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**Description of A New Species of the Gudgeon Genus *Microphysogobio* Mori 1934
(Cypriniformes: Cyprinidae) from Guangdong Province, Southern China**

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Shih-Pin Huang, I-Shiung Chen, Yahui Zhao, and Kwang-Tsao Shao (2018) *Microphysogobio luhensis* n. sp., a new cyprinid species, is described from the Rongjiang River in eastern Guangdong Province, China. Morphological characters and molecular evidence based on mitochondrial DNA Cytochrome *b* (Cyt *b*) and Cytochrome oxidase subunit I (*COI*) sequences were used to compare this new species with other related species from mainland China, Vietnam and Taiwan. The present molecular evidences revealed that this new species is closely related to *M. kachekensis* and *M. yunnanensis*. However, these three species can be well distinguished based on the number of small pearl-like papillae on their inside papillae, lip papillae shape, barbel length, barbel width and color pattern. Furthermore, the morphometric comparison between *M. kachekensis* and the poorly known species *M. yunnanensis* is also discussed in this study for the first time. In addition, a diagnostic key to all 14 valid species of *Microphysogobio* from southern mainland China, Hainan Island and

Taiwan is also provided.

Key words: Freshwater Fish, Taxonomy, Rongjiang River, Gudgeon, Cytochrome *b*.

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BACKGROUND

Microphysogobio Mori, 1934 is a genus of small benthic gudgeons under the subfamily Gobioninae (Cypriniformes: Cyprinidae), which is widely distributed in eastern Asia, including Russia, Korea, mainland China, Hainan Island, Mongolia, Taiwan, Vietnam and Laos, and usually occurs in the upper and middle reaches of river systems (Cheng and Zheng 1987; Kottelat 2001a b).

Thirty species of *Microphysogobio* have been considered valid in the world (Eschmeyer et al. 2018). Twenty of which are found in China (Jiang et al. 2012; Huang et al. 2017). The Yangtze River is the longest river in China, and forms a natural boundary between northern and southern China. Among 20 species found in China, six species are considered as endemic to northern China, including *M. amurensis* (Taranez, 1937), *M. liaohensis* (Qin, 1987), *M. linghensis* Xie, 1986, *M. hsinglungshanensis* Mori, 1934, *M. wulonghensis* Xing, Zhao, Tang and Zhang, 2011, and *M. chinssuensis* (Nichols, 1926a).

There were 14 species distributed in southern China (Wu 1977; Chen 1998; Jiang et al. 2012; Huang et al. 2016), including *M. tungtingensis* (Nichols, 1926a), *M. fukiensis* (Nichols, 1926b), *M. kachekensis* (Oshima, 1926), *M. kiatingensis* (Wu, 1930), *M. tafangensis* (Wang, 1935), *M. chenhisenensis* (Fang, 1938), *M. elongatus* (Yao and Yang, 1977), *M. yunnanensis* (Yao and Yang, 1977), *M. microstomus* Yue, 1995, *M. pseudoelongatus* Zhao and Zhang, 2001, *M. nudiventris* Jiang, Gao and Zhang, 2012, *M. exilicauda* (Jiang and Zhang, 2013), *M. xianyouensis* Huang, Chen and Shao, 2016, and *M. zhangii* Huang, Zhao, Chen and Shao, 2017. Among them, *M. labeoides* (Nichols and Pope, 1927) is regarded as a junior synonym of *M. kachekensis* (Kottelat 2001b), and *M. suiifuensis* (Wu, 1930) as a junior synonym of *M. kiatingensis* (Wu 1977). In addition, *M.*

chinssuensis multipapillatus Bănărescu and Nalbant, 1973 is regarded as a junior synonym of *M. kiatingensis* (Huang et al. 2017).

A few species of *Microphysogobio* were recently described based on combined morphological and molecular evidence; these molecular phylogenetic studies not only provide molecular evidence, but also show the phylogenetic relationships between members of the subfamily (Huang et al. 2016 2017). Four species of *Microphysogobio* from southern China and one from northern China have been described as new species in the past ten years. These surveys greatly promote our understanding of biodiversity and distribution of *Microphygobio* in China.

Microphysogobio kachekensis was reported to be widely distributed in Guangdong Province in southern mainland China, Vietnam and its type locality, Hainan Island, which lies off the coast of Guangdong Province (Pan 1991; Kottelat 2001b). An additional nominal species, *M. labeoides*—also described from Hainan Island—was regarded as a junior synonym of *M. kachekensis* (Kottelat 2001b).

Huang et al.'s (2017) molecular analysis of the phylogenetic relationships among *Microphysogobio* species found significant genetic divergence between materials identified as *M. kachekensis* from Hainan Island and mainland China, indicating that further study of the taxonomic status of the two populations is necessary.

In order to reassess the taxonomic status of material previously identified as *M. kachekensis*, this study analyzed more samples of *M. yunnanensis* and populations identified as *M. kachekensis* from Hainan Island and mainland China. Our morphological and molecular analyses results indicate that populations of *M. kachekensis* from the Rongjiang drainage of southern China belong to a previously unnamed, distinct species described herein.

MATERIALS AND METHODS

Sample collection

All examined specimens were collected by casting net or bought from local markets. The

sampling localities are shown in figure 1. Specimens used for morphological studies were fixed in 10% formalin solution for three to five days, followed by 70% ethanol for long-term preservation. Tissue samples used for molecular analysis were preserved in 95% ethanol.

Morphological studies

All morphometric measurements followed Hosoya et al. (2002), and meristic counts followed Chen et al. (2009). Most of the morphometric measurements and the definition of lip papillae followed Huang et al. (2017). In this study, the proportion of the eye diameter, snout length, entire papillae lobe length, posterior papillae lobe length, inside papillae lobe length, medial pad length, barbel length and maximum barbel diameter were respectively measured as diagnostic features and given in table 1. The lip papillae system consisted of three parts, including anterior papillae on upper lip, a pair of inside papillae on lower lip, and single medial pad on lower lip. The inside papillae on lower lip comprised dozens of pearl-like small papillae (Fig. 4), the number of pearl-like small papillae were counted using a microscope and is given in table 1. An illustration for morphometric measurements of lip papillae is given in figure 4c. All lengths used in this study are standard length (SL).

Table 1. Comparison of morphometric measurements of lip papillae of *Microphysogobio luhensis* n. sp. and its related species *M. kachekensis* and *M. yunnanensis*

Species	<i>M. luhensis</i>		<i>M. kachekensis</i>		<i>M. yunnanensis</i> *	
Locality	Rong River, China		Hainan Island, China		Red River, Vietnam	
Number	5	Ave.	6	Ave.	6	Ave.
Standard length (mm SL)	48.4 - 62.1		50.7 - 75.1		37.8 - 47.9	
% of head length						
Eye diameter	25.2	- 27.9 (26.9)	24.4	- 28.0 (26.3)	-	
Snout length	40.2	- 45.0 (42.9)	42.1	- 46.4 (44.0)	36.6	- 39.0 (37.8)
Entire papillae lobe length	26.5	- 29.8 (28.2)	30.8	- 33.7 (31.9)	-	
Posterior papillae lobe length	15.1	- 19.7 (17.2)	21.3	- 25.2 (22.9)	-	
Inside papillae lobe length	10.5	- 13.6 (12.3)	13.5	- 16.5 (15.1)	-	

Medial pad length	10.4 - 11.0 (10.7)	10.0 - 11.3 (10.6)	8.9 - 9.7 (9.3)
Barbel length	16.2 - 18.4 (17.9)	21.0 - 23.8 (22.6)	-
% of barbel length Maximum barbel diameter	27.0 - 29.8 (28.3)	15.0 - 18.8 (17.2)	-
Number of small pearl-like papillae on inside papillae	20 - 26 (23.3)	32 - 40 (34.8)	30 - 41 (36.2)

*Several items for morphometric measurement of *M. yunnanensis* collected from northern Vietnam were absented due to the deformation of partial lip papillae and barbel.

All examined specimens were deposited at the Biodiversity Research Museum, Academia Sinica, Taipei (ASIZP); National Taiwan Ocean University, Keelung (NTOUP); National Museum of Natural Science, Taichung (NMNS); the Institute of Zoology, Chinese Academy of Sciences, Beijing (ASIZB); American Museum of Natural History, New York (AMNH); United States National Museum, Washington, D. C. (USNM); British Museum of Natural History (BMNH); and Zoologisches Museum Berlin (ZMB). Abbreviated name of all institution codes followed Fricke and Eschmeyer (2018). All comparative *Microphysogobio* species used as materials were listed in table S1.

Molecular phylogenetic studies

Full length mitochondrial DNA Cytochrome *b* (*Cyt b*) sequences were used as molecular evidence. DNA extractions of the samples used a high purity product preparation kit (Roche, USA). Sequences were amplified by PCR using two primers: (cytbF1: 5'-TGA CTT GAA GAA CCA CCG TTG TA-3' for forward primer; cytbR1: 5'-CGA TCT TCG GAT TAC AAG ACC GAT G-3' for reverse primer) following Huang et al. (2016).

In order to strengthen the reliability of molecular evidence in the present analysis, another molecular phylogenetic analysis was performed using the partial *COI* gene, all examined species of *Microphysogobio* in the present study were included. The *COI* gene was amplified by PCR using the following two primers: (FishF1: 5'- TCAACCAACCACAAAGACATTGGCAC -3'; FishR1: 5'- TAGACTTCTGGGTGGCCAAAGAATCA -3'), following Ward et al. (2005). All primers were also used as the primers for DNA sequencing. PCR was done in a MODEL 2700 or 9700 thermal

cycler (Perkin-Elmer) for 35-40 cycles. Double-stranded PCR products were purified using a high pure product purification kit (Roche, USA) before undergoing direct cycle sequencing with dye-labeled terminators (ABI Big-Dye kit). Labeled fragments were analyzed using an ABI PRISM Model 377-64 DNA Automated sequencer (ABI, USA).

Sequence alignment was carried out using BIOEDIT version 5.9 (Hall 2001), and then verified manually. Aligned mutation sites was analyzed using Molecular Evolutionary Genetics Analysis (MEGA) version 7.0 (Kumar et al. 2016). The best-fit model for reconstructing the phylogenetic tree was determined using jModelTest v.2.1.3 (Darriba et al. 2012). The Bayesian inference (BI) analyses were performed using MrBayes 3.0 (Ronquist and Huelsenbeck 2003). The posterior probabilities of each node were computed from the remaining 75% of all sampled trees.

RESULTS

TAXONOMY

Family Cyprinidae

Microphysogobio luhensis n. sp.

(Figs. 2; 3a b; 4a)

urn:lsid:zoobank.org:act:855FE0C8-8BF1-48E8-B29E-56C39F68863C

Material examined: Holotype: NTOUP 2013-10-119, 56.2 mm SL, Rong River (Rongjiang), Dongkeng Town, Luhe County, Guangdong Province, China (latitude: 23°18'15.4"; longitude: 115°42'51.4"E), coll. S.P. Huang, 2 April 2009.

Paratypes: ASIZP 0080740, 2 specimens, 48.3-60.8 mm SL. NTOUP 2010-11-545, 1 specimen, 52.7 mm SL. ASIZB 204717, 1 specimen, 57.3 mm SL. Paratypes were collected with holotype.

Non-types: NTOUP 2013-10-116, 3 specimens, 46.7-53.7 mm SL, collected with holotype.

Diagnosis: This new species can be distinguished from other congeners by the following unique combination of features: (1) meristic accounts: anal fin rays 3, 6; pectoral fin rays 1, 12-13 (modally 13); lateral-line scales 37-38 (modally 37); transverse scales 7; predorsal scales 10-11

(modally 10); gill rakers 16; vertebral counts 4+33-34; inside papillae lobes covered with clusters of 20-26 well-developed pearl-like papillae; (2) lip papillae: A pair of barbels flat and slightly short, 16.2-18.4% of head length, maximum barbel diameter was measured as 27.0-29.8% of barbel length; the medial pad on lower lip divided; (3) color patterns: Body with five distinct black crossbars; Two horizontally aligned black dashes above and below each lateral-line scale; caudal fin membrane with two rows of distinct vertically-aligned black lines.

Description: Body elongated and compressed laterally. Snout pointed. Eye moderately large and located in dorsal half of head, eye diameter was measured as 25.2-27.9% of head length. Belly flatted in males, and slightly rounded in females. Body covered with moderately small cycloid scales which are larger posteriorly. The morphometric measurements of this new species are provided in table 2.

Table 2. Morphometric measurements of *Microphysogobio luhensis* n. sp.

Types	<i>Microphysogobio luhensis</i> n. sp.			
	Holotype	Holotype+Paratypes		
Number		5		Ave.
Percentage of standard length (%)				
Head length	24.6	22.9 - 24.6		(23.6)
Body depth	17.3	15.0 - 18.2		(16.7)
Body width	14.2	12.7 - 14.5		(13.7)
Depth of caudal peduncle	8.2	7.9 - 8.4		(8.1)
Length of caudal peduncle	21.4	19.4 - 21.4		(20.6)
Predorsal length	43.9	41.5 - 43.9		(42.6)
Preanal length	52.7	51.6 - 53.4		(52.6)
Prepelvic length	44.7	44.6 - 45.2		(44.7)
Height of dorsal fin	20.3	19.2 - 20.3		(19.7)
Length of depressed dorsal	21.7	21.2 - 22.5		(21.6)
Length of dorsal fin base	13.2	11.2 - 13.2		(12.3)
Height of anal fin	12.8	12.8 - 14.7		(13.7)
Length of depressed anal	17.1	16.3 - 17.1		(16.7)
Length of anal fin base	7.8	7.8 - 8.9		(8.2)
Pectral fin length	20.1	20.1 - 21.6		(20.8)
Pelvic fin length	15.3	15.3 - 16.6		(16.0)
Percentage of head length (%)				
Head depth	53.6	51.1 - 55.9		(53.0)
Head width	52.2	50.4 - 53.1		(51.6)
Snout length	44.2	40.2 - 45.0		(42.9)

Orbit diameter	26.1	25.2	-	27.9	(26.9)
Interorbital width	23.2	22.1	-	25.0	(23.5)

Inter-pectoral fin basal region always naked, but rear margin of which, backward extending to anal fin anterior base always covered with cycloid scales. Lateral line complete and running slightly downward abruptly above pectoral fin and along the ventral profile into middle of caudal fin base.

Vertebral counts 4+33 (in 1 individual)-4+34 (1). Gill rakers 16 (1). Dorsal fin rays 3, 7 (8), anal fin rays 3, 6 (8), pectoral fin rays 1, 12 (2)-1, 13 (11), pelvic fin rays 1, 7 (15). Lateral line scales 37 (11)-38 (5), transverse scales 7(8), predorsal scales 10 (6)-11 (2). Pectoral fin maximum reach anterior margin of pelvic fin when compressed in both sexes. Pelvic fin rounded. Anterior margin of pelvic fin inserted below second branched ray of dorsal fin. Caudal fin deeply forked and rear margin of caudal fin lobe rounded. Snout is prominent, 40.2-45.0% of head length.

Lip papillae: Mouth horseshoe-shaped. Upper and lower lip thick, covered with pearl papillae. Lip papillae consists of three parts: a pair of papillae on upper lip, and backward extending to the base of barbel; a pair of inside papillae lobes on lower lip; and a heart-shaped medial pad on lower lip. On upper lip, anterior papillae covered with one row of large pearl-like papillae, both posterior lobes covered with clusters of well-developed, small pearl-like papillae. Posterior margin of both inside papillae lobes rounded, and covered with clusters of 20-26 well-developed, small pearl-like papillae (Table 1). The medial pad on lower lip completely divided. The lip papillae of *M. luhensis* is shown in figure 4.

The morphometric measurements of papillae and barbell were shown in table 1. Entire papillae lobe was measured as 26.5-29.8% of head length. A pair of posterior lobes and inside papillae lobes were measured as 15.1-19.7% and 10.5-13.6% of head length, respectively. Medial pad was measured as 10.4-11.0% of head length. A pair of barbels flat and slightly short, located at corners of mouth and rooted at posterior edge of lower jaw, 16.2-18.4% of head length. Maximum barbel diameter was measured as 27.0-29.8% of barbel length.

Coloration in fresh specimen: Head and body generally pale yellowish brown (Fig. 2). Belly pale grayish white. Body with five distinct black crossbars (four bars on trunk and one bar on neck) (Fig. 3). Cheek and lower opercular regions bright sliver-white, and with a few indistinct small black spots. Upper opercular region grayish brown. A distinct bars is present on anterior margin of

eye and extending to upper lip. Two horizontally aligned black dashes above and below each lateral-line scale. Dorsal fin membrane with two rows of indistinct, longitudinally-aligned black lines. Pectoral fin and pelvic fin membranes having some tiny black spots. Anal fin membrane clean. Caudal fin base with a distinct small black mark. Caudal fin membrane with two rows of distinct vertically-aligned black lines.

Distribution: Known only from the upper reaches of the Rongjiang River (Rong River), a river located in eastern Guangdong Province, southern China (Fig. 1).



Fig. 1. The Sampling localities of *Microphysogobio luhensis* n. sp., and comparative materials. +, *M. luhensis* n. sp.; ◆, *M. alticorpus*; ●, *M. brevirostris*; ■, *M. chenhsienensis*; △, *M. elongatus*; ◇, *M. exilicauda*; ▼, *M. fukiensis*; □, *M. kacheakensis*; ▽, *M. kiatingensis*; ▲, *M. microstomus*; ○, *M. pseudoelongatus*; ★, *M. tafangensis*; ⊙, *M. tungtingensis*; ⊕, *M. xianyouensis*; ☆, *M. yunnanensis*; *, *M. zhangii*.



Fig. 2. The specimen photographs of *Microphysogobio luhensis* n. sp., holotype, NTOUP 2013-10-119, 56.2 mm SL.



Fig. 3. The dorsal view of *Microphysogobio luhensis* n. sp. (a, b) and *Microphysogobio kachekensis* (c, d), standard length was measured as 56.2, 57.3, 50.3 and 68.2 mm SL for individual a, b, c and d, respectively.

Etymology: The Latinized specific name, “*luhensis*” is refers to “Luhe County”, located in northeastern Guangdong Province, China, wherein lies the type locality.

Remarks: On the aspect of morphological feature, compared to all 22 valid *Microphysogobio* species from related areas, *M. luhensis* can be immediately distinguished from five valid species (*M. chenhsienensis*, *M. chinssuensis*, *M. exilicauda*, *M. tafangensis* and *M. wulonghensis*) by the different types of medial pad on lower lip (centrally divided vs. undivided).

As to the remaining 17 species, *M. luhensis* can be discriminated from *M. hsinglungshanensis*, *M. liaohensis*, *M. linghensis* and *M. nudiventris* by the different pattern of scale distribution (midventral region covered with scales vs. midventral region naked).

Out of the remaining 13 species, this new species can be distinguished from *M. tungtingensis* and *M. zhangii* by having more anal fin rays (3, 6 vs. 3, 5). Compared to the remaining 11 species, *M. luhensis* can be distinguished from *M. alticorpus*, *M. amurensis*, *M. microstomus*, *M. kiatingensis* and *M. xianyouensis* by having different frequency distribution of lateral-line scale series (37-38 vs. 39-42 for *M. amurensis*, 35-36 for *M. alticorpus*, *M. kiatingensis* and *M. xianyouensis*; 34 for *M. microstomus*).

As to the remaining five species, *M. luhensis* can be discriminated from *M. yunnanensis* by having fewer pearl-like papillae on inside papillae (20-26 vs. 30-41), and this new species can be distinguished from *M. elongatus* by having more vertebral counts (4+33-34 vs. 4+32).

Compared to the remaining four species, *M. luhensis* can be distinguished from *M. brevirostris*, *M. fukiensis*, and *M. pseudoelongatus* by having different type of medial pad (heart-shaped for *M. luhensis* vs. rectangular form for the rest) (Fig. 4).

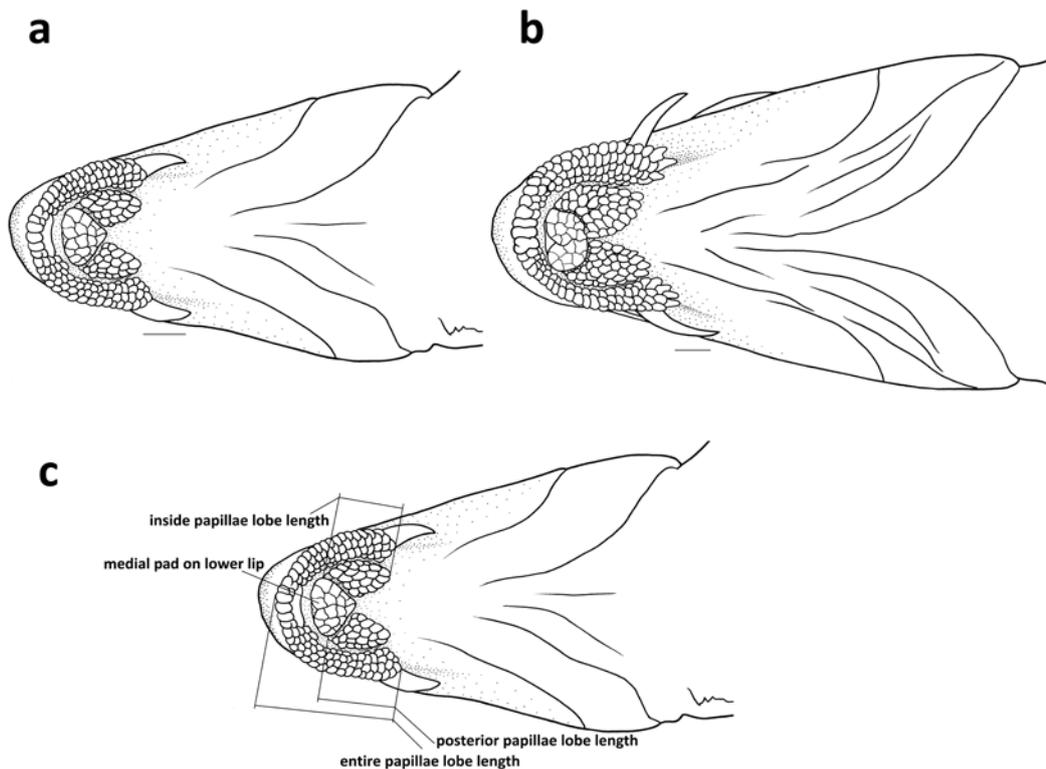


Fig. 4. Lip papillae of a, *Microphysogobio luhensis* n. sp., holotype; b, *Microphysogobio kachekensis*, NTOUP 2013-10-117, 64.4 mm SL; c, an illustration for morphometric measurements of lip papillae. Scale bar = 1 mm.

Of all the valid species of *Microphysogobio*, the new species appears to be most closely related to *M. kachekensis* based on molecular evidence and some morphological features. Both species share similar anal fin rays (3, 6), predorsal scale series (10-11), spotted dorsal fin and caudal fin, and these two species with two horizontally aligned black dashes above and below each lateral-line scale. However, *M. luhensis* still can be distinguished from *M. kachekensis* based on following morphological difference: (1) *M. luhensis* has shorter and broader barbel (16.2-18.4% of head length and 27.0-29.8% of barbel length, respectively) when compared to *M. kachekensis* (21.0-23.8% of head length and 15.0-18.8% of barbel length, respectively); (2) *M. luhensis* has fewer pearl-like papillae on inside papillae (20-26 vs. 32-40); (3) the rear margin of posterior papillae lobe always in arc-shaped for *M. luhensis*, and in tassel-shaped for *M. kachekensis*; (4) different color patterns (presence vs. absence of five distinct black crossbars at body); and (5) fewer lateral-line scale series (modally 37 vs. 38).

Pseudogobio labeoides Nichols and Pope, 1927 which was described from Nodda, Hainan Island, China, was previously regarded as a junior synonym of *Microphysogobio kachekensis*

(Kottelat 2001b). In order to confirm the taxonomic status of *P. labeoides*, its holotype specimen was examined. The result revealed that *P. labeoides* should be conspecific with *M. kachekensis*, both share the same type of medial pad on lower lip, the rear margin of medial pads always in arc-shaped (Fig. 4b). In addition, *P. labeoides* and *M. kachekensis* share the longer and slender barbel. *P. labeoides* can be immediately distinguished from *M. luhensis* by the latter species having an acuminate rear margin and having shorter and broader barbel (Fig. 4a). Our result was consistent with Kottelat's taxonomic treatment, suggesting the *P. labeoides* should be treated as a junior synonym of *M. kachekensis*. Under the subfamily Gobioninae, both *Microphysogobio* and *Pseudogobio* are common benthic gudgeon distributed in East Asia. In addition, these two genera share similar characters, including have a pair of barbels and lip covered with pearl papillae. However, *Pseudogobio* can be distinguished from *Microphysogobio* by having flatter snout and longer snout (longer than two times of eye diameter) (Pan 1991).

Otherwise, *Microphysogobio luhensis* is further compared with several nominal species of *Microphysogobio* distributed in Vietnam, Mongolia, and the Yalu River, which forms the border between China and North Korea. The results are discussed as follows. When compared to the *M. yaluensis* (Mori, 1928) known from the Yalu River, *M. luhensis* can be distinguished by having more pectoral fin rays (1, 12-13 vs. 1, 11).

Microphysogobio luhensis differs from *M. anudarini* Holcík and Pivnicka, 1969, a species known from Mongolia, by having significantly shorter distance between the anus and anal fin origin (17.2-17.6% of SL, averaged 17.4%, measured from five individuals including holotype versus 19.0-20.8% of SL, using previous data from the literature reported by Kottelat in 2006). *M. luhensis* has two irregular lines on caudal fin membrane, but it is rather regular in *M. anudarini* (based on its detailed specimen photograph from the literature reported by Kottelat in 2006).

Microphysogobio luhensis can also be discriminated from two nominal species of *Microphysogobio* known from Vietnam. At the first, it differs from *M. nikolskii* (Dao and Mai, 1959) by having fewer lateral-line scales (37-38 vs. 43). Kottelat (2001b) reported that the taxonomic assignment of *M. vietnamica* Mai, 1978, another nominal species from Vietnam still remains unclear. Nevertheless, *M. luhensis* can be discriminated from *M. vietnamica* by having smaller dorsal fin and longer pectoral fin (versus a dorsal fin reaching backward almost to the anal fin base,

and pectoral fin never reach base of pelvic fin in *M. vietnamica*).

Molecular phylogenetic analysis: The code of each species and GenBank accession numbers used in this study were given in table 3. *Carassius auratus langsdorfi* was used as outgroup species. The Cyt *b* and partial *COI* sequences from *M. luhensis* and 10 species of *Microphysogobio* were analyzed. A total of 20 haplotypes from 42 individuals for Cyt *b* gene, and 15 haplotypes from 26 individuals for *COI* gene were included in this analysis. The length of Cyt *b* and *COI* sequence are 1141 bp and 636 bp in total, respectively. The alignment contains 390 and 232 total mutations, and 329 and 177 polymorphic (segregating) sites for Cyt *b* and *COI* genes, respectively. The phylogenetic analysis using the Bayesian inference (BI) was provided. The phylogenetic trees were reconstructed by the BI method based on the HKY+G model.

The Cyt *b* phylogenetic tree (Fig. 5) revealed that *M. luhensis* - *M. kachekensis* - *M. yunnanensis* clade is the earliest offshoot. Out of all taxa, *M. alticorpus* and *M. zhangii* formed respective clades. *M. tafangensis* and *M. chenhsienensis* formed a related sister group, which is sister to *M. brevirostris* - *M. xianyouensis* + *M. elongatus* - *M. fukiensis* clade. Inter-specific nodes between *M. luhensis* and the closely related species *M. kachekensis* and *M. yunnanensis* with high posterior probability reach to 1.00. All inter-specific nodes were supported by high bootstrap values (as high as 0.95-1.00). However, an inter-clades node between two clades (clade *M. brevirostris* + *M. xianyouensis* + *M. elongatus* + *M. fukiensis*, and clade *M. tafangensis* + *M. chenhsienensis*) had a lower posterior probability of 0.67. The genetic distances of relationships among *M. luhensis* and ten valid species of *Microphysogobio* were analyzed based on Kimura 2 parameter model (K2P), ranged from 1.2-16.1% for Cyt *b* gene, and ranged from 2.9-20.2% for *COI* gene.

Although the *COI* phylogenetic tree (Fig. 6) revealed clades different from those on the Cyt *b* tree, the *COI* tree also strongly supported that the *M. luhensis* can be discriminated from *M. kachekensis* and *M. yunnanensis*, and that this phylogenetic tree had high posterior probability reaching 1.00.

Table 3. OTU codes, sampling localities and accession numbers of examined *Microphysogobio* species and outgroup for molecular analysis

Code	Species	Locality	Cyt <i>b</i>			COI		
			No.	Accession no.	Source	No.	Accession no.	Source
MALKP1	<i>M. alticorpus</i>	Kaoping River, Pingtung, Taiwan	2	KM999925	Huang et al. 2016	1	MK139889	This study
MBRKL1	<i>M. brevisrostris</i>	Keelung River, Tamsui River, Keelung, Taiwan	2	KM999926	Huang et al. 2016	2	MK139899	This study
MCHOJ1	<i>M. chenhsienensis</i>	Ojiang River, Yantou Township, Zhejiang, China	1	KT075097	Huang et al. 2016	1	MK139894	This study
MCHOJ2	<i>M. chenhsienensis</i>	Ojiang River, Youngjia County, Zhejiang, China	1	KT075098	Huang et al. 2016	1	MK139895	This study
MELQZ1	<i>M. elongatus</i>	Quanzhou County market, Guangxi, China	5	KU356199	Huang et al. 2016	2	MK139897	This study
MFUMJ1	<i>M. fukiensis</i>	Shaowu City market, Fujian, China	1	KM999927	Huang et al. 2016	1	MK139898	This study
MFUMJ2	<i>M. fukiensis</i>	Min River, Shaowu City, Fujian, China	1	KM999928	Huang et al. 2016	1	MK139898	This study
MFUMJ3	<i>M. fukiensis</i>	Min River, Xinquan, Fujian, China	1	KM999929	Huang et al. 2016	1	MK139898	This study
MFUJL1	<i>M. fukiensis</i>	Pinghe County market, Fujian, China	2	KT877353	Huang et al. 2016	-	-	This study
MKAND1	<i>M. kacheakensis</i>	Nandu River, Nankai Township, Hainan, China	6	KM999930	This study / Huang et al. 2016	3	MK139891	This study
MLURJ1	<i>M. luhensis</i> n. sp.	Rongjiang River, Luhe County, Guangdong, China	3	KT877355	This study	3	MK139890	This study
MTAQT1	<i>M. tafangensis</i>	Changhua Township market, Zhejiang, China	1	KT075099	Huang et al. 2016	1	MK139893	This study
MXIML1	<i>M. xianyouensis</i>	Mulan River, Xianyou County, Fujian, China	3	KM999931	Huang et al. 2016	2	MK139896	This study
MYUVN1	<i>M. yunnanensis</i>	Lixian River, Red River, Dien Bien, Vietnam	5	MK133329	This study	3	MK139892	This study
MZHGC1	<i>M. zhangii</i>	Gongcheng County market, Guangxi, China	2	KT877354	Huang et al. 2017	1	MK139888	This study
MZHGL1	<i>M. zhangii</i>	Guilin City market, Guangxi, China	1	KU356194	Huang et al. 2017	1	MK139887	This study
MZHGL2	<i>M. zhangii</i>	Guilin City market, Guangxi, China	1	KU356195	Huang et al. 2017	-	-	This study
MZHQZ1	<i>M. zhangii</i>	Quanzhou County market, Guangxi, China	1	KU356196	Huang et al. 2017	1	MK139885	This study
MZHQZ2	<i>M. zhangii</i>	Quanzhou County market, Guangxi, China	1	KU356197	Huang et al. 2017	1	MK139886	This study
MZHQZ3	<i>M. zhangii</i>	Quanzhou County market, Guangxi, China	2	KU356198	Huang et al. 2017	-	-	This study
CAURA1	<i>C. auratus langsdorfi</i>	Japan	1	NC002079	Murakami et al. 1998	1	NC002079	Murakami et al. 1998

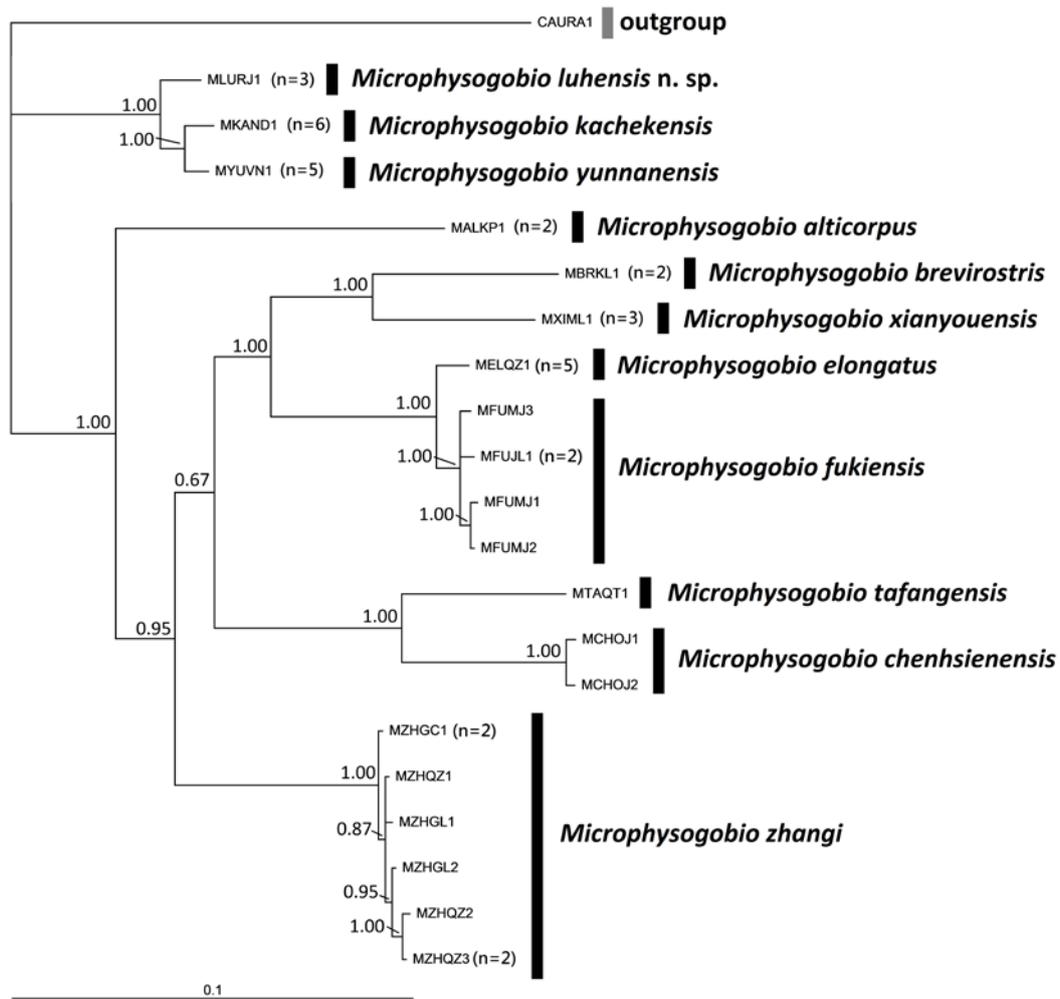


Fig. 5. Molecular phylogenetic tree of *Microphysogobio luhensis* n. sp. and other comparative materials based on *Cyt b* sequence reconstructed by Bayesian analysis method (values above the branch: posterior probabilities). The sample size of each haplotype is shown behind the OTU.

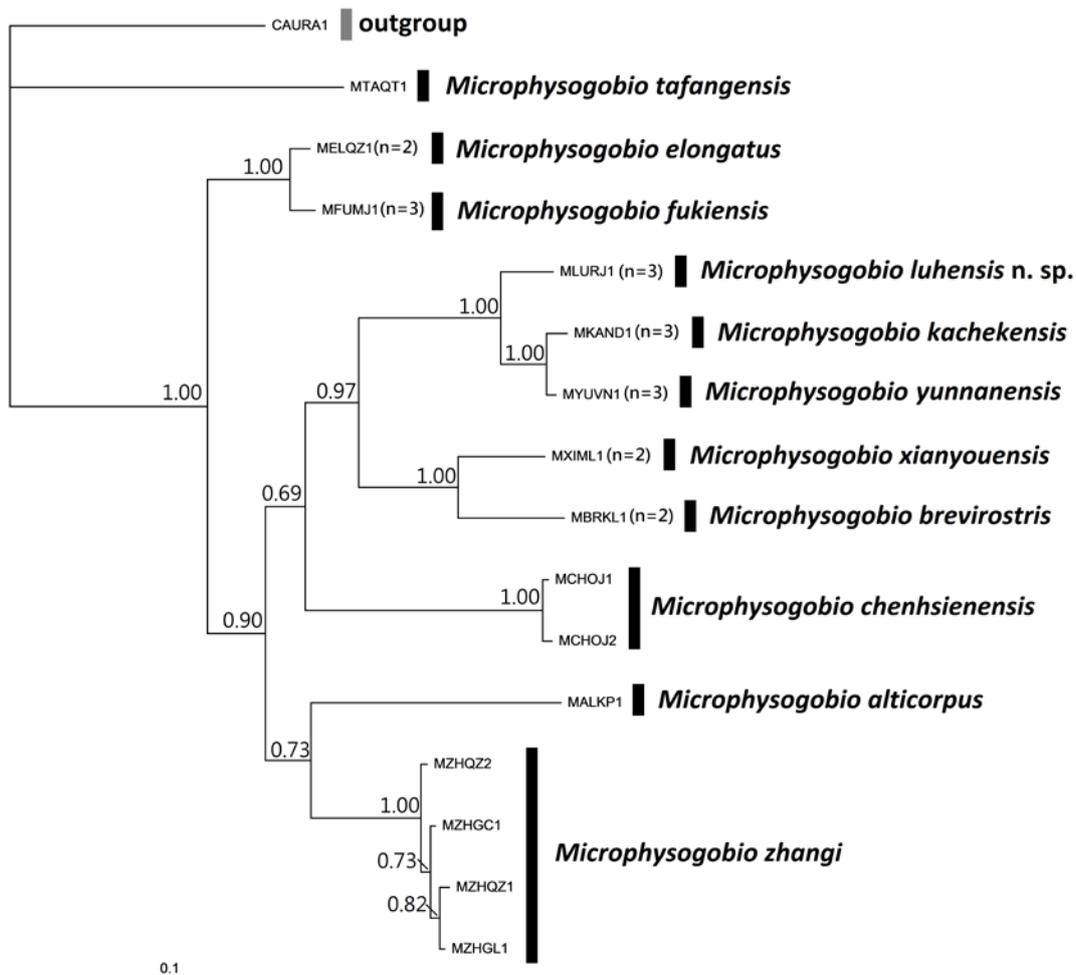


Fig. 6. Molecular phylogenetic tree of *Microphysogobio luhensis* n. sp. and other comparative materials based on partial *COI* sequence reconstructed by Bayesian analysis method (values above the branch: posterior probabilities). The sample size of each haplotype is shown behind the OTU.

DISCUSSION

The specific feature of the lip papillae is considered a diagnostic character for defining the genus *Microphysogobio* and distinguishing it from other related genera under subfamily Gobioninae (Wu 1977). Subsequently, Huang et al. (2017) suggested that feature can not only be used to identify at the inter-generic level, it can also be used for species identification. Recently, Huang et al. (2016) discriminated *M. xianyouensis* from its related species *M. brevirostris* based on the lip feature, this result is also supported by molecular evidence. The morphological feature of lip papillae should be considered as an important diagnostic character to define an independent species.

Genetic divergence was frequently used as good molecular evidence for verifying the validity of new species or reconstructing their phylogenetic relationship (Costagliola et al. 2004; Mukai et al. 2005; Chen et al. 2009). The mitochondrial *Cyt b* sequences have been applied to identify species of freshwater cyprinids and brackish water gobies, and are regarded as an ideal marker (Jang-Liaw and Chen 2013; Huang et al. 2013; Huang et al. 2017). *COI* sequences were also used for fish species identification (Ward et al. 2005). The present *COI* tree (Fig. 6) revealed that each species could be well recognized, showing that the validity of *M. luhensis* can be strongly supported by molecular evidence based on both *Cyt b* and *COI* genes.

The range of the inter-specific genetic distances of *M. luhensis* and two related species *M. kachekensis* and *M. yunnanensis* are 2.1% and 2.0%, respectively, for *Cyt b*, and 3.2% and 2.9%, respectively, for *COI* based on the K2P model. It is clearly higher than the genetic distance between the two valid species *M. fukiensis* and *M. elongatus* (1.4-1.5% for *Cyt b*, and 1.1% for *COI*), also higher than its two sister species *M. kachekensis* and *M. yunnanensis* (1.2% for *Cyt b*, and 0.6% for *COI*).

Furthermore, the intra-species genetic distance of *M. fukiensis* is from 0.1-0.5% for *Cyt b* (0.4-0.5% for the cross-river populations); in addition, the genetic distance is from 0.1-0.4% for *Cyt b* and 0.2-0.5% for *COI* (cross-river populations were included for those two genes) in *M. zhangii*. These intra-species genetic distances were apparently lower than in the inter-species genetic distances between *M. luhensis*, *M. kachekensis* and *M. yunnanensis*.

Yao and Yang (1977) reported that *M. yunnanensis* is most similar to *M. kachekensis*, but these two species can be separated by the different medial pad size, shorter snout and different color pattern (Yao and Yang, 1977). However, this supposition has never been verified by molecular evidence. The present molecular evidence is the first to verify that these two species formed a sister group.

The morphometric differences between *M. yunnanensis* and *M. kachekensis* have been previously mentioned (Yao and Yang 1977); however, none have expounded their morphological differences in detail. Our results revealed that *M. yunnanensis* can be distinguished from *M. kachekensis* by its smaller medial pad (9.3% vs. 10.6% of head length in average, Table 1), shorter snout length (37.8% vs. 44.0% of head length in average, Table 1), and three distinct black

crossbars at the posterior section of the body (versus without black crossbars).

CONCLUSIONS

The present morphological and molecular evidence strongly support *Microphysogobio luhensis* n. sp. is an independent and valid species, this new species can be well distinguished from its related species *M. kachekensis* and *M. yunnanensis*. Furthermore, the morphological features of lip papillae, including the type of medial pad, number of small pearl-like papillae on their inside papillae, lip papillae shape, barbel length, and barbel width should be considered as important diagnostic character, these features will be rather beneficial for specific identification of genus *Microphysogobio*.

A diagnostic key to all valid species of *Microphysogobio* from southern mainland China, Hainan Island and Taiwan

- 1a. Medial pad on lower lip undivided 2
- 1b. Medial pad on lower lip centrally divided 4
- 2a. Lateral-line scales 34; dorsal fin rather long, reach caudal fin base in mature males *M. tafangensis*
- 2b. Lateral-line scales more than 36; dorsal fin medium, do not reach caudal fin base in both sexes 3
- 3a. Dorsal and caudal fin membranes with two rows of black line; posterior lobe short, 46.8-55.4% of eye diameter *M. chenhisenensis*
- 3b. Dorsal and caudal fin membranes without any black lines; posterior lobe long, 79.4-96.3% of eye diameter *M. exilicauda*
- 4a. Two-third ventral region of belly naked *M. nudiventris*
- 4b. Ventral region covered with scales 5
- 5a. Anal fin rays 3, 5 6

5b. Anal fin rays 3, 6	7
6a. Vertebral counts 4+30-31; interorbital region with a black crossbar	<i>M. zhangii</i>
6b. Vertebral counts 4+34; interorbital region without crossbar	<i>M. tungtingensis</i>
7a. A “<” shaped black mark on the base of the caudal fin	<i>M. xianyouensis</i>
7a. A circular or rectangular mark on the base of the caudal fin	8
8a. Lateral-line scales 34; pearl papillae of the posterior lobe reduced, posterior tip smoothed	<i>M. microstomus</i>
8b. Lateral-line scales 35-38; pearl papillae of the posterior lobe well-developed, posterior tip free formed	9
9a. No distinct crossbar present on dorsal side	10
9b. Dorsal side with distinct crossbars	11
10a. Pectoral fin rays 1, 12-13; vertebral counts 4+33	<i>M. kachekensis</i>
10b. Pectoral fin rays 1, 11; vertebral counts 4+32	<i>M. pseudoelongatus</i>
11a. The upper nasal region observed as slightly flatted	<i>M. elongatus</i>
11b. The upper nasal region can be easily observed as recessed.....	12
12a. Medial pad heart shaped	13
12b. Medial pad square shaped	14
13a. Inside papillae lobes covered with 20-26 well-developed pearl-like papillae; snout length 40.2-45.0% of head length	<i>M. luhensis</i> n. sp.
13b. Inside papillae lobes covered with 30-41 well-developed pearl-like papillae; snout length 36.6-39.0% of head length	<i>M. yunnanensis</i>
14a. Meddle line of the body side with a longitudinal thick greyish black band	<i>M. fukiensis</i>
14a. Meddle line of the body side with several independent blotches, and do not form a longitudinal band	<i>M. kiatingensis</i>

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REFERENCES

- Bănărescu PM, Nalbant TT. 1973. Pisces, Teleostei. Cyprinidae (Gobioninae). Das Tierreich **93**:i-vii+1-304.
- Chen IS, Wu JH, Huang SP. 2009. The taxonomy and phylogeny of the cyprinid genus *Opsariichthys* Bleeker (Teleostei: Cyprinidae) from Taiwan, with description of a new species. Environ Biol Fish **86**:165-183. doi: 10.1007/s10641-009-9499-y.
- Chen YY. 1998. Fauna Sinica, Osteichthyes, Cypriniformes II. Science Press, Beijing.
- Cheng Q, Zheng B. 1987. Systematic synopsis of Chinese fishes. Vol. 1. Science Press, Beijing. (in Chinese)

- Costagliola D, Robertson DR, Guidetti P, Stefanni S, Wirtz P, Heiser JB, Bernardi G. 2004. Evolution of coral reef fish *Thalassoma* spp. (Labridae). 2. Evolution of the eastern Atlantic species. Mar Biol **144**:377-383. doi: 10.1007/s00227-003-1200-y.
- Dao VT, Mai DY. 1959. Preliminary data on the fishes of Nego Thai, a feeder river of the Red River Yen Bai province—nothern Vietnam. Report of the West Pacific Fishery Conference IV. Hanoi. 1-30. (in Vietnamese)
- Darriba D, Taboada GL, Doallo R, Posada D. 2012. jModelTest 2: more models, new heuristics and parallel computing. Nat Methods **9**:772-772. doi:10.1038/nmeth.2109.
- Eschmeyer WN, Fricke R, Laan R van der (eds). Catalog of Fishes: Genera, Species, Rferences. <http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp>. Electronic version Accessed 20 March 2018.
- Fang PW. 1938. On *Huigobio chenhsiensis*, gen. & sp. nov. Bull Fan Memorial Inst Biol Peiping (Zool Ser) **8**:237-243.
- Fricke R, Eschmeyer WN. Guide to Fish Collections. <http://researcharchive.calacademy.org/research/ichthyology/catalog/collections.asp>. Electronic version Accessed 20 March 2018.
- Hall TA. 2001. Bioedit: a user-friendly biological sequence alignment editor and analysis, version 5.09. Raleigh, North Carolina: Department of Microbiology, North Carolina State University, Raleigh.
- Holčík J, Pivnička K. 1969. Notes on a collection of fishes from Mongolia with description of *Microphysogobio tungtingensis anudarini* ssp. n. and discovery of some new or little known fishes. Annot Zool Botan **56**:1-25.
- Hosoya K, Ashiwa H, Wayanabe M, Mizunguchi K, Okazaki T. 2002. *Zacco sieboldii*, a species distinct from *Zacco temminckii*. Ichthyol Res **50**:1-8. doi:10.1007/s102280300000.
- Huang SP, Chen IS, Shao KT. 2013. The molecular phylogeny of genus *Hemigobius* (Teleostei: Gobiidae), with the confirmation of validity of *Hemigobius crassa* (Herre, 1945). J Mar Sci Tech Suppl **21**:86-93. doi: 10.6119/JMST-013-1220-4.
- Huang SP, Chen IS, Shao KT. 2016. A new species of *Microphysogobio* (Cypriniformes: Cyprinidae) from Fujian Province, China, and a molecular phylogenetic analysis of

Microphysogobio species from southeastern China and Taiwan. Proc Biol Soc Wash
129:195-211. doi:10.2988/0006-324X-129.Q3.195.

Huang SP, Zhao YH, Chen I-S, Shao KT. 2017. A new species of *Microphysogobio* (Cypriniformes: Cyprinidae) from Guangxi Province, southern China. Zoological Studies, **56**:8.
doi:10.6620/ZS.2017.56-08.

Jang-Liaw NH, Chen IS. 2013. *Onychostoma minnanensis*, a new cyprinid species (Teleostei: Cyprinidae) from Fujian, southern mainland China, with comments on the genetic differentiation among related species. Ichthyol Res **60**:62-74. doi:10.1007/s10228-012-0312-0.

Jiang ZG, Gao EH, Zhang E. 2012. *Microphysogobio nudiventris*, a new species of gudgeon (Teleostei: Cyprinidae) from the middle Chang-Jiang (Yangtze River) basin, Hubei Province, South China. Zootaxa **3586**:211-221. doi:10.2988/0006-324X-129.Q3.195.

Jiang ZG, Zhang E. 2013. Molecular evidence for taxonomic status of the gudgeon genus *Huigobio* Fang, 1938 (Teleostei: Cypriniformes), with a description of a new species from Guangdong Province, South China. Zootaxa **3731**:171-182. doi:10.11646/zootaxa.3731.1.8.

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

Kottelat M. 2001b. Freshwater fishes of northern Vietnam. A preliminary check-list of the fishes known or expected to occur in northern Vietnam with comments on systematics and nomenclature. Environment and Social Development Unit, East Asia and Pacific Region, The World Bank.

Kottelat M. 2006. Fishes of Mongolia. A check-list of the fishes known to occur in Mongolia with comments on systematics and nomenclature. The World Bank.

Mai DY. 1978. Identification of the fresh-water fishes of North Viet Nam. Ha Noi, Scientific & Technology Publisher. (in Vietnamese)

Mori T. 1928. On the fresh water fishes from the Yalu River, Korea, with descriptions of new species. J Chosen Nat Hist Soc **6**:54-70.

Mori T. 1934. The fresh water fishes of Jehol. In: Tokunaga S (ed) Report of the first scientific expedition to Manchoukuo, Tokyo.

Mukai T, Nakamura S, Suzuki T, Nishida M. 2005. Mitochondrial DNA divergence in yoshinobori gobies (*Rhinogobius* species complex) between the Bonin Islands and the Japan-Ryukyu

- Archipelago. Ichthyol Res **52**:410-413. doi:10.1007/s10228-005-0295-1.
- Nichols JT. 1926a. Some Chinese fresh-water fishes. XV. Two apparently undescribed catfishes from Fukien. XVI. Concerning gudgeons related to *Pseudogobio*, and two new species of it. XVII. Two new Rhodeins. Am Mus Novit **214**:1-7.
- Nichols JT. 1926b. Some Chinese fresh-water fishes. XVIII. New species in recent and earlier Fukien collections. Am Mus Novit **224**:1-7.
- Nichols JT, CH Pope. 1927. The fishes of Hainan. Bulletin of the American Museum of Natural History **54**:321-394.
- Oshima M. 1926. Notes on a collection of fishes from Hainan, obtained by Prof. S. F. Light. Annotnes Zool Jap **11**:1-25.
- Pan, JH. 1991. The freshwater fishes of Guangdong Province. Guangdong Science and Technology Press, Guangzhou, 589 pp.
- Qin KJ. 1987. Fauna Liaoningica. Pisces. In: Liu CX, Qin KJ. Liaoning Science and Technology Press, pp 1-552. (in Chinese)
- Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics **19**:1572-1574. doi:10.1093/bioinformatics/btg180.
- Kumar S, Stecher G, Tamura K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Mol Bio Evol **33**:1870-1874. doi:10.1093/molbev/msw054.
- Taranetz AY. 1937. A note on a new genus of gudgeons from the Amur Basin. Izv. Tikh Nauch-Issl Inst Rybn Khoz Okean Núm **23**:113-115. (in Russian)
- Wang KF. 1935. Preliminary notes on the fishes of Chekiang (Isospondyli, Apodes & Plectospondyli). Contr Biol Lab Sci Soc China Zool Ser **11**:1-65.
- Ward RD, Zemplak TS, Innes BH, Last PR, Hebert PDN. 2005. DNA barcoding Australia's fish species. Phil Trans R Soc B **360**:1847-1857. doi:10.1098/rstb.2005.1716.
- Wu HW. 1930. On some fishes collected from the upper Yangtse Valley. Sinensis **1**:65-86.
- Wu HW. 1977. The cyprinid fishes of China. Volume 2. People's Press, Shanghai. (in Chinese)
- Xie YH. 1986. On a new cyprinid fish of the genus *Microphysogobio* from Liaoning, China (Cypriniformes: Cyprinidae). Acta Zootamn Sinica **11**:220-222.
- Xing YC, Zhao YH, Tang WQ, Zhang CG. 2011. A new species, *Microphysogobio wulonghensis*

(Teleostei: Cypriniformes: Cyprinidae), from Shandong Province, China. *Zootaxa* **2901**:59-68.
doi:10.5281/zenodo.206492.

Yao PQ, Yang GR. 1977. Gobioninae. Volume 2. *In*: Wu XW. The cyprinid fishes of China. Science Press, Peking, pp. 439-549. (in Chinese)

Yue PQ. 1995. Description of a new species of the genus *Microphysogobio* Mori from China (Cypriniformes: Cyprinidae). *Acta Zootaxon Sin* **20**:495-498.

Zhao YH, Zhang CG. 2001. A new species of the genus *Microphysogobio* Mori from Guangxi, China (Cypriniformes: Cyprinidae). *Acta Zootaxon Sin* **26**:589-592.

Supplementary Material

Table S1. Other comparative materials. (download)