A new species of Microphysogobio (Cypriniformes: Cyprinidae) from Fujian Province, China, and a molecular phylogenetic analysis of Microphysogobio species from southeastern China and Taiwan

Authors: Huang, Shih-Pin, Chen, I-Shiung, and Shao, Kwang-Tsao

Source: Proceedings of the Biological Society of Washington, 129(1) : 195-211

Published By: Biological Society of Washington

URL: https://doi.org/10.2988/0006-324X-129.Q3.195

PROCEEDINGS OF THE BIOLOGICAL SOCIETY OF WASHINGTON
129:195–211. Published 29 September 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

Shih-Pin Huang**, I-Shiung Chen**, and Kwang-Tsao Shao*

(SPH, KTS) Biodiversity Research Center, Academia Sinica, 128 Academia Road, Section 2, Nankang, Taipei 11529, Taiwan;
(ISC) Institute of Marine Biology, National Taiwan Ocean University, 2 Beining Road, Jhongjheng, Keelung 20224, Taiwan.

Abstract.—A new species of gudgeon, *Microphysogobio xianyouensis*, is described from Mulan River in Fujian Province, southeastern China. *M. xianyouensis* appears to be closely related to *M. brevirostris* (Günther, 1868) of Taiwan. However, it can be well distinguished based on a combination of meristics, morphometric measurements, color pattern and molecular data. A molecular phylogenetic analysis of species of *Microphysogobio* from southeastern China and Taiwan based on concatenated mitochondrial Cyt *b* and D-loop genes is presented and tree topology strongly supports that *M. xianyouensis* as a distinct species and sister to *M. brevirostris*. Our molecular evidence agreed with Bănărescu's proposed taxonomic viewpoint, the tree topology reveals that the type species of the genus *Huigobio*, *H. chenhsienensis*, is nested within several valid species of *Microphysogobio*, and *Huigobio* is confirmed to be a junior synonym of the genus *Microphysogobio*.

Keywords: Gobioninae, taxonomy, gudgeon, molecular phylogeny, Mulan River

The family Cyprinidae contains 16 subfamilies and about 3042 species (Eschmeyer et al. 2016). Almost all cyprinids are restricted to freshwater habitats; only a few species are found in brackish water habitats (Nelson 2006). Cyprinids are important dominant species of the freshwater fish fauna in China and Taiwan (Chen 1998, Chen & Fang 1999, Chen et al. 2008).

Among cyprinids, the subfamily Gobioninae consists of about 29 genera and 200 species of benthic fishes widely distributed in Eurasian freshwater bodies (Nelson 2006, Jiang et al. 2012). Among these, the genus of small gudgeons *Microphysogobio* Mori, 1934, was described based on *M. hsinglungshanensis* Mori, 1934 (Jiang et al. 2012). Species of *Microphysogobio* are widely distributed in eastern Asia, including Korea, China, Mongolia, Taiwan, Vietnam and Laos, and usually occur in upper and middle reaches of rivers (Wu 1977, Xie 1986, Cheng & Zheng 1987, Pan 1991, Kottelat 2001a, b; Zhao & Zhang 2001, Kottelat 2006, Jiang et al. 2012). A total of 27 species of *Microphysogobio* are considered valid, 17 of which are found in China. Six of these are considered restricted to northern China and the remaining 11 to southern China (Wu 1977, Chen 1998,

* Corresponding author (KTS).
Address: 128 Academia Road, Section 2, Nankang, Taipei 11529, Taiwan, Biodiversity Research Center, Academia Sinica.
E-mail: zoskt@gate.sinica.edu.tw
Tel: +886-2-2789-9545 #200
** Both SPH & ISC have equal contributions to this paper.
DOI: 10.2988/0006-324X-129.Q3.195

Jiang et al. 2012). In this region, the Yangtze River is the longest river in China and its basin encompasses the type localities of four species of *Microphysogobio*, including *M. kiatingensis* (Wu, 1930), *M. nudiventris* Jiang, Gao & Zhang, 2012, *M. microstomus* Yue, 1995, and *M. tungtingensis* (Nichols, 1926a). To the south of the Yangtze River, the Pearl River is the third longest river in China, flowing through Guangxi and Guangdong Provinces (Fig. 1). One valid species of *Microphysogobio*, *M. elongatus* (Yao & Yang, 1977) is described from this basin.

An additional six valid species were described or recorded in different basins in southern China. *M. tafangensis* (Wang, 1935) was described in the Qiantang River, Zhejiang Province; *M. chenhisenensis* (Fang, 1938) was recorded in independent rivers of Zhejiang Province; *M. fukiensis* (Nichols, 1926b) was described in the Min River, Fujian Province; *M. kachekensis* (Oshima, 1926) was recorded in Hainan and Guangdong Provinces; *M. pseudoelongatus* Zhao & Zhang, 2001 was described in the Fangcheng River, Guangxi Province; *M. yunnanensis* (Yao & Yang, 1977) was described in the Yuan River, Yunnan Province. An additional nominal species, *M. labeoides* (Nichols & Pope, 1927) was regarded as a junior synonym of *M. kachekensis* (Kottelat 2001b) and *M. suifuensis* (Wu, 1930) was regarded as a junior synonym of *M. kiatingensis* (Wu, 1977). However, rigorous comprehensive studies of the genus have been lacking and the taxonomy of the group is still poorly understood (Jiang et al. 2012).

Previous studies have strongly indicated that the Taiwanese freshwater fish fauna is most similar to that of the southeastern mainland China region, including Zhejiang, Fujian and Guangdong Provinces (Chen & Fang 1999). Recently, several cyprinids have been described as new species in Fujian Province, China and Taiwan based on morphological and molecular evidence (Chen & Chang 2007, Jang-Liaw & Chen 2013). Compared to Taiwan's two valid species of *Microphysogobio*, Fujian Province is three times greater in area, but only a single species of *Microphysogobio*, *M. fukiensis*, was previously known and further investigation seemed warranted.

Although a molecular phylogeny of the subfamily Gobioninae has been published (Tang et al. 2011). Only a few valid species of *Microphysogobio* were included in that study and a comprehensive hypothesis of relationships is lacking. The validity of the genus *Huigobio* Fang, 1938 is still controversial. In earlier studies, the genus *Huigobio* was regarded as independent, though closely related to *Microphysogobio* based on an entire versus medially dissected heart-shaped central pad on the lower lip (Fang 1938, Wu 1977, Cheng & Zheng 1987, Chen 1998). However, Bănărescu (1992) regarded *Huigobio* as a junior synonym of *Microphysogobio*. The molecular study of the subfamily Gobioninae by Tang et al. (2011) resulted in a tree topology indicating that six valid species of *Microphysogobio* and *Huigobio chinssuensis* (Nichols, 1926a) formed a monophyletic group. Based on this evidence, Jiang et al. (2012) agreed with Bănărescu and suggested that the genus *Huigobio* might be a junior synonym of *Microphysogobio*. Subsequently, Jiang and Zhang (2013) proposed that the genus *Huigobio* is an independent clade, and therefore regarded it as a valid genus. A new species, *Huigobio exilicauda* Jiang & Zhang, 2013 also was described in that study. A new species of *Microphysogobio* was discovered during a survey of freshwater fish in Fujian Province, China, and is described herein. A new molecular phylogenetic study, expanded over previous efforts, includes this new species, as well as the type species of the genus *Huigobio*, *H. chenhisenensis* Fang, 1938 and other putatively related valid species from southeastern China and Taiwan.

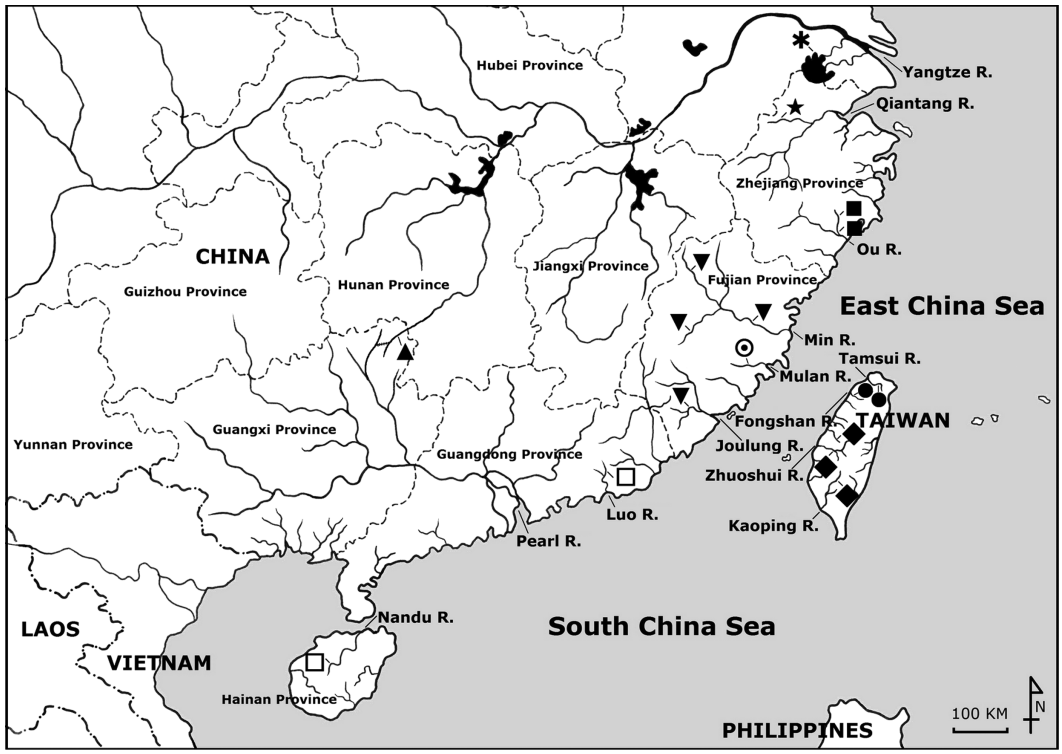

Fig. 1. The sampling localities of *Microphysogobio xianyouensis* and comparative species from southern China and Taiwan. ⊙, *M. xianyouensis*; ◆, *M. alticorpus*; ●, *M. brevirostris*; ■, *M. chenhsienensis*; ▲, *M. elongatus*; ▼, *M. fukiensis*; □, *M. kachekensis*; ✻, *M. microstomus*; ★, *M. tafangensis*.

Materials and Methods

All examined specimens were collected by cast net. 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 and then transferred to 70% ethanol for long-term preservation. All sampling localities of species of *Microphysogobio* are shown in Fig. 1. The morphological measurements followed Hosoya et al. (2002) and meristic counts followed Chen et al. (2009). The meristic abbreviations are as follows: A, anal fin; D, dorsal fin; LL, lateral line scales; P1, pectoral fin; P2, pelvic fin; PreD, predorsal scales; TR, transverse scale series; VC, vertebral count. Standard length (SL) is used for measurement of specimens. Number of vertebrae was based on X-ray photographs. Values for barbel length and length of posterior lobe of papillae (Figs. 3, 4) are presented as percent of length of eye diameter (Table 4).

All examined materials were deposited at the National Taiwan Ocean University, Keelung, Taiwan (NTOUP); 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); and National Museum of Natural Science, Taichung, Taiwan (NMNS). Our molecular study was based on the full length of mtDNA sequence Cytochrome *b* (Cyt *b*) and the control region (D-loop). DNA extractions were performed using the High Pure Product Preparation kit (Roche). Cyt *b* was ampli-

Fig. 2. Specimen photographs of *Microphysogobio xianyouensis* and its sister species, *M. brevirostris*. A, *M. xianyouensis*, holotype, NTOUP 2010-11-533, 61.3 mm SL. B, *M. brevirostris*, NTOUP 2010-05-258, 62.7 mm SL.

fied using the primers: cytbF1 (5′-TGA CTT GAA GAA CCA CCG TTG TA-3′) and cytbR1 (5′-CGA TCT TCG GAT TAC AAG ACC GAT G-3′); D-loop was amplified using the primers: P-CPTHRA (5′-AAA GCA TCG GTC TTG TAA TCC GAA G-3′) and CB-Phe-SR1 (5′-CAT CTT CAG TGC TAT GCT TT-3′).

PCR amplifications were performed on a MODEL 2700 or 9700 thermocycler (Perkin-Elmer) in a final 50 μL reaction volume containing: 33.5 μL of sterile distilled water, 5 μL of 10X PCR buffer (Takara), 4 μL of dNTP (2.5 mM each), 3 μL of Mgcl2 (2.5 mM each), 1 μL of each primer, 0.5 μL of 0.5 unit Ex *Taq* (Takara) and 2 μL of template. The therm cycle procedure (35 cycles) was as follows: denaturation at 94°C for 60 seconds, annealing at 52–58°C for 60 seconds and extension at 72°C for 120 seconds. A negative control without template accompanied each run of PCR. The results are visualized on ethidium-bromide stained agarose gels.

Double-stranded PCR products were purified using a kit (Roche, High Pure Product Purification kit), before undergoing direct cycle sequencing with dye-labeled terminators (ABI Big-Dye kit). The sequencing primers used were same as those listed for PCR. All sequencing reactions were performed according to the manufac-

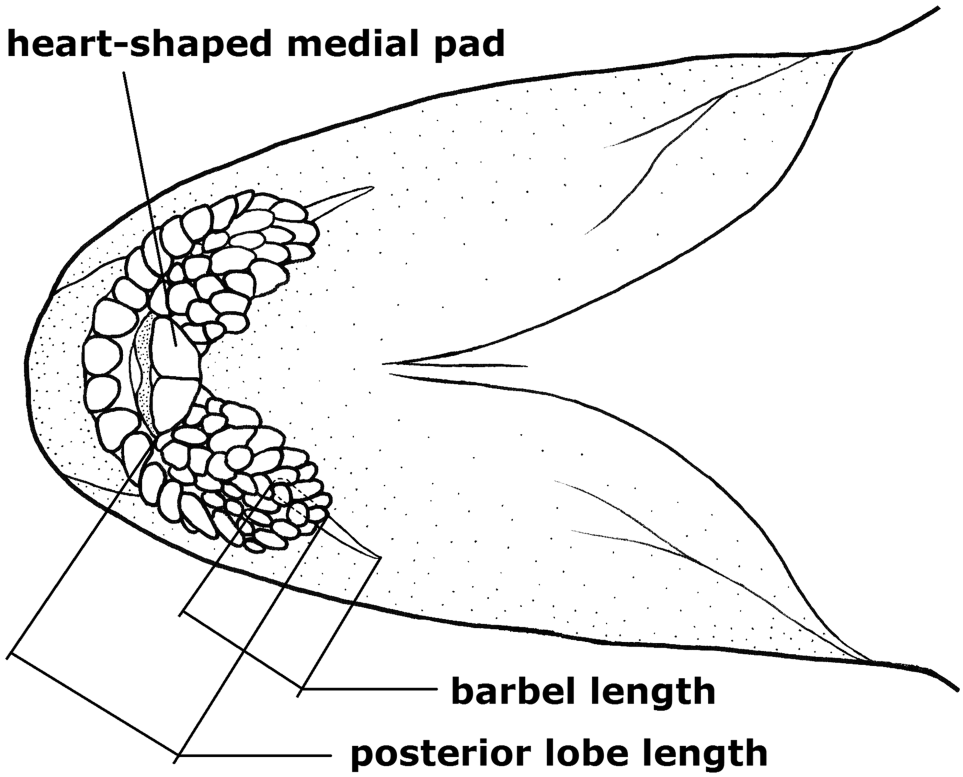

Fig. 3. An illustration for morphometric measurements of lip papillae system.

turer's instructions. Labeled fragments were analyzed using as ABI PRISM Model 377-64 DNA Automated sequencer (ABI).

Nucleotide sequence alignment was verified visually using BIOEDIT version 5.9 (Hall 2001). The analyses of the sequences were conducted using Molecular Evolutionary Genetics Analysis (MEGA) version 6.0 (Tamura et al. 2013). MEGA 6.0 was also used for aligning unequal length sequences, and then manual modifications were performed before phylogenetic analysis.

The ML trees reconstructed by concatenated Cyt *b* and D-loop sequences, and single Cyt *b* and D-loop sequences were based on HKY+G, HKY+G and T92+G models, respectively. The HKY+G model was selected as the best-fit model for the BI trees reconstructed by concatenated Cyt *b* and D-loop sequences, and single Cyt *b* and D-loop sequences calculated by jModelTest v.2.1.3.

The molecular clock has often been used for estimating divergence times in biogeographic studies (Smith et al. 2002, Wang et al. 2007, Liao et al. 2008). Bayesian inference (BI) and Maximum likelihood (ML) were employed for phylogenetic analyses in this study. The ML analyses were carried out using MEGA 6.0. Branch support for ML trees were established via bootstrap analyses (1000 replications). The best-fit model for sequence evolution was selected by the jModelTest v.2.1.3 (Darriba et al. 2012) in the BI analyses. The best-fit model was selected by the MEGA 6.0 in the ML analyses.

Concatenated Cyt *b* and D-loop sequences and each single gene were analyzed and reconstructed phylogenetic tree by the BI and ML methods. The BI analyses were performed using MrBayes 3.0 (Ronquist & Huelsenbeck 2003), a total of 1,000,000 replications were performed. The posterior probabilities of each

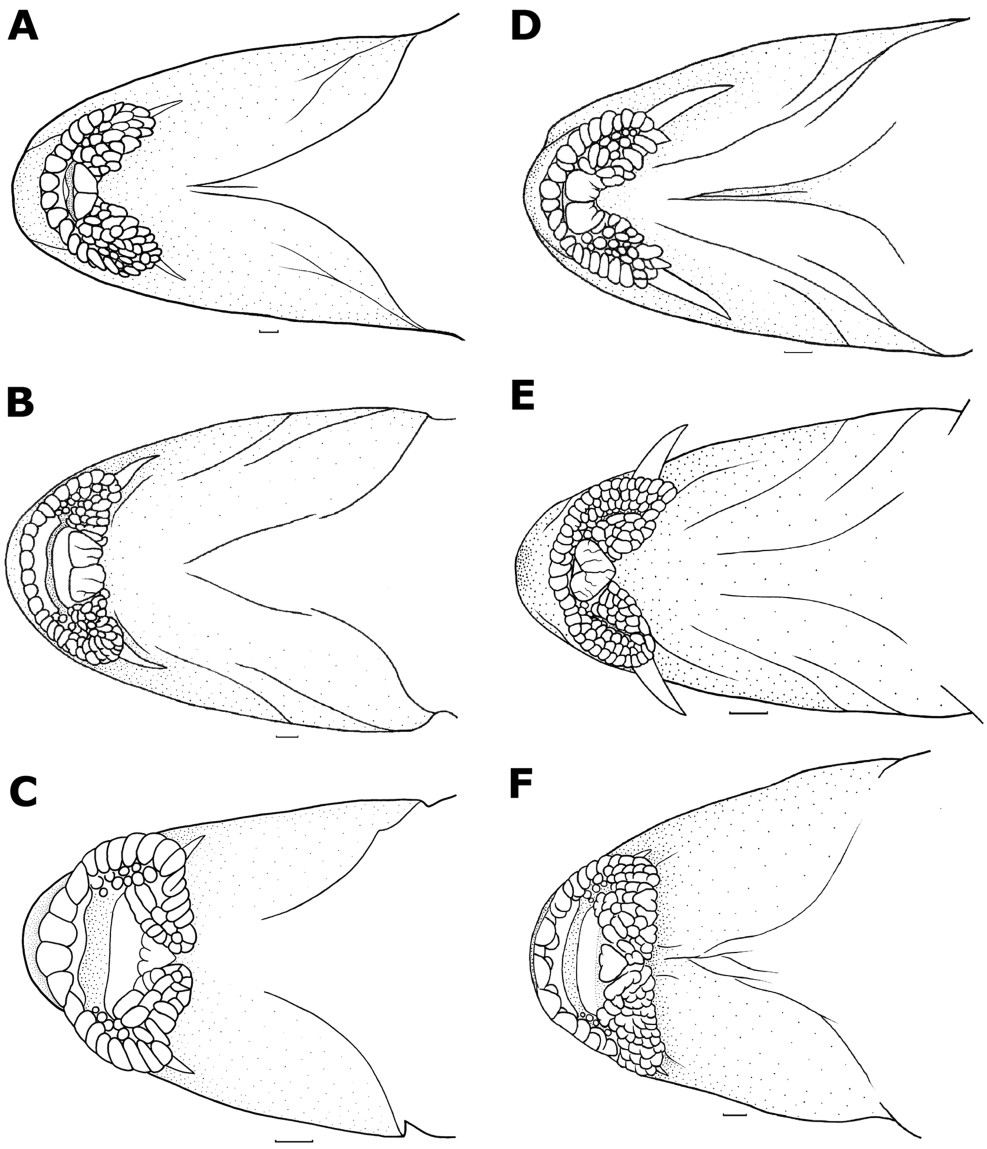

Fig. 4. Lip papillae system of *Microphysogobio xianyouensis* and five other comparative species. A, *M. xianyouensis*, holotype, NTOUP 2010-11-533, 61.3 mm SL. B, *M. brevirostris*, NTOUP 2010-11-540, 75.7 mm SL. C, *M. chenhsienensis*, NTOUP 2010-11-550, 52.5 mm SL. D, *M. fukiensis*, NTOUP 2010-11-535, 73.0 mm SL. E, *M. kachekensis*, NTOUP 2010-11-545, 61.9 mm SL. F, *M. tafangensis*, NMNSF01653, 62.0 mm SL. Bar = 1mm.

node were computed from the remaining 75% of all sampled trees. The molecular clock was calculated using MEGA 6.0 for estimating divergence time, and the molecular clock was tested using the Tajima's Relative Rate Test in MEGA 6.0.

Results

Molecular phylogenetic analysis.—Codes of each species and GenBank accession numbers are given in Table 1. *Carassius auratus langsdorfi* was used as the outgroup species obtained from GenBank

Table 1.–Sampling localities, OTU codes and accession numbers of *M. xianyouensis* and other valid species of *Microphysogobio* for molecular analysis from southeastern China and Taiwan.

Code	Sample size	Species	Locality	Accession number		Source
				Cytb	D-loop	
MALKP1	1	*Microphysogobio alticorpus*	Kaoping River, Ligang Township, Pingtung County, Taiwan	KM999925	KM999933	This study
MALKP2	1	*Microphysogobio alticorpus*	Kaoping River, Shanlin District, Kaohsiung City, Taiwan	KM999925	KM999934	This study
MBRKL1	1	*Microphysogobio brevirostris*	Keelung River, Tamsui River, Sijiaoting, Keelung City, Taiwan	KM999926	KM999935	This study
MBRFS1	1	*Microphysogobio brevirostris*	Fongshan River, Hsinchu County, Taiwan	KM999926	KM999936	This study
MCHOJ1	1	*Microphysogobio chenhsienensis*	Ojiang River, Yantou, Youngjia County, Zhejiang Province, China	KT075097	KT075100	This study
MCHOJ2	1	*Microphysogobio chenhsienensis*	Ojiang River, Yantou Township, Youngjia County, Zhejiang Province, China	KT075098	KT075100	This study
MFUMJ1	1	*Microphysogobio fukiensis*	Shaowu City market, Fujian Province, China	KM999927	KM999937	This study
MFUMJ2	1	*Microphysogobio fukiensis*	Min River, Shaowu City, Fujian Province, China	KM999928	KM999937	This study
MFUMJ3	1	*Microphysogobio fukiensis*	Min River, Xinquan, Fujian Province, China	KM999929	KM999937	This study
MKAND1	2	*Microphysogobio kachekensis*	Nandujiang River, Nankai Township, Hainan Province, China	KM999930	KM999938	This study
MXIML1	3	*Microphysogobio xianyouensis*	Mulan River, Daji Township, Xianyou County, Fujian Province, China	KM999931	KM999939	This study
MTAQT1	1	*Microphysogobio tafangensis*	Changhua Township market, Lin'an City, Zhejiang Province, China	KT075099	KT075101	This study
CAURA1	1	*Carassius auratus langsdorfi*	Japan	NC002079	NC002079	Murakami et al. 1998

(NC002079) (Murakami et al. 1998). The aligned Cyt *b* and D-loop sequence consisted of 9 haplotypes which were from seven species of *Microphysogobio* with 15 individuals as the in-group. The length of the combined sequence of Cyt *b* and D-loop sequence is 2030–2076 bp (1141 bp and 889–935 bp in Cyt *b* and D-loop,

Table 2.–Morphometric measurements of *M. xianyouensis*.

	Holotype	Holotype + Paratypes	Ave.
n		6	
Percentage of standard length (%)			
Head length	21.4	20.9–22.3	(21.7)
Body depth	15.7	15.7–16.8	(16.2)
Body width	13.9	12.9–14.7	(13.9)
Depth of caudal peduncle	9.0	8.4–9.0	(8.8)
Length of caudal peduncle	19.9	19.4–21.3	(20.3)
Predorsal length	43.8	42.7–44.4	(43.7)
Preanal length	60.4	59.7–61.2	(60.3)
Prepelvic length	50.5	49.2–50.5	(49.9)
Height of dorsal fin	20.8	20.8–22.3	(21.7)
Length of depressed dorsal	23.5	23.4–24.5	(23.9)
Length of dorsal fin base	14.6	13.2–14.7	(14.3)
Height of anal fin	15.2	15.0–16.7	(15.8)
Length of depressed anal	16.6	15.3–17.4	(16.8)
Length of anal fin base	9.2	8.4–9.2	(9.0)
Pectoral fin length	22.6	22.1–24.4	(23.3)
Pelvic fin length	18.0	17.8–20.1	(18.9)
Percentage of head length (%)			
Head depth	62.5	60.0–62.5	(61.4)
Head width	64.0	63.5–65.5	(64.4)
Snout length	46.3	43.3–46.3	(45.1)
Orbit diameter	27.7	27.4–29.5	(28.4)
Interorbital width	28.9	28.4–30.2	(29.1)

respectively). This alignment contains 582 total mutations and 510 polymorphic (segregating) sites, calculated by DNA sequence polymorphism (DnaSP v5) (Librado & Rozas 2009).

The phylogenetic trees reconstructed by either ML or BI methods produced very similar tree topologies based on concatenated Cyt *b* and D-loop sequences or single D-loop sequence, respectively (Figs. 5, 6). The phylogenetic trees reconstructed by BI and ML methods

Table 3.–Frequency distribution of meristic features of *M. xianyouensis* and other comparative species of *Microphysogobio* from southern China and Taiwan.

	D		A			P1			P2				LL			TR			PreD				VC			
	3,7	x	3,5	3,6	x	1,10	1,11	1,12	1,13	x	1,6	1,7	1,8	x	34	35	36	37	38	39	x	6	7	8	x	9	10	11	12	x	4+30	4+31	4+32	4+33	4+34	x
M. xianyouensis	12	7.0	–	12	6.0	–	4	12	–	11.8	–	20	–	7.0	–	–	6	14	–	–	35.7	–	12	–	7.0	–	12	–	–	10.0	–	–	5	1	–	30.6
M. alticorpus	14	7.0	–	14	6.0	5	20	3	–	10.9	2	22	–	6.9	–	10	18	–	–	–	35.6	–	–	14	8.0	–	–	12	2	11.1	7	3	–	–	–	30.3
M. brevirostris	13	7.0	–	13	6.0	–	12	16	–	11.6	–	32	2	7.1	–	–	–	–	10	16	38.6	–	–	13	8.0	–	4	8	1	10.8	–	–	2	6	33.8
M. chenhsienensis	12	7.0	12	–	5.0	–	15	2	–	11.1	–	12	–	7.0	–	–	3	17	4	–	37.0	–	12	–	7.0	7	5	–	–	9.4	–	–	–	6	2	33.3
M. elongatus	6	7.0	–	6	6.0	–	–	8	4	12.3	–	6	–	7.0	–	–	–	9	3	–	37.3	–	6	–	7.0	2	4	–	–	9.7	–	–	4	–	–	32.0
M. fukiensis	18	7.0	3	15	5.8	–	14	10	–	11.4	4	26	–	6.9	–	14	13	1	–	–	35.5	15	–	–	6.0	10	5	–	–	9.3	2	5	–	–	–	30.7
M. kachekensis	10	7.0	–	10	6.0	–	–	4	11	12.7	–	19	–	7.0	–	–	–	14	6	–	37.3	–	10	–	7.0	–	7	3	–	10.3	–	–	–	2	–	33.0
M. microstomus	1	7.0	–	1	6.0	2	–	–	–	10.0	–	2	–	7.0	2	–	–	–	–	–	34.0	–	1	–	7.0	–	1	–	–	10	1	–	–	–	–	30.0
M. tafangensis	1	7.0	1	–	5.0	–	–	2	–	12.0	–	2	–	7.0	2	–	–	–	–	–	34.0	–	1	–	7.0	1	–	–	–	9.0	–	–	–	1	–	33.0

Table 4.–Morphometric measurements of lip papillae system of *M. xianyouensis* and other six valid species of *Microphysogobio* from southeastern China and Taiwan.

Species / Characters	n	Barbel length in percentage of eye diameter	Ave.	Posterior lobe length in percentage of eye diameter	Ave.
M. xianyouensis	6	66.6–72.6	69.7	75.2–79.4	76.6
M. alticorpus	8	51.6–63.0	57.8	53.7–69.0	62.0
M. brevirostris	6	72.3–81.3	75.4	50.1–57.7	54.8
M. chenhsienensis	10	40.3–48.4	44.9	46.8–55.4	51.8
M. fukiensis	6	100.3–119.3	106.9	80.3–94.2	84.7
M. kachekensis	6	68.1–77.6	71.2	69.6–78.1	74.4
M. tafangensis	1	30.8		72.5	

based on concatenated Cyt *b* and D-loop sequences show similar topology with three major clades (Fig. 5). The tree topology reveals that the *M. kachekensis* is the earliest offshoot (clade I), *M. xianyouensis* and *M. brevirostris* formed a sister pair (clade II) sister to clade III, which consists of two sister groups (*M. tafangensis* and *M. chenhsienensis*, and *M. fukiensis* - *M. alticorpus*). Trees for *M. xianyouensis* and its sister species *M. brevirostris* have strong bootstrap support (ML) and a high posterior probability (BI) at most nodes. Nodes between *M. xianyouensis* and *M. brevirostris* were as high as 1.00 and 90 in BI and ML trees.

The Cyt *b* tree reconstructed by ML and BI methods produced a different tree topology. Although bootstrap values at nodes between *M. fukiensis* - *M. alticorpus* and *M. xianyouensis* - *M. brevirostris* were lower than 50 in ML tree (Fig. 8), bootstrap values at nodes between *M. xianyouensis* and *M. brevirostris* or *M. tafangensis* and *M. chenhsienensis* were as high as 90 and 99, respectively.

Genetic distances of relationships among *M. xianyouensis* and the other six species range from 11.9–23.3% and 2.9–24.0% based on Cyt *b* and D-loop sequences, respectively, using Kimura 2 parameter model (K2P). The molecular evidence strongly supports *M. xianyouensis* as a distinct species.

In Cyt *b* sequence, a divergence rate of 1% per million years has been hypothesized for cyprinids in a previous study (Smith et al. 2002) and that rate is adopted for the present analysis. Based on this divergence rate, a divergence time of 11,900,000 years ago is implied between *M. xianyouensis* and its sister species *M. brevirostris*.

A test using Tajima's relative rate test in MEGA 6.0 was performed for testing reliability of the molecular clock. In this test, the equality of evolutionary rate between *M. xianyouensis* (MXIML1) and *M. brevirostris* (MBRKL1) was calculated, and *M. fukiensis* (MFUMJ1) was used as an outgroup in Tajima's relative rate test. A P-value less than 0.05 is used to reject the null hypothesis of equal rates between lineages. Our results yield a χ^2 test statistic of 0.47 (P = 0.49130 with 1 degree of freedom). The present molecular clock is regarded as reliable.

Systematics

Microphysogobio xianyouensis sp. nov.
(Figs. 1, 2A, 4A)

Holotype.—NTOUP 2010-11-533, 61.3 mm SL, collected on 20 Dec 2009 by J. C. Liu.

Type locality.—Mulan River, Daji Township, Xianyou County, Fujian Province, China (latitude: 25.367720, longitude: 118.641998).

Paratypes.—NTOUP 2010-11-534, 8, 53.4–60.7 mm SL. ASIZP78398, 2, 56.1–59.5 mm SL, all paratypes collected with holotype.

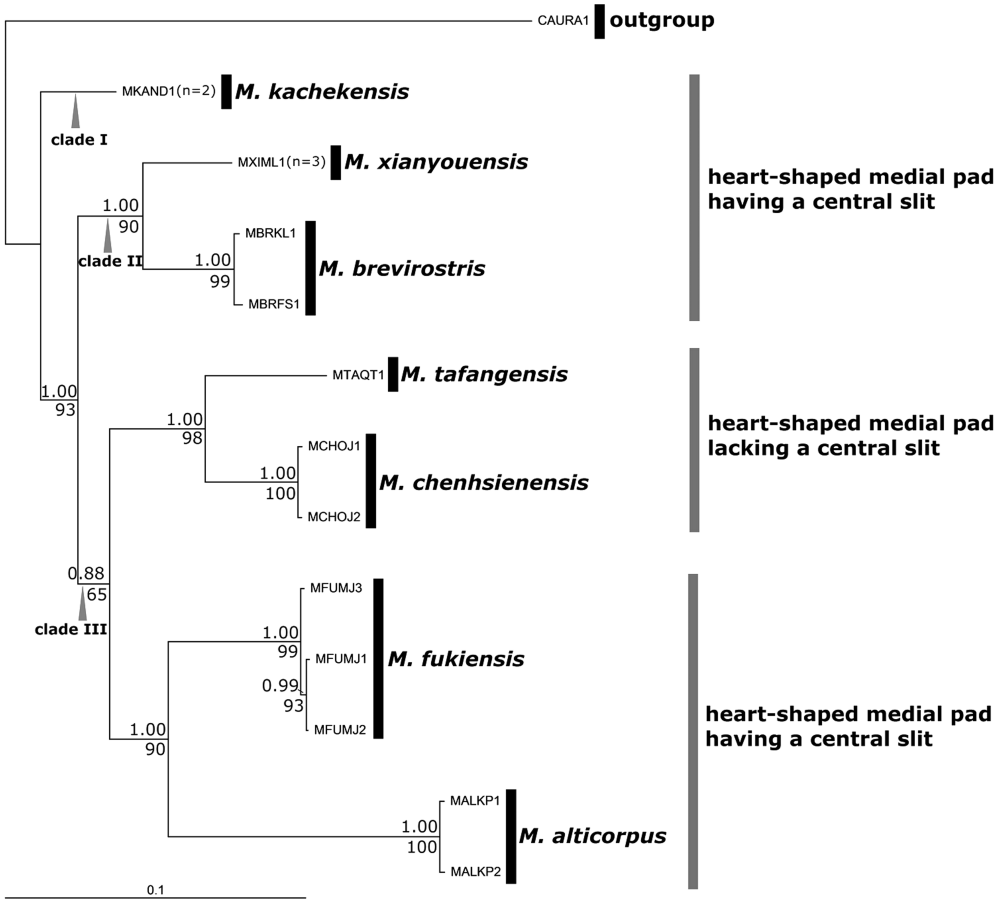

Fig. 5. Molecular phylogenetic tree of species of *Microphysogobio* from southeastern China and Taiwan based on concatenated Cyt *b* and D-loop sequences reconstructed by Bayesian inference (values above the branch: posterior probability). Bootstrap values of ML tree only given below the branch. The sample sizes of each of the haplotypes are shown following the OTU.

Diagnosis.—This new species can be distinguished from congeners by the combination of characteristics: anal-fin rays 3, 6; pectoral-fin rays 1, 11–12 (modally 12); lateral-line scales 35–36 (modally 36); transverse scales 7; predorsal scales 10; vertebral counts 4+32–33 (modally 4+32); two horizontally-aligned black dishes at and bottom of each lateral-line scale; caudal-fin base with a small "<" shaped black mark, caudal-fin membrane with 2–3 rows of vertically aligned dashes; and posterior lip-papillae lobes moderately long, 75.2–79.4% of eye diameter, barbel length medium, 66.6–72.6% of eye diameter.

Description.—Body elongate and compressed laterally. Belly flattened, snout pointed. Eyes moderately large and placed dorsolaterally on head. Numerous tiny papillae distributed on cheek, preoperculum, operculum and inter-orbital space in adult male, and covered with very few tiny papillae in female. Vertebral counts 4+32–33 (modally 4+32). Total gill rakers 14–15. The morphometric measurements of *M. xianyouensis* and congeners are given in Table 2.

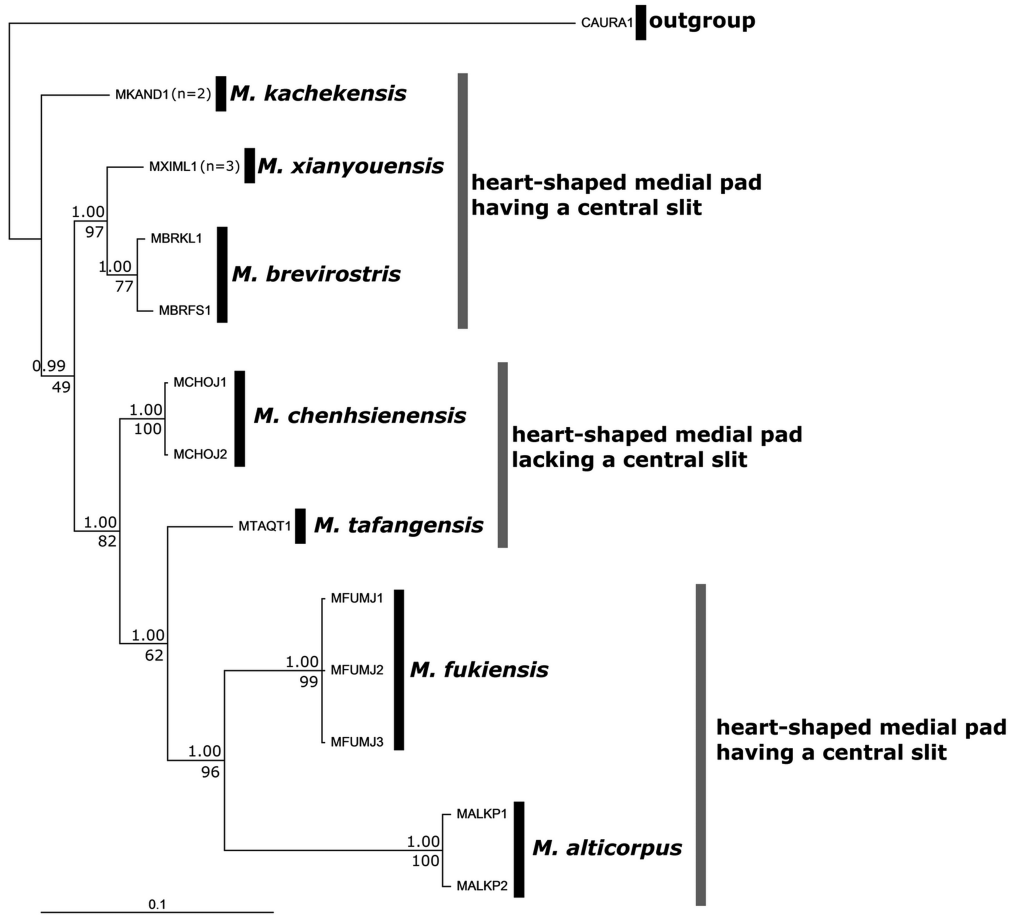

Fig. 6. Molecular phylogenetic tree of species of *Microphysogobio* based on D-loop sequences reconstructed by Bayesian inference (values above the branch: posterior probability). Bootstrap values of ML tree only given below the branch. The sample sizes of each of the haplotypes are shown following the OTU.

Dorsal-fin rays 3, 7, anal-fin rays 3, 6, pectoral-fin rays 1, 11–12 (modally 12), pelvic-fin rays 1, 7. Pectoral-fins reach anterior margin of anal-fin when compressed. Pelvic-fins rounded. Anterior margin of anal-fin inserted below fifth branched ray of dorsal-fin. Caudal-fin deeply forked, lower lobe slightly longer than upper lobe.

Lateral-line scales 35–36 (modally 36), transverse scales 7, predorsal scales 10. Body covered with moderate-sized cycloid scales, belly covered with cycloid scales, inter-pectoral fin region naked. Lateral-line complete and dipping slightly downward above pectoral fin to run along the ventral profile onto middle of caudal fin base. The frequency distributions of fin ray counts and scale series of *M. xianyouensis* and other congeners are given in Table 3.

Lip papillae arrangement.—Mouth horseshoe-shaped. Upper and lower lips thick, and covered with pearl-like papillae. The lip arrangement consists of an anterior lip with a row of large papillae, rear lip with two posterior lobes, and a heart-shaped medial pad. The posterior lobes are covered with many well-developed papillae, forming a grape-shaped cluster of papillae in each lobe. The heart-shaped

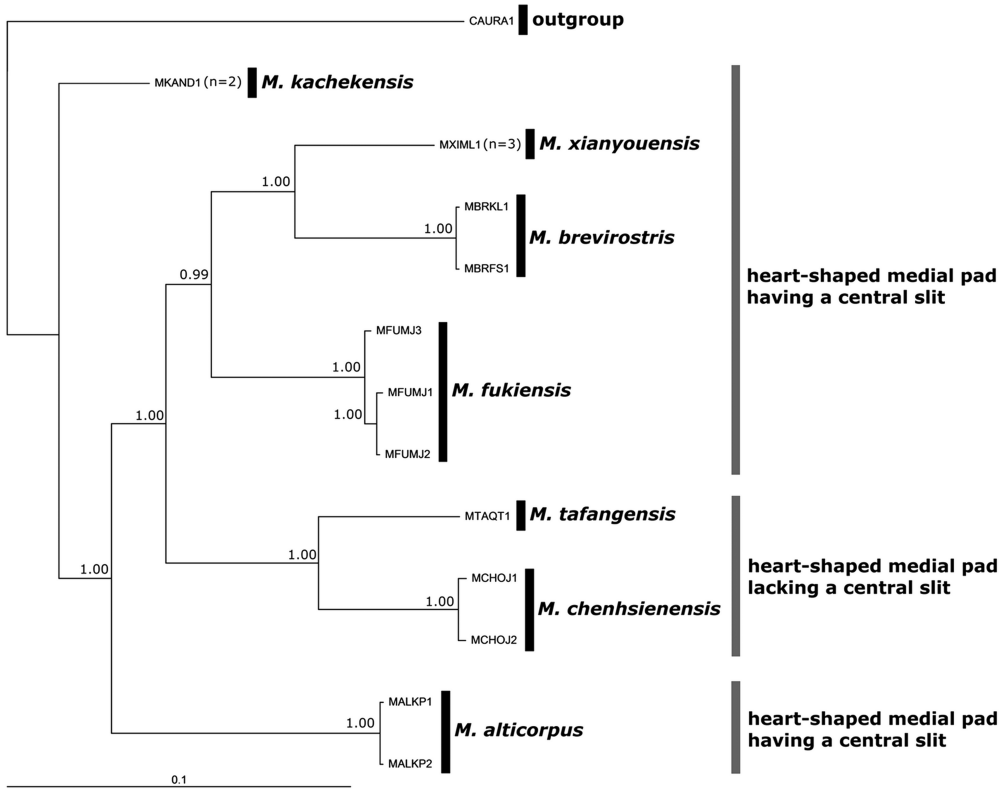

Fig. 7. Molecular phylogenetic tree of species of *Microphysogobio* based on Cyt *b* sequences reconstructed by Bayesian inference. The sample sizes of each of the haplotypes are shown behind the OTU.

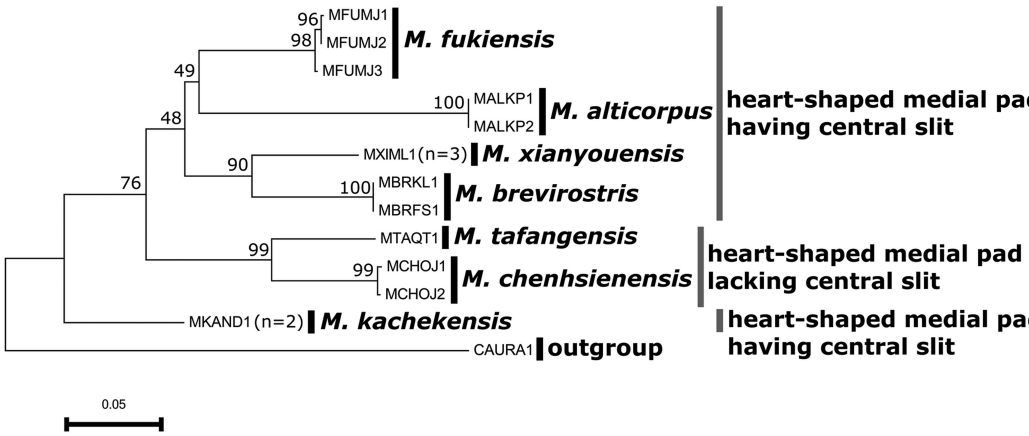

Fig. 8. Molecular phylogenetic tree of species of *Microphysogobio* based on Cyt *b* sequences reconstructed by Maximum likelihood. The sample sizes of each of the haplotypes are shown behind the OTU.

medial pad is dissected medially. Posterior lobes long, 75.2–79.4% of eye diameter. A single barbel rooted at posterior corner of each jaw, barbel length medium, 66.6–72.6% of eye diameter. Comparisons of lip papillae structure and morphometric measurements of *M. xianyouensis* and other species are given in Fig. 4 and Table 4.

Coloration in fresh specimen.—Head and upper side of body generally yellowish brown, belly pale grayish white. Two horizontally aligned black dashes above and below the each lateral-line scale. Dorsum with four distinct black saddles, first positioned beneath dorsal-fin origin, second posterior to dorsal fin, and two on peduncle.

Operculum and suborbital areas with distinct black blotches. Dorsal-fin membrane with three rows of horizontally aligned black dashes. Upper portion of pectoral-fin base with horizontal black bar, pectoral-fin membrane with small irregular black spots. Pelvic-fin and anal-fin membranes translucent. Caudal-fin base with a small "<" shaped black mark. Caudal-fin membrane with two or three rows of vertically aligned black dashes. Photographs of *M. xianyouensis* and its sister species, *M. brevirostris* are given in Fig. 2.

Distribution.—Known only from middle reaches of the Mulan River in eastern Fujian Province, China.

Etymology.—The Latin specific name, "*xianyouensis*" refers to "Xianyou County", in eastern Fujian Province, China, wherein lies the type locality.

Comparisons.—Compared to all 20 valid species of *Microphysogobio* from China and Taiwan, *M. xianyouensis* can be readily distinguished from *M. chenhsienensis*, *M. chinssuensis*, *M. exilicauda*, *M. tafangensis* and *M. wulonghensis* Xing, Zhao, Tang & Zhang, 2011 by the medially dissected lower lip pad (vs. pad entire). *Microphysogobio xianyouensis* can be distinguished from *M. hsinglungshanensis*, *M. liaohensis* (Qin, 1987), *M. linghensis* Xie, 1986 and *M. nudiventris* by having the midventral region covered with scales (vs. naked). *Microphysogobio xianyouensis* can be distinguished from the following six species by having 35–36 lateral-line scales vs. 38–39 for *M. tungtingensis*, 39–42 for *M. amurensis* (Taranetz, 1937), 38–40 for *M. yunanensis*, and 37–38 for *M. elongatus*, *M. pseudoelongatus* and *M. kachekensis*). *Microphysogobio xianyouensis* can be distinguished from *M. alticorpus* Bănărescu & Nalbant, 1968, *M. fukiensis* and *M. microstomus* by having higher vertebral counts (4+32–33 vs. 4+30 for *M. microstomus*; 4+30–31 for *M. alticorpus* and *M. fukiensis*). *Microphysogobio xianyouensis* can be distinguished from *M. kiatingensis* by having more total gill rakers (14–15 vs. 4–5), fewer pectoral-fin rays (1, 11–12 vs. 1, 13), and two horizontally aligned black dashes at top and bottom of each lateral-line scale vs. no horizontally aligned black dashes on each lateral line scale.

Of all the valid species of *Microphysogobio*, *M. xianyouensis* appears to be most closely related to *M. brevirostris* based on molecular evidence and some morphological features and pigmentation. Both species share the similar spotted fin membranes and lateral body coloration. However, *M. xianyouensis* can be distinguished from *M. brevirostris* by having fewer lateral line scale series: 35–36 vs. 38–39, fewer transverse scale series: 7 vs. 8; and a small "<" shaped black mark vs. a large circular black mark on the base of the caudal fin.

This work has been registered in ZooBank with the registration number LSID: 07F161 94-0532-4CBE-8666-44FE2D5C7BD3

Discussion

Molecular and morphological evidences strongly support *M. xianyouensis* as a distinct species, these evidences also provide well resolved phylogeny for the genus *Microphysogobio*. Furthermore, the molec-

ular clock provide a clue for speciation to connect with paleogeography. All tree topologies strongly support *M. xianyouensis* and *M. brevirostris* formed a sister pair (clade II). Molecular clock results based on the ML tree showed that divergence time of *M. xianyouensis* of mainland China and its sister species, *M. brevirostris* of Taiwan is 11,900,000 years ago, it means that two sister species were separated during the Miocene.

The genetic distance can be regarded as a reference for assessing the taxonomic status at the specific level (Costagliola et al. 2004, Mukai et al. 2005, Chen et al. 2009, Huang et al. 2013). Compared to other Taiwanese inland freshwater cyprinids, the genetic distance of D-loop sequences between *M. xianyouensis* and other species of *Microphysogobio* (2.9–24.0%) are generally equal to or higher than that of two other species of Taiwanese cyprinids, *Opsariichthys pachycephalus* (Günther, 1868) and *Opsariichthys kaopingensis* Chen & Wu, 2009 (2.6–3.8%) (Chen et al. 2009).

All tree topologies showed *M. kachekensis* to be the earliest offshoot. Although the present analyses produced different tree topologies, the "heart-shaped medial pad lacking a central dissection" (clade II) is always nested among other species of *Microphysogobio* with a dissected pad (Figs. 5–8). Jiang and Zhang (2013) considered the genus *Huigobio* to be an independent clade, however, *M. kachekensis*, *M. brevirostris* and *M. xianyouensis* were not included in their analysis.

Similar to other species of *Microphysogobio*, *M. kachekensis* and *M. brevirostris* have a typical dissected heart-shaped medial pad, lip papillae pattern, and small sized swim bladder, and are recognized as species of *Microphysogobio* (Chen 1998, Chen & Fang 1999). Our molecular evidence provides a new taxonomic viewpoint to propose that heart-shaped medial pad lacking a central slit could be regarded as a derived feature, supporting the conclusion that the genus *Huigobio* should be regarded as a junior synonym of *Microphysogobio*.

Comparative Materials

Microphysogobio alticorpus.—Holotype: USNM 192926, 1, 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, 36.0–60.7 mm SL, collected with holotype; NTOUP 2007-12-198, 2, 41.5–49.8 mm SL, Kaoping River, Ligang Township, Pingtung County, Taiwan, 30 Dec 2007, S. P. Huang; NTOUP 2007-12-199, 2, 36.7–39.6 mm SL, Zhuoshui River, Xiluo Township, Yunlin County, Taiwan, 28 Dec 2007, S. P. Huang; NTOUP 2009-10-112, 1, 44.2 mm SL, Kaoping River, Shanlin District, Kaohsiung City, Taiwan, 6 Aug 2009, S. P. Huang; NTOUP 2010-05-305, 1, 36.2 mm SL, Wu River, Caotun Township, Changhua County, Taiwan, 6 Jan 2010, S. P. Huang; NTOUP 2010-11-542, 3, 37.1–55.0 mm SL, Bazhang River, Fanlu Township, Chiayi County, Taiwan. 15 Feb 2003; NTOUP 2010-11-543, 5, 33.4–58.1 mm SL, Zhuoshui River, Jiji Township, Nantou County, Taiwan, 26 Dec 2003, C. W. Wang.

Microphysogobio brevirostris.—Lectotype: BMNH 1865.5.2.49, 1, Formosa, from Consul Swinhoe's collection. Paralectotypes: BMNH 1865.5.2.50–53, 4; ZMB 6305, 1; NTOUP 2006-09-724, 3, 35.6–53.3 mm SL, Keelung River, Tamsui River, Sijiaoting, Keelung City, Taiwan, 5 Sep 2006, S. P. Huang; NTOUP 2007-10-007, 1, 59.2 mm SL, Dahan River, Tamsui River, Daxi Township, Taoyuan City, Taiwan, 22 Oct 2007, S. H. Su; NTOUP 2010-05-258, 1, 62.7 mm SL, Keelung River, Tamsui River, Sijiaoting, Keelung City, Taiwan, 6 Aug 2006, S. P. Huang; NTOUP 2010-10-518, 1, 59.7 mm SL,

Keelung River, Tamsui River, Yourui, Keelung City, Taiwan, 4 Aug 2010, S. P. Huang; NTOUP 2010-10-530, 3, 42.7–48.0 mm SL, Keelung River, Tamsui River, Ruifang District, New Taipei City, Taiwan, 29 Feb 2008, S. P. Huang; NTOUP 2010-11-539, 2, 66.7–68.9 mm SL, Keelung River, Tamsui River, Ruifang District, New Taipei City, Taiwan, 10 Jul 2008, S. P. Huang; NTOUP 2010-11-540, 1, 75.7 mm SL, Keelung River, Tamsui River, Nuannuan, Keelung City, Taiwan, 18 Jun 2008, S. P. Huang; NTOUP 2010-11-541, 1, 62.2 mm SL, Shuangxi River, Gongliao Township, Taipei County, Taiwan, 17 Feb 2008, S. P. Huang.

Microphysogobio chenhsienensis.—NTOUP 2010-11-550, 6,50.7–52.5 mm SL, Ou River, Yantou Township, Youngjia County, Zhejiang Province, China, 7 Jan 2008, S. P. Huang; NTOUP 2010-11-551, 8, 53.3–65.9 mm SL, Ou River, Wenzhou City, Zhejiang Province, China, 18 Oct 2010, I-S. Chen.

Microphysogobio elongatus.—ASIZP78399, 6, 69.0–77.3 mm SL, Quanzhou County market, Guangxi Province, China, 6 Nov 2015, S. P. Huang.

Microphysogobio fukiensis.—NTOUP 2010-11-535, 1, 73.0 mm SL, Shaowu City market, Fujian Province, China, 8 Dec 2008, S. P. Huang; NTOUP 2010-11-536, 1, 55.5 mm SL, Xinquan market, Fujian Province, China, 25 Jun 2006, I-S. Chen; NTOUP 2010-11-537, 2, 43.8–61.6 mm SL, Datian County market, Fujian Province, China, 27 Jun 2006, I-S. Chen; NTOUP 2010-11-538, 3, 43.4–51.2 mm SL, Shaowu City market, Fujian Province, China, 11 Sep 2007, I-S. Chen; NMNSF01803, 23, 68.9–77.9 mm SL, Min River, Minhou County, Fujian Province, China, 23 Nov 2007, N. H. Jang-Liaw; NTOUP 2015-10-001, 8, 59.0–71.5 mm SL, Pinghe County market, Fujian Province, China, 11 Mar 2015, I-S. Chen.

Microphysogobio kachekensis.—NTOUP 2010-11-544, 2, 63.7–66.9 mm SL, Nandu River, Nankai Township, Hainan Province, China, 15 Sep 2005, S. P. Huang; NTOUP 2010-11-545, 8, 48.4–61.9 mm SL, Luo River, Dongkeng, Luhe County, Guangdong Province, China, 2 Apr 2009, S. P. Huang.

Microphysogobio microstomus.—NTOUP 2010-11-546, 1, 43.7 mm SL, Taihu lake, Lujiaxiang, Dongshan Township, Suzhou City, Jiangsu Province, China, 13 Dec 2008, M. C. Chiang.

Microphysogobio tafangensis.—NMNSF01653, 1, 62.0 mm SL, Changhua Township market, Lin'an City, Zhejiang Province, China, 16 Aug 2006, N. H. Jang-Liaw.

Acknowledgements

We are very grateful to Mr. Chien-Chin Liu for his great contribution in the collection of this new species in the field. We also appreciated Dr. Tzi-Yuan Wang for great assistance in molecular clock analysis. Thanks also to three anonymous reviewers for constructive improvements to the manuscript.

Literature Cited

Bănărescu, P. M. 1992. A critical updated checklist of Gobioninae (Pisces, Cyprinidae). Travaux du Museum d'Histoire Naturelle Grigore Antipa 32:303–330.

Bănărescu, P. M., & T. T. Nalbant. 1968. Some new Chinese minnows (Pisces, Cypriniformes). Proceedings of the Biological Society of Washington 81:335–346.

Costagliola, D., D. R. Robertson, P. Guidetti, S. Stefanni, P. Wirtz, J. B. Heiser, & G. Bernardi. 2004. Evolution of coral reef fish *Thalassoma* spp. (Labridae). 2. Evolution of the eastern Atlantic species. Marine Biology 144:377–383.

Chen, I. S., & Y. C. Chang. 2007. Taxonomic revision and mitochondrial sequence evolution of the cyprinid genus *Squalidus* (Teleostei: Cyprinidae) in Taiwan with description of a new species. The Raffles Bulletin of Zoology Supplement 14:69–76.

Chen, I-S., & L. S. Fang. 1999. The freshwater and estuarine fishes of Taiwan. The Preparatory Office of National Museum of Marine Biology & Aquarium Press, Pingtung, 287 pp.

Chen, I-S., J. H. Wu, & C. H. Hsu. 2008. The taxonomy and phylogeny of the cyprinid genus, *Candidia* (Teleostei: Cyprinidae) from Taiwan, with description of a new species and comments on a new genus. The Raffles Bulletin of Zoology Supplement 19:203–214.

Chen, I-S., J. H. Wu, & S. P. Huang. 2009. The taxonomy and phylogeny of the cyprinid genus *Opsariichthys* Bleeker (Teleostei: Cyprinidae) from Taiwan, with description of a new species. Environmental Biology of Fishes 86:165–183.

Chen, Y. Y. 1998. Fauna Sinica, Osteichthyes, Cypriniformes II. Science Press, Beijing, 531 pp.

Cheng, Q., & B. Zheng. 1987. Systematic synopsis of Chinese fishes. Vol. 1. Science press, Beijing, 593 pp.

Darriba, D., G. L. Taboada, R. Doallo, & D. Posada. 2012. jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9:772–772.

Eschmeyer, W. N., R. Fricke, & R. van der Laan. 2016. Catalog of Fishes: genera, species, references. http://researcharchive.calacademy.org/research/ichthyology/catalog/SpeciesByFamily.asp. (last accessed on 18 March 2016)

Fang, P. W. 1938. On *Huigobio chenhsienensis*, gen. & sp. nov. Bulletin of the Fan Memorial Institute of Biology, Peiping (Zoology Series) 8:237–243.

Günther, A. 1868. Catalogue of the fishes in the British Museum. Catalogue of the Physostomi, containing the families Heteropygii, Cyprinidae, Gonorhynchidae, Hyodontidae, Osteoglossidae, Clupeidae, Halosauridae, in the collection of the British Museum. Catalogue of the Fishes 7:1–512.

Hall, T. A. 2001. Bioedit: a user-friendly biological sequence alignment editor and analysis, version 5.09. Department of Microbiology, North Carolina State University, Raleigh, North Carolina.

Hosoya, K., H. Ashiwa, M. Wayanabe, K. Mizunguchi, & T. Okazaki. 2002. *Zacco sieboldii*, a species distinct from *Zacco temminckii*. Ichthyological Research 50:1–8.

Huang, S. P., I-S. Chen, & K. T. Shao. 2013. The molecular phylogeny of genus *Hemigobius* (Teleostei: Gobiidae), with the confirmation of validity of *Hemigobius crassa* (Herre, 1945). Journal of Marine Science and Technology Supplement 21:86–93.

Jang-Liaw, N. H., & I-S. Chen. 2013. *Onychostoma minnanensis*, a new cyprinid species (Teleostei: Cyprinidae) from Fujian, southern mainland China, with comments on the mitogenetic differentiation among related species. Ichthyological Research 60:62–74.

Jiang, Z. G., E. H. Gao, & E. Zhang. 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.

Jiang, Z. G., & E. Zhang. 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.

Kottelat, M. 2001a. Fishes of Laos. Wildlife Heritage Trust Publications, Colombo, 198 pp.

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, i–iii+123 pp.+1–18.

Kottelat, M. 2006. Fishes of Mongolia, a check-list of the fishes known to occur in Mongolia with comments on systematics and nomenclature. World Bank Report (NEMO), Washington, D. C., i–xi+103 pp.

Liao, T. Y., T. Y. Wang, H. D. Lin, S. C. Shen, & C. S. Tzeng. 2008. Phylogeography of the endangered species, *Sinogastromyzon puliensis* (Cypriniformes: Balitoridae), in southwestern Taiwan based on mtDNA. Zoological Studies 47:383–392.

Librado, P., & J. Rozas. 2009. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25:1451–1452.

Mori, T. 1934. The fresh water fishes of Jehol. Report of the first scientific expedition to Manchoukuo, Tokyo, 61 pp.

Mukai, T., S. Nakamura, T. Suzuki, & M. Nishida. 2005. Mitochondrial DNA divergence in yoshinobori gobies (*Rhinogobius* species complex) between the Bonin Islands and the Japan-Ryukyu Archipelago. Ichthyological Research 52:410–413.

Murakami, M., Yamashita, Y., & H. Fujitani. 1998. The Complete Sequence of Mitochondrial Genome from a Gynogenetic Triploid "Ginbuna" (*Carassius auratus langsdorfi*). Zoological Science 15:335–337.

Nelson, J. S. 2006. Fishes of the World, fourth edition. John Wiley & Sons, Hoboken, 624 pp.

Nichols, J. T. 1926a. Some Chinese fresh-water fishes. XV. Two apparently undescribed cat-

fishes from Fukien. XVI. Concerning gudgeons related to *Pseudogobio*, and two new species of it. XVII. Two new Rhodeins. American Museum Novitates 214:1–7.

Nichols, J. T. 1926b. Some Chinese fresh-water fishes. XVIII. New species in recent and earlier Fukien collections. American Museum Novitates 224:1–7.

Nichols, J. T., & C. H. 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. Annotationes Zoologicae Japonenses 11:1–25.

Pan, J. H. 1991. The freshwater fishes of Guangdong Province. Guangdong Science and Technology Press, Guangzhou, 589 pp.

Qin, K. J. 1987. Pp.1–552 *in* C. X. Liu, K. J. Qin. 1987. Fauna Liaoningica. Pisces. Liaoning Science and technology Press, Shenyang, 552 pp.

Ronquist, F., & J. P. Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574.

Smith, G. R., T. Dowling, K. W. Gobalet, T. Lugaski, D. Shiazawa, & R. P. Evans. 2002. Biogeography and timing of evolutionary events among Great Basin fishes. Pp. 175–234 *in* R. Hershler, D. B. Madsen, & D. R. Currey, Great Basin Aquatic Systems History Smithsonian Contributions to Earth Sciences, Washington, D. C., 405 pp.

Taranetz, A. Ya. 1937. A note on a new genus of gudgeons from the Amur Basin. Bulletin of the Far Eastern Branch of the Academy of Sciences of the USSR 23:113–115.

Tamura, K., G. Stecher, D. Peterson, A. Filipski, & S. Kumar. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution 30:2725–2729.

Tang, K. L., K. A. Mary, W. J. Chen, M. V. Hirt, E. R. Morgan, S. Tetsuya, M. S. Leah, L. Yang, L. B. Henry, S. P. He, H. Z. Liu, M. Masaki, S. Kenji, M. S. Andrew, M. W. Robert, & L. M. Richard. 2011. Phylogeny of the gudgeons (Teleostei: Cyprinidae: Gobioninae). Molecular Phylogenetics and Evolution 61:103–124.

Wang, K. F. 1935. Preliminary notes on the fishes of Chekiang (Isospondyli, Apodes & Plectospondyli). Contributions from the Biological Laboratory of the Science Society of China (Zoological Series) 11:1–65.

Wang, T. Y., T. Y. Liao, & C. S. Tzeng. 2007. Phylogeography of the Taiwanese endemic hillstream loaches, *Hemimyzon formosanus* and *H. taitungensis* (Cypriniformes: Balitoridae). Zoological Studies 46:547–560.

Wu, H. W. 1930. On some fishes collected from the upper Yangtse Valley. Sinensis 1:65–86.

Wu, H. W. 1977. The cyprinid fishes of China. Volume 2. People's Press, Shanghai, 229–598 pp.

Xie, Y. H. 1986. On a new cyprinid fish of the genus *Microphysogobio* from Liaoning, China (Cypriniformes: Cyprinidae). Acta Zootaxonomica Sinica 11:220–222.

Xing, Y. C., Y. H. Zhao, W. Q. Tang, & C. G. Zhang. 2011. A new species, *Microphysogobio wulonghensis* (Teleostei: Cypriniformes: Cyprinidae), from Shandong Province, China. Zootaxa 2901:59–68.

Yao, P. Q., & G. R. Yang. 1977. The cyprinid fishes of China. Gobioninae. Volume 2. Pp. 524–528 *in* X. W. Wu, ed., Science Press, Peking, pp. 439–549.

Yue, P. Q. 1995. Description of a new species of the genus *Microphysogobio* Mori from China (Cypriniformes: Cyprinidae). Acta Zootaxonomica Sinica 20:495–498.

Zhao, Y. H., & C. G. Zhang. 2001. A new species of the genus *Microphysogobio* Mori from Guangxi, China (Cypriniformes: Cyprinidae). Acta Zootaxonomica Sinica 26:589–592.