangensis sp. nov. can be distinguished from *M. fukiensis*, *M. kachekensis* (Oshima, 1926), *M. elongatus* (Yao & Yang, 1977), *M. yunnanensis* (Yao & Yang, 1977), *M. vietnamica* Mai, 1978, *M. pseudoelongatus* Zhao & Zhang, 2001, and *M. luhensis* by having wider horny sheathed cutting edge on upper jaw (equal or larger than half mouth width vs. smaller than half mouth width, Figure 1E, H). *Microphysogobio oujiangensis* sp. nov. differs from *M. microstomus* and *M. jeoni* Kim & Yang, 1999 by having well-developed lip papillae (vs. undeveloped lip papillae). In addition, the new species differs from *M. nikolskii* (Dao & Mai, 1959) by having 36–38 lateral line scales (vs. 43 (Huang et al., 2018)).

The new species is similar to *M. longidorsalis* Mori, 1935, *M. koreensis* Mori, 1935, and *M. alticorpus* regarding squamation patterns on midventral region, width of cutting edge on upper jaw, and number of anal-fin rays. However, *Microphysogobio oujiangensis* sp. nov. differs from *M. longidorsalis* by having a different dorsal fin shape (distal margin slightly concave vs. distal margin strongly convex). *Microphysogobio oujiangensis* sp. nov. differs from *M. koreensis* by number of lateral line scales (36–38 vs. 39–41 (Kim & Yang, 1999)). The new species can be distinguished from *M. alticorpus* by having 36–38 (average 37) lateral line scales (vs. 35–36 (average

36)), two scales below lateral line (vs. three), deeper concavity on top of snout before nostrils (vs. smooth or shallow concavity), and different lip papillae pattern (medal pad disconnected from two lateral lobes vs. medal pad connected to two lateral lobes on anterior side of medal pad to posterior side of lateral lobes by elongated papillae).

The new species is very similar to *M. brevirostris* and *M. xianyouensis*, with all sharing a similar lip papillae pattern, equal number of scales below lateral line, and apparent concavity on top of snout before nostrils. However, the new species can be distinguished from *M. xianyouensis* by having 10–11 (average 11) branched pectoral-fin rays (vs. 11–12 (average 12)), interorbital width 21.3%–27.6% of HL (vs. 28.4%–30.2% of HL), and barbel length 81.3%–90.4% of eye diameter (vs. 66.6%–72.6% of eye diameter). The new species can also be distinguished from *M. brevirostris* by having 36–38 (average 37) lateral line scales (vs. 38–39 (average 38)), 8–10 (average 9) pre-dorsal scales (vs. 11), interorbital width 21.3%–27.6% of HL (vs. 29.9%–32.6% of HL), and eye diameter 30.9%–36.9% of HL (vs. 25.3%–30.9% of HL). Detailed morphological data are shown in Supplementary Table S2.

Based on phylogenetic analysis, the new species formed a monophyletic lineage with *M. brevirostris*, in agreement with their morphological similarities. The sister lineage containing *M. xianyouensis*, *M. longidorsalis*, and *M. koreensis* also showed some morphological similarities with the *Microphysogobio oujiangensis* sp. nov.–*M. brevirostris* lineage. The new species is currently known only from the Oujiang River Basin, whereas *M. brevirostris* is only found in independent rivers north of the Miaoli Plateau on Taiwan Island (Chang et al., 2016), *M. xianyouensis* is only distributed in the Mulanxi River Basin, and *M. longidorsalis* and *M. koreensis* are only distributed in the South Korean Peninsula. These five species indicate a potential relationship among mainland China, Taiwan Island, and the Korean Peninsula. For example, although the distribution of the new species exhibits no overlap with *M. brevirostris* or *M. xianyouensis*, the distribution patterns of these three species suggest a close historic connection between the river basins, especially between the Tamsui River Basin on Taiwan Island and the coastal rivers in Zhejiang and Fujian. In fact, land bridges between the Asian continent and Taiwan emerged three or four times during the Pliocene and Pleistocene epochs, and many terrestrial species could have migrated from the mainland to the island during these glaciations as a result of lowered sea levels (Huang et al., 1995; Yu, 1995). Previous studies have shown that freshwater fish species such as *Squalidus argentatus*, *Hemibarbus labeo*, *Sinibrama macrops*, and *Opsariichthys evolans*, which are distributed on the Miaoli Plateau along the Tamsui River in Taiwan, have a close relationship with congeners found in the coastal rivers of Zhejiang (Hsu et al., 2005; Lin et al., 2010; Yang et al., 2012). Two north and south routes connecting Taiwan and mainland China have also been suggested in previous studies based on freshwater fish fauna similarity (Oshima, 1923; Tzeng, 1986), and *M. brevirostris* is considered a north-originated species (Chang et al., 2016). The discovery of *Microphysogobio oujiangensis* sp. nov. provides additional evidence to support this view.

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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Nomenclature act LSID:

urn:lsid:zoobank.org:act:9BBE5614-DDAD-4AA9-B216-3AC495D84F05

SCIENTIFIC FIELD SURVEY PERMISSION INFORMATION

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

COMPETING INTERESTS

The authors declare that they have no competing interests.

ACKNOWLEDGEMENTS

Many thanks to Prof. Phillip S. Lobel (Boston University) for suggestions regarding color pattern description.

AUTHOR CONTRIBUTIONS

Y.H.Z. and Z.X.S. designed the study. Z.X.S. contributed to fieldwork, collected data, performed data analyses, and wrote the manuscript with input from Y.H.Z. and J.W. B.Q.Z. contributed to fieldwork. Y.H.Z. revised the manuscript. All authors read and approved the final version of the manuscript.

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