

A New Species of *Microphysogobio* (Cypriniformes: Cyprinidae) from Guangxi Province, Southern China

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(Received 6 January 2017; Accepted 29 March 2017; Published 21 April 2017; Communicated by Benny K.K. Chan)

Shih-Pin Huang, Yahui Zhao, I-Shiung Chen, and Kwang-Tsao Shao (2017) *Microphysogobio zhangi* n. sp., a new cyprinid species is described from Guangxi Province, China. Morphological and molecular evidence based on mitochondrial DNA Cytochrome *b* (Cyt *b*) sequence were used for comparing this new species and other related species. The phylogenetic tree topology revealed that this new species is closely related to *M. elongatus* and *M. fukiensis*. We also observed the existence of a peculiar trans-river gene flow in the Pearl River and the Yangtze River populations, and speculated that it was caused by an ancient artificial canal, the Lingqu Canal, which forming a pathway directly connecting these two rivers.

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

BACKGROUND

Microphysogobio Mori, 1934 is a genus of small gudgeons under the subfamily Gobioninae which was widely distributed in eastern Asia, including Korea, China, Mongolia, Taiwan, Vietnam and Laos, and usually occurs in upper and middle reaches of river basins (Cheng and Zheng 1987; Kottelat 2001a, b; Jiang et al. 2012; Huang et al. 2016).

Twenty-seven species of *Microphysogobio* were considered valid in the world (Eschmeyer et al. 2017). Eighteen of which were found in China (Jiang et al. 2012; Huang et al. 2016). Six of them were considered as endemic species to northern China, including *M. amurensis* (Taranetz, 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 are 13 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), and *M. xianyouensis* Huang, Chen and Shao, 2016. The distribution of all valid species of *Microphysogobio* from China is shown in table 1.

A new species of *Microphysogobio* was discovered during a field survey of freshwater fish in northern Guangxi Province, southern China, and

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is described herein. This new species is confirmed on the basis of morphological and molecular evidences. A diagnostic key to this new species and other valid species from southern China and Taiwan is also provided.

MATERIALS AND METHODS

Sample collection

All examined specimens from China were collected by casting net or bought from local markets. Specimen tissues used for molecular analysis were preserved in 95% ethanol. Specimens used for morphological studies were fixed in 10% formalin solution for three days, followed by 70% ethanol for long-term preservation.

Morphological studies

Methods for taking measurements followed Hosoya et al. (2002) and meristics followed Chen et al. (2009). All lengths are standard length (SL). All examined materials were deposited at the National Zoological Museum, Institute of Zoology, Chinese Academy of Sciences, Beijing, China (ASIZB); United States National Museum, Washington, D. C., USA (USNM), British Museum of Natural History, UK (BMNH); Zoologisches Museum Berlin, Germany (ZMB); Biodiversity Research Museum, Biodiversity Research Center, Academia Sinica, Taipei, Taiwan (ASIZP); National Taiwan Ocean University, Keelung, Taiwan (NTOUP) and National Museum of Natural Science, Taichung, Taiwan (NMNS). All abbreviated name of institution codes followed Fricke and Eschmeyer (2017).

The species of *Microphysogobio* was known as having various lip papillae types in different species (Wu 1977). Thus, the proportion of the eye diameter and the barbel length, posterior lobe length and whole papillae length were respectively measured as diagnostic features. An illustration for morphometric measurements of lip papillae system is given in figure 1.

The meristic abbreviations used in this study as follows: A, anal fin; D, dorsal fin; LL, lateralline scale; P1, pectoral fin; P2, pelvic fin; PreD, predorsal scales; TR, transverse scale series; VC, vertebral count.

Molecular phylogenetic studies

The phylogenetic relationships were studied using the full length of Cyt *b* sequence. All DNA extractions of the samples used a high purity product preparation kit (Roche, USA). D-loop

Table 1. Distribution of valid species of Microphysogobio from China

Species	Distribution				
M. chinssuensis (Nichols, 1926a)	Yellow River, northern China				
<i>M. tungtingensis</i> (Nichols, 1926a)	middle reaches of Yangtze River, Hunan Province, southern China				
<i>M. fukiensis</i> (Nichols, 1926b)	Min River and Joulung River, Fujian Province, southern China				
<i>M. kachekensis</i> (Oshima, 1926)	Guangdong Province and Hainan Island, southern China				
M. kiatingensis (Wu, 1930)	upper reaches of Yangtze River, Sichuan Province, southern China				
M. hsinglungshanensis Mori, 1934	Hebei Province, northern China				
<i>M. tafangensis</i> (Wang, 1935)	Qiantang River, Zhejiang Province, southern China				
M. amurensis (Taranetz, 1937)	Amur River, northern China				
M. chenhisenensis (Fang, 1938)	Caoer River and Ou River, Zhejiang Province, southern China				
M. elongatus (Yao and Yang, 1977)	Pearl River and Yangtze River, Guangxi Province, southern China				
M. yunnanensis (Yao and Yang, 1977)	Yuan River, Yunnan Province, southern China				
M. linghensis Xie, 1986	Liao River, northern China				
<i>M. liaohensis</i> (Qin, 1987)	Liao River, northern China				
M. microstomus Yue, 1995	lower reaches of Yangtze River, Jiangsu Province, southern China				
<i>M. pseudoelongatus</i> Zhao and Zhang, 2001	Fangcheng River, Guangxi Province, southern China				
M. wulonghensis Xing, Zhao, Tang and Zhang, 2011	Wulong River, Shandong Province, northern China				
M. nudiventris Jiang, Gao and Zhang, 2012	middle reaches of Yangtze River, Hubei Province, southern China				
M. exilicauda (Jiang and Zhang, 2013)	Pearl River and Yangtze River, Guangdong and Jiangxi Provinces, southern China				
M. xianyouensis Huang, Chen and Shao, 2016	Mulan River, Fujian Province, southern China				
<i>M. zhangi</i> n. sp.	Pearl River and Yangtze River, Guangxi Province, southern China				

region were amplified by polymerase chain reaction (PCR) using following 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). PCR was done in a MODEL 2700 or 9700 thermal cycler (Perkin-Elmer) and 40 cycles were carried out. 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). The sequencing primers were the same as the primers used in PCR. Labeled fragments were analyzed using an ABI PRISM Model 377-64 DNA Automated sequencer (ABI, USA).

Nucleotide sequence alignment was verified manually after running through BIOEDIT version 5.9 (Hall 2001). The analysis of aligned mutation sites was conducted using Molecular Evolutionary Genetics Analysis (MEGA) version 6.0 (Tamura et al. 2013) for aligned mutation sites analysis. The maximum parsimony (MP) analysis was carried out using PAUP* version 4.0b10 (Swofford 2003) using heuristic search. Branch support was established via bootstrap analysis (2000 replications). For the Bayesian (BI) analysis, the best-fit model for sequence evolution was determined using jModelTest v.2.1.3 (Darriba et al. 2012). The BI analyses were performed using MrBayes 3.0 (Ronguist and Huelsenbeck 2003). The posterior probabilities of each node were computed from the remaining 75% of all sampled trees.

page 3 of 12

RESULTS

Family Cyprinidae

Microphysogobio zhangi n. sp. urn:lsid:zoobank.org:act:C1F8D275-44E3-4E24-BAF4-AE90DE8C3248

Material examined: Holotype: ASIZB 204677, 77.1 mm SL, Xiang River, a tributary of Yangtze River, Quanzhou County, Guangxi Province, China (latitude: 25°56'00.0"; longitude: 111°04'49.4"), coll. S.P. Huang and J.C. Huang, 6 November 2015.

Paratypes: ASIZP 0078398, 3 specimens, 65.2-69.1 mm SL, collected with holotype. ASIZP 0078397, 2 specimens, 62.3-63.7 mm SL, Guilin City market, Guangxi Province, China, coll. S.P. Huang and J.C. Huang, 5 November 2015. NTOUP 2010-11-547, 12 specimens, 53.0-64.1 mm SL, Gongcheng County market, Guangxi Province, China, coll. I-S. Chen, 8 August 2009.

Diagnosis: This new species can be distinguished from other congeners by the following unique combination of features: (1) meristic accounts: anal fin rays 3, 5; pectoral fin rays 11-12; lateral-line scales 35-36; transverse scales 7; predorsal scales 9-10; vertebral counts 4+30-31; (2) lip papillae: barbel length medium, 53.5-69.9% of eye diameter; the medial pad on lower lip divided; and (3) coloration patterns: body with 6-7 indistinct black horizontally-aligned crossbars; interorbital region with a black crossbar;

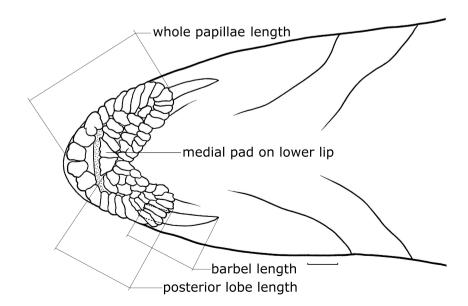


Fig. 1. The lip papillae system of Microphysogobio zhangi n. sp., paratype, NTOUP 2010-11-547, 55.6 mm SL. Scale bar = 1 mm.

a thin black vertical stripe throughout the cheek; dorsal fin and caudal fin membranes with indistinct vertically-aligned black lines.

Description: The morphometric measurements of this new species are provided in table 2. Body elongated and compressed laterally. Belly flatted. Snout pointed. Eye moderately large, located dorsal half of head. Lateral-line complete and running slightly downward abruptly above the pectoral fin and along the ventral profile into the middle of the caudal fin base. Gill rakers 11-13. Vertebral counts 4+30-31. Dorsal fin rays 3, 7; anal fin rays 3, 5; pectoral fin rays 1, 11-12 (modally 12); pelvic fin rays 1, 7; lateral-line scales 35-36 (modally 35); transverse scales 7; predorsal scales 9-10 (modally 10) (Table 3). Adult males with pectoral fin reaching anterior margin of anal fin base when compressed. Anterior margin of pelvic fin inserted below second branched ray of dorsal fin. Caudal fin deeply forked, lower lobe slightly longer than upper lobe. Body covered with large cycloid scales. Belly covered with cycloid scales, inter-pectoral fin basal region always naked.

Lip papillae: Mouth horseshoe-shaped. Upper

and lower lip thick, covered with pearl papillae. Lip papillae consist of anterior papillae, two posterior lobes and a medial pad on lower lip. Anterior papillae covered with one row of large pearl papillae. Both posterior lobes covered with clusters of well-developed, small pearl-like papillae. The medial pad on lower lip completely divided (Fig. 1). A pair of barbels located at corners of mouth and rooted at posterior edge of lower jaw, 53.5-69.9% length of eye diameter. Posterior lobes medium length, 60.8-72.5% of eye diameter. Whole papillae long, 89.5-106.4% length of eye diameter.

Coloration in fresh specimen: Head and body generally pale yellowish brown (Fig. 2A). Interorbital region with a black cross-band (Fig. 2B). Belly grayish to pale white. Body with 6-7 indistinct, black horizontally-aligned crossbands. Operculum region with a deep brown mark. A thin black stripe running vertically across the cheek. Dorsal fin membrane with one or two rows of indistinct, longitudinally-aligned black lines. Pectoral fin, pelvic fin and anal fin membranes having some tiny black spots. Caudal fin membrane with two or three rows of indistinct

type	Holotype			Holotype + Para	types		
catalogue number	ASIZB204677	ASIZB204677+ASIZP0078397			~8+NTOUP2010-11-547		
n			10		Average		
Percentage of standard length (%)							
Head length	22.5	20.5	-	22.5	(21.4)		
Body depth	19.1	18.5	_	21.8	(20.2)		
Body width	14.4	13.2	_	14.7	(14.3)		
Depth of caudal peduncle	9.7	8.5	-	9.8	(9.3)		
Length of caudal peduncle	17.5	16.6	_	18.3	(17.7)		
Predorsal length	43.2	41.9	_	43.4	(42.8)		
Preanal length	58.4	58.4	_	60.4	(59.3)		
Prepelvic length	50.0	48.3	_	52.6	(50.7)		
Height of dorsal fin	23.9	20.9	_	23.9	(22.0)		
Length of depressed dorsal	25.8	21.9	_	25.8	(24.3)		
Length of dorsal fin base	14.9	13.6	_	14.9	(14.3)		
Height of anal fin	15.2	14.7	_	15.5	(15.2)		
Length of depressed anal	19.5	17.3	_	19.5	(18.1)		
Length of anal fin base	8.9	7.6	_	8.9	(8.0)		
Pectral fin length	23.8	22.6	_	23.8	(23.1)		
Pelvic fin length	19.9	17.7	_	20.2	(19.3)		
Percentage of head length (%)							
Head depth	56.5	55.8	_	58.9	(57.5)		
Head width	58.4	55.6	_	58.4	(56.9)		
Snout length	42.1	41.6	_	44.9	(43.6)		
Orbit diameter	34.7	32.6	_	35.5	(34.3)		
Interorbital width	15.0	14.9	_	16.6	(15.5)		

Table 2. Morphometric measurements of *Microphysogobio zhangi* n. sp. from southern China

vertically-aligned black lines.

Distribution: Known only from the middle reaches of the Yu River and the Li River, two tributaries of the Pearl River, and the Xiang River, a tributary of the Yangtze River, located in Guangxi and Hunan Provinces of China (Fig. 3).

Etymology: The Latinized specific name, *"zhangi"* is derived from the name of the Chinese

Table 3. Frequency distribution of meristic features of *Microphysogobio zhangi* n. sp. and comparativematerials from southern China and Taiwan

	[C		Α			P1			
	3,7	x	3,5	3,6	х	1,10	1,11	1,12	1,13	х
<i>M. zhangi</i> n. sp.	20*	7.0	20*	_	5.0	-	10	25*	2	11.8
M. alticorpus	14	7.0	-	14	6.0	5	20	3	-	10.9
M. brevirostris	13	7.0	-	13	6.0	-	12	16	-	11.6
M. chenhsienensis	12	7.0	12	-	5.0	-	15	2	-	11.1
M. elongatus	6	7.0	-	6	6.0	-	-	8	4	12.3
M. exilicauda	8	7.0	8	-	5.0	-	7	2	-	11.2
M. fukiensis	18	7.0	3	15	5.8	-	14	10	-	11.4
M. kachekensis	10	7.0	-	10	6.0	-	-	4	11	12.7
M. microstomus	1	7.0	-	1	6.0	2	-	-	-	10.0
M. tafangensis	1	7.0	1	-	5.0	-	-	2	-	12.0
M. xianyouensis	12	7.0	-	12	6.0	-	4	12	-	11.8

		P2				LL						
	1,6	1,7	1,8	x	34	35	36	37	38	39	х	
<i>M. zhangi</i> n. sp.	-	35*	-	7.0	-	23	17*	-	-	-	35.4	
M. alticorpus	2	22	-	6.9	-	10	18	-	-	-	35.6	
M. brevirostris	-	32	2	7.1	-	-	-	-	10	16	38.6	
M. chenhsienensis	-	12	-	7.0	-	-	3	17	4	-	37.0	
M. elongatus	-	6	-	7.0	-	-	-	9	3	-	37.3	
M. exilicauda	-	16	-	7.0	-	-	-	10	6	-	37.4	
M. fukiensis	4	26	-	6.9	-	14	13	1	-	-	35.5	
M. kachekensis	-	19	-	7.0	-	-	-	14	6	-	37.3	
M. microstomus	-	2	-	7.0	2	-	-	-	-	-	34.0	
M. tafangensis	-	2	-	7.0	2	-	-	-	-	-	34.0	
M. xianyouensis	-	20	-	7.0	-	6	14	-	-	-	35.7	

		TR				PreD					VC				
	6	7	8	x	9	10	11	12	x	4+30	4+31	4+32	4+33	4+34	х
<i>M. zhangi</i> n. sp.	-	20*	-	7.0	7	13*	-	-	9.7	5	9*	-	-	-	30.6
M. alticorpus	-	-	14	8.0	-	-	12	2	11.1	7	3	-	-	-	30.3
M. brevirostris	-	-	13	8.0	-	4	8	1	10.8	-	-	-	2	6	33.8
M. chenhsienensis	-	12	-	7.0	7	5	-	-	9.4	-	-	-	6	2	33.3
M. elongatus	-	6	-	7.0	2	4	-	-	9.7	-	-	4	-	-	32.0
M. exilicauda	-	8	-	7.0	-	6	2	-	10.3	-	-	2	4	2	33.0
M. fukiensis	15	-	-	6.0	10	5	-	-	9.3	2	5	-	-	-	30.7
M. kachekensis	-	10	-	7.0	-	7	3	-	10.3	-	-	-	2	-	33.0
M. microstomus	-	1	-	7.0	-	1	-	-	10.0	1	-	-	-	-	30.0
M. tafangensis	-	1	-	7.0	1	-	-	-	9.0	-	-	-	1	-	33.0
M. xianyouensis	-	12	-	7.0	-	12	-	-	10.0	-	-	5	1	-	30.6

*means data from the holotype.

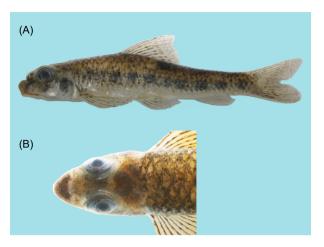


Fig. 2. The specimen photographs of *Microphysogobio zhangi* n. sp., (A) holotype, ASIZB 204677, 77.1 mm SL. (B) the same individual, dorsal view of head.

ichthyologist "Professor Chunguang Zhang" in recognition of his great contribution to the fish taxonomic studies in China.

Remarks: The molecular phylogenetic evidence of the new species is provided in the next section. Comparing all 19 valid Microphysogobio species from China and two valid species from Taiwan, M. zhangi can be easily distinguished from four valid species (M. chenhsienensis, M. chinssuensis, 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. zhangi can be distinguished 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, M. zhangi can be distinguished from M. amurensis, M. exilicauda, M. tungtingensis and M. yunanensis by having fewer lateral-line scale series (35-

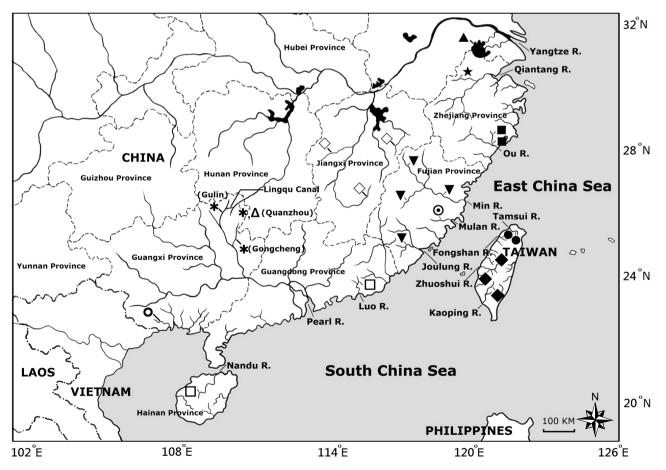


Fig. 3. The Sampling localities of *Microphysogobio zhangi* n. sp., and comparative materials from southern China and Taiwan. *, *Microphysogobio zhangi* n. sp.; ◆, *M. alticorpus*; ●, *M. brevirostris*; ■, *M. chenhsienensis*; △, *M. elongatus*; ◇, *M. exilicauda*; ▼, *M. fukiensis*; □, *M. kachekensis*; ▲, *M. microstomus*; ○, *M. pseudoelongatus*; ★, *M. tafangensis*; ⊙, *M. xianyouensis*.

36 vs. 38-39 for *M. tungtingensis*, 39-42 for *M. amurensis*, 37-38 for *M. exilicauda* and 38-40 for *M. yunanensis*). Finally, this new species can be distinguished from *M. alticorpus*, *M. brevirostris*, *M. fukiensis*, *M. kachekensis*, *M. kiatingensis*, *M. microstomus*, *M. pseudoelongatus* and *M. xianyouensis* by having fewer anal fin rays (3, 5 vs. 3, 6).

Of all the valid species of *Microphysogobio* from China and Taiwan, *M. zhangi* appears to be most closely related to *M. elongatus* based on molecular evidence and some morphological features. Both species share similar pectoral fin rays (modally 1, 12), predorsal scale series (9-10), spotted dorsal fin and caudal fin. In addition, both species are sympatric (Fig. 3). However, *M. zhangi* still can be distinguished from *M. elongatus* by having (1) fewer anal fin rays (3, 5 vs. 3, 6); (2) fewer lateral-line scale series (modally 35 vs. 37); and (3) different color pattern (presence vs. absence of a black cross-band at interorbital region).

The type specimen of the poorly known species, *Microphysogobio chinssuensis multipapillatus* Bănărescu and Nalbant, 1973 which was described from Chengtu, China, was also re-examined. Since it shared the same lateral-line scales (37), spotted fins, cheek spot pattern and lip papillae as *M. kiatingensis*, it should be regarded as a junior synonym of *M. kiatingensis*.

M. zhangi 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) and the results are discussed as follows. *M. zhangi* can be distinguished from *M. yaluensis* known from the Yalu River (Mori, 1928) by having fewer anal fin rays (3, 5 vs. 3, 6).

M. zhangi differs from *M. anudarini* Holcík and Pivnička, 1969, a species known from Mongolia, by having significantly shorter distance between the anus and anal fin origin (15.4-17.3% of SL, averaged 16.6%, measured from 15 individuals including holotype, 16.3% in the holotype versus 19.0-20.8% of SL, using previous data from the literature reported by Kottelat in 2006). The upper nasal region in *M. zhangi* can also be easily observed as recessed vs. the region in *M. anudarini* (based on its detailed specimen photograph from the literature reported by Kottelat in 2006).

M. zhangi differs from *M. nikolskii* (Dao and Mai, 1959), one of the two species known from Vietnam, by having fewer lateral-line scales (35-

36 vs. 43). Kottelat (2001b) reported that the taxonomic assignment of *M. vietnamica* Mai, 1978, the other species from Vietnam, remains unclear. Nevertheless, *M. zhangi* can be discriminated from *M. vietnamica* by having fewer branched soft rays in the anal fin (5 vs. 6) and smaller dorsal fin (versus a dorsal fin reaching backward almost to the anal fin base).

Molecular phylogenetic analysis

The code of each species and GenBank accession numbers used in this study are given in table 4. Pseudogobio vaillanti (Sauvage, 1878) was used as outgroup species. The Cyt b sequence from M. zhangi and five closely related species of Microphysogobio. A total of 15 haplotypes from 27 individuals were included in this analysis. The length of Cyt b sequence is 1141 bp in total. This alignment contains 308 total mutations, and 273 polymorphic (segregating) sites. The phylogenetic analyses using both the Bayesian inference (BI) and maximum parsimony (MP) were provided. The phylogenetic tree was reconstructed by the BI analysis based on the HKY+G model. The MP analysis by heuristic search resulted in only one tree, with tree length 507; the consistency index (CI) being 0.7100, retention index (RI) being 0.8088 and homoplasy index (HI) being 0.2840.

The phylogenetic trees reconstructed by the BI and MP methods showed the same tree topology (Fig. 4) and revealed that *M. kachekensis* is the earliest offshoot. *M. alticorpus* and *M. brevirostris* belong to two independent clades. *M. elongatus* and *M. fukiensis* were formed a related sister group, and is sister to *M. zhangi*. Interspecific nodes between *M. zhangi* and the closely related species *M. elongatus* and *M. fukiensis* sister group with high bootstrap value reach to 100 in MP tree. The posterior probabilities were as high as 1.00 in the BI tree. The interspecific nodes between *M. alticorpus* and *M. brevirostris* were supported by lower bootstrap values (51 and 0.69-0.73 in MP and BI, respectively).

The genetic distances of relationships among *M. zhangi* and five other species were analyzed based on Kimura 2 parameter model (K2P), and ranged from 10.8-15.6%. The genetic distances from 10.8-11.7% and 11.2-11.4% when compared with *M. fukiensis* and *M. elongatus*, respectively. The genetic evidence strongly supported *M. zhangi* to be a distinct species. At the intraspecific level, the genetic distances of *M. zhangi* from different localities are from 0.1-0.4%. The results are similar

to 0.1-0.5% for *M. fukiensis* which were collected from the Min River and the Joulung River.

DISCUSSION AND CONCLUSIONS

The specific feature of the lip papillae was considered a diagnostic character for defining the genus *Microphysogobio* and for distinguishing the genus from other related genera under subfamily Gobioninae (Wu 1977). Huang et al. (2016) discriminated *M. xianyouensis* from its related species *M. brevirostris* based on the feature. The present study also reveals that different species of *Microphysogobio* have different characters of lip papillae. We suggest that the feature not only can be used to identify to the inter-generic level, it can also be used for species identification.

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; Huang et al. 2016). The mitochondrial Cyt *b* sequences have been applied to the species identification of freshwater cyprinids and brackish water gobies, and are regarded as an ideal marker (Jang-Liaw and Chen 2013; Huang et al. 2013). The range of the inter-specific genetic distance of *M. zhangi*, the new species, and *M. fukiensis*, a related species, is 10.8-11.7% for Cyt *b* sequences based on the K2P model. It is obviously higher than the distance between *M. fukiensis* and *M. elongates*, another related species (1.4-1.5%).

Most species of *Microphysogobio* found in China probably only occur in a few rivers since they were restricted to their type localities and adjacent regions or rivers. According to our overall surveys of the species of *Microphysogobio* in southern China, *M. fukiensis* is probably restricted to the Min River and the Joulung River of Fujian Province (Fig. 3), and is the only species to be found in the Min River so far. *M. elongatus* is only known from the Pearl River basin and the Xiang River, a tributary of the Yangtze River in Guangxi Province. *M. kachekensis* is probably restricted to Guangdong Province in southern mainland

Table 4. OTU codes, sampling localities and accession numbers of examined *Microphysogobio* species and outgroup for molecular analysis from southern China and Taiwan

Code	Species	Locality	Sample size	Accession number
MALKP1	Microphysogobio alticorpus	Kaoping River, Ligang Township, Pingtung County, Taiwan	2	KM999925
MBRKL1	Microphysogobio brevirostris	Keelung River, Tamsui River, Sijiaoting, Keelung City, Taiwan	2	KM999926
MELQZ1	Microphysogobio elongatus	Quanzhou County market, Guangxi Province, China	5	KU356199
MFUMJ1	Microphysogobio fukiensis	Shaowu City market, Fujian Province, China	1	KM999927
MFUMJ2	Microphysogobio fukiensis	Min River, Shaowu City, Fujian Province, China	1	KM999928
MFUMJ3	Microphysogobio fukiensis	Min River, Xinquan, Fujian Province, China	1	KM999929
MFUJL1	Microphysogobio fukiensis	Pinghe County market, Fujian Province, China	2	KT877353
MKAND1	Microphysogobio kachekensis	Nandu River, Nankai Township, Hainan Province, China	3	KM999930
MKALR1	Microphysogobio kachekensis	Luo River, Dongkeng, Luhe County, Guangdong Province, China	2	KT877355
MZHGC1	<i>Microphysogobio zhangi</i> n. sp.	Gongcheng County market, Guangxi Province, China	2	KT877354
MZHGL1	<i>Microphysogobio zhangi</i> n. sp.	Guilin City market, Guangxi Province, China	1	KU356194
MZHGL2	Microphysogobio zhangi n. sp.	Guilin City market, Guangxi Province, China	1	KU356195
MZHQZ1	Microphysogobio zhangi n. sp.	Quanzhou County market, Guangxi Province, China	1	KU356196
MZHQZ2	<i>Microphysogobio zhangi</i> n. sp.	Quanzhou County market, Guangxi Province, China	1	KU356197
MZHQZ3	<i>Microphysogobio zhangi</i> n. sp.	Quanzhou County market, Guangxi Province, China	2	KU356198
PVAHJ1	Pseudogobio vaillanti	Hanjiang basin, Changting County, Fujian Province, China	1	KM999932

China and Hainan Island which lies off the coast of Guangdong. However, although the two populations have similar external morphological features, a distinct genetic divergence (2.1%) has been observed of these two populations. Further comparisons to reassess their taxonomic status may be necessary. Different individuals of *M. zhangi* from the Yangtze River and the Pearl River were used for genetic analysis in this study (Table 2). Two of the sampling sites were located in different tributaries of the Pearl River (Guilin and Gongcheng, codes = MZHGL1-2 and MZHGC1, respectively) and one was located in the Yangtze River (Quanzhou,

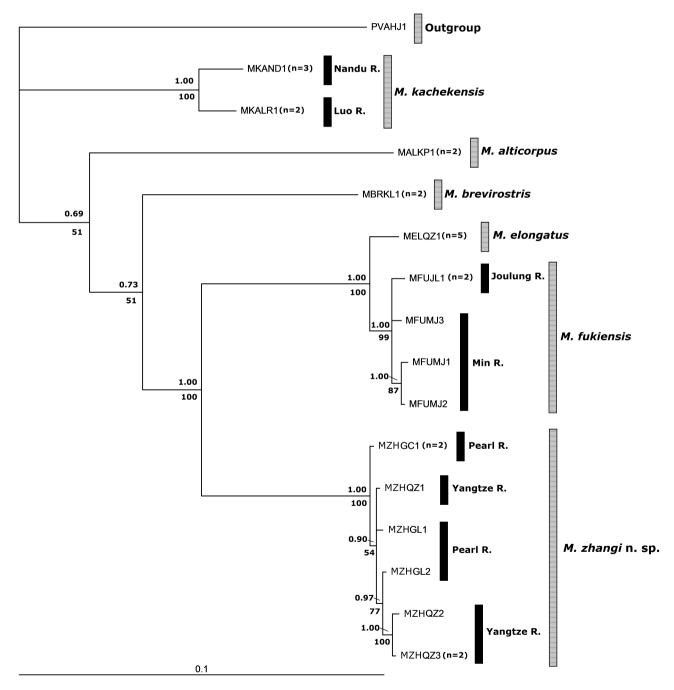


Fig. 4. Molecular phylogenetic tree of *Microphysogobio zhangi* n. sp., and other comparative materials from southern China and Taiwan based on Cyt *b* sequence reconstructed by Bayesian analysis method (values above the branch: posterior probabilities). The similar topology for bootstrap consensus tree by maximum parsimony method (values below the branch) list only the bootstrap. The sample size of each haplotypes were shown behind the OTU.

code = MZHQZ1-3). The tree topology (Fig. 4) revealed that the Guilin populations (MZHGL1-2, in the Pearl River) were closer to the Quanzhou populations (MZHQZ1-3, in the Yangtze River) than the Gongcheng populations (MZHGC1, in another tributary of the Pearl River). These results revealed that the trans-river gene flow has occurred in the Pearl River and the Yangtze River populations. The gene flows between the intraspecies populations from tributaries of the Yangtze River and the Pearl River have also been observed in several Chinese cyprinids such as *Opsariichthys bidens* and *Squalidus argentatus* (Perdices and Coelho 2006; Yang et al. 2012).

This peculiar trans-river gene flow between the Pearl River and the Yangtze River populations might be caused by the Lingqu Canal (Fig. 1), an artificial canal built in the third century B. C. connecting the Yangtze River and the Pearl River. Since the dispersal of freshwater fish was closely related to the history of river connections (Yang et al. 2012), the molecular results suggest that the Lingqu Canal might have formed a pathway directly connecting these two rivers and, consequently, resulting in dispersal and gene flow.

A diagnostic key to all valid species of *Microphysogobio* from southern China and Taiwan

	Medial pad on lower lip undivided 2
	Medial pad on lower lip centrally divided 4
	Lateral-line scales 34 M. tafangensis
	Lateral-line scales more than 36 3
3a.	Dorsal and caudal fin membranes with two rows of black
2h	lines <i>M. chenhisenensis</i> Dorsal and caudal fin membranes without any black lines
50.	<i>M. exilicauda</i>
4a.	Two-third ventral region of belly naked M. nudiventris
4b.	Ventral region covered with scales 5
	Anal fin rays 3, 5 6
	Anal fin rays 3, 6 7
6a.	Lateral-line scales 35-36; vertebral counts 4+30-31
6h	
00.	<i>M. tungtingensis</i>
7a	A "<" shaped black mark on the base of the caudal fin
ra.	<i>M. xianyouensis</i>
7a.	A circular or rectangular mark on the base of the caudal fin
8a.	Lateral-line scales 34; the pearl papillae of posterior lobe
	reduced, posterior tip smoothed M. microstomus
8b.	Lateral-line scales 35-38; the pearl papillae of posterior lobe well-developed, posterior tip free formed
9a.	No crossbar present on dorsal side M. pseudoelongatus
9b.	Dorsal side with distinct crossbars 10
10a	. Vertebral counts 4+30-31 M. fukiensis
10b	. Vertebral counts 4+32-36 11

11a. Gill rakers 4-5	M. kiatingensis
11b. Gill rakers 14-17	12
12a. Vertebral counts 4+34-36, gill rakers 14-15	
	M. yunnanensis
12a. Vertebral counts 4+32-33	13
13a. Vertebral counts 4+32, gill rakers 14	M. elongatus
13b. Vertebral counts 4+33, gill rakers 16-17	M. kachekensis

Other comparative materials

Microphysogobio alticorpus: Holotype, USNM 192926, 63.0 mm SL, small stream and roadside ditch near Chia-I-Hsien (Chia-yi), western coastal plain of Taiwan Agriculture area, Mar. 1961, R. Kunts and W. Wells. Paratypes, USNM 202592, 66 specimens, 36.0-60.7 mm SL, collected with holotype; NTOUP 2007-12-198, 2 specimens, 41.5-49.8 mm SL, Kaoping River, Ligang Township, Pingtung County, Taiwan. NTOUP 2007-12-199, 2 specimens, 36.7-39.6 mm SL, Zhuoshui River, Xiluo Township, Yunlin County, Taiwan. NTOUP 2009-10-112, 1 specimen, 44.2 mm SL, Kaoping River, Shanlin District, Kaohsiung City, Taiwan. NTOUP 2010-05-305, 1 specimen, 36.2 mm SL, Wu River, Caotun Township, Changhua County, Taiwan. NTOUP 2010-11-542, 3 specimens, 37.1-55.0 mm SL, Bazhang River, Fanlu Township, Chiayi County, Taiwan. NTOUP 2010-11-543, 5 specimens, 33.4-58.1 mm SL, Zhuoshui River, Jiji Township, Nantou County, Taiwan.

Microphysogobio brevirostris: Lectotype, BMNH 1865.5.2.49, Formosa, from Consul Swinhoe's collection. Paralectotypes, BMNH 1865.5.2.50-53, 4 specimens; ZMB 6305, 1 specimen; ASIZP 0066723, 2 specimens, 70-73 mm SL, Tamsui River, Pinglin District, New Taipei City, Taiwan; NTOUP 2006-09-724, 3 specimens, 35.6-53.3 mm SL, Keelung River, Tamsui River, Sijiaoting, Keelung City, Taiwan, 5 September 2006. NTOUP 200710-007, 1 specimen, 59.2 mm SL, Dahan River, Tamsui River, Daxi District, Taoyuan City, Taiwan. NTOUP 2010-05-258, 1 specimen, 62.7 mm SL, Keelung River, Tamsui River, Sijiaoting, Keelung City, Taiwan. NTOUP 2010-10-518, 1 specimen, 59.7 mm SL, Keelung River, Tamsui River, Yourui, Keelung City, Taiwan. NTOUP 2010-10-530, 3 specimens, 42.7-48.0 mm SL, Keelung River, Tamsui River, Ruifang District, New Taipei City, Taiwan. NTOUP 2010-11-539, 2 specimens, 66.7-68.9 mm SL, Keelung River, Tamsui River, Ruifang District, New Taipei City, Taiwan. NTOUP 2010-11-540, 1 specimen, 75.7 mm SL, Keelung River, Tamsui River, Nuannuan, Keelung City, Taiwan. NTOUP 2010-11-541, 1 specimen, 62.2 mm SL, Shuangxi River, Gongliao District, New Taipei City, Taiwan.

Microphysogobio chenhsienensis: NTOUP 2010-11-550, 6 specimens, 50.7-52.5 mm SL, Ou River, Yantou Township, Youngjia County, Zhejiang Province, China. NTOUP 2010-11-551, 8 specimens, 53.3-65.9 mm SL, Ou River, Wenzhou City, Zhejiang Province, China.

Microphysogobio elongatus: ASIZP 0078399, 6 specimens, 69.0-77.3 mm SL, Quanzhou County market, Guangxi Province, China.

Microphysogobio exilicauda: NTOUP 2010-11-552, 54.3 mm SL, Gangjiang River, a tributary of the Yangtze River, Ningdu County, Jiangxi Province, China; NTOUP 2010-11-553, 4 specimens, 54.9-58.3 mm SL, Gangjiang River, a tributary of the Yangtze River, Hongjiang Township, Yichun City, Jiangxi Province, China; NTOUP 2010-11-554, 3 specimens, 49.1-53.7 mm SL, Shinjiang River, a tributary of Yangtze River, Zixi County, Jiangxi Province, China.

Microphysogobio fukiensis: NTOUP 2010-11-535, 1 specimen, 73.0 mm SL, Shaowu City market, Fujian Province, China. NTOUP 2010-11-536, 1 specimen, 55.5 mm SL, Xinquan market, Fujian Province, China, 25 June 2006. NTOUP 2010-11-537, 2 specimens, 43.8-61.6 mm SL, Datian County market, Fujian Province, China. NTOUP 2010-11-538, 3 specimens, 43.4-51.2 mm SL, Shaowu City market, Fujian Province, China. NMNSF 01803, 23 specimens, 68.9-77.9 mm SL, Min River, Minhou County, Fujian Province, China. NTOUP 2015-10-001, 8 specimens, 59.0-71.5 mm SL, Pinghe County market, Fujian Province, China.

Microphysogobio kachekensis: NTOUP 2010-11-544, 2 specimens, 63.7-66.9 mm SL, Nandu River, Nankai Township, Hainan Province, China. NTOUP 2010-11-545, 8 specimens, 48.4-61.9 mm SL, Luo River, Dongkeng, Luhe County, Guangdong Province, China.

Microphysogobio kiatingensis: ASIZB 44906, 1 specimen, 74.8 mm SL, Sichuan Province, China.

Microphysogobio microstomus: NTOUP 2010-11-546, 1 specimen, 43.7 mm SL, Taihu lake, Lujiaxiang, Dongshan Township, Suzhou City, Jiangsu Province, China.

Microphysogobio anudarini: Data from Kottelat (2006).

Microphysogobio pseudoelongatus: Holotype, ASIZB 70974, 81.5 mm SL, Fangcheng River, Dali Town, Fangcheng District, Guangxi Province, China; Paratype, ASIZB 70975, 86.5 mm SL. ASIZB 70976, 71.0 mm SL, remaining data same as holotype.

Microphysogobio tafangensis: NMNSF 01653, 1 specimen, 62.0 mm SL, Changhua Township market, Lin'an City, Zhejiang Province, China.

Microphysogobio xianyouensis: Holotype, NTOUP 2010-11-533, 61.3 mm SL, Mulan River, Daji, Xianyou, Fujian Province, China; Paratypes, NTOUP 2010-11-534, 8 specimens, 53.4-60.7 mm SL. ASIZP 0078398, 2 specimens, 56.1-59.5 mm SL, collected with holotype.

Acknowledgments: This work and new species name have been registered with ZooBank under urn:lsid:zoobank.org:pub:3B0A31DB-6880-4AF3-9D79-9F1D464A8967. The authors appreciated to Ms. Jinching Huang for providing great assistances in the field during collecting trip in Guangxi Province, China. We thank Mr. Radford Arrindell who gave a great assistance for taking detailed photographs of Microphysogobio chinssuensis multipapillatus holotype specimen in the American Museum of Natural History. We are grateful to Prof. Mai Dinh Yen for providing the original description of the Rostrogobio nikolskii. Mr. Thien Huynh provided a great help in this study, we also appreciated here. Thanks also to two anonymous reviewers for constructive improvements to the manuscript.

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