Supplementary Materials

Comparative materials

Microphysogobio brevirostris

ASIZP 0066723, 2 specimens, 59.0–72.5 mm SL, from the Beishi River, Tamsui River, Pinglin District, New Taipei City, Taiwan Province, China, 9 November 2002, collected by J.-Y. Chen (for lip papillae illustration, Figure 1F); NTOUP 2007–10–007, 1 specimen, 59.0 mm SL, from the Dahan River, Tamsui River, Daxi Township, Taoyuan City, Taiwan Province, China, 22 October 2007, collected by S.-H. Su; NTOUP 2010–11–539, 2 specimens, 65.6–69.3 mm SL, from the Keelung River, Tamsui River, Ruifang District, New Taipei City, Taiwan Province, China, 10 July 2008, collected by S.-P. Huang; ASIZP 0074398, 1 specimen, 67.6 mm SL, from the Shuangxi River, Shuangxi District, New Taipei City, Taiwan Province, China, 8 April 1992, collected by M.-X. Zheng; ASIZP 0080953–5, 3 specimens, 55.4–64.7 mm SL, from the Keelung River, Tamsui River, Ruifang District, New Taipei City, Taiwan Province, China, 4 September 2006.

Microphysogobio xianyouensis

ASIZP 0078398, 2 specimens, 56.1–59.5 mm SL, from the Mulanxi River, Daji Township, Xianyou County, Putian City, Fujian Province, China, 20 December 2009, collected by J.-C. Liu (for lip papillae system illustration, Figure 1G). Additional data from (S.-P. Huang *et al*., 2016).

Microphysogobio alticorpus

ASIZP 0059636, 2 specimens, 55.2–62.5 mm SL, from the Zengwen River, Alishan Township, Chiayi County, Taiwan Province, China, 9 April 1998 (for lip papillae illustration, Figure 1J); NTOUP 2010–11–542, 3 specimens, 36.8–54.2 mm SL, from the Bazhang River, Fanlu Township, Chiayi County, Taiwan Province, China, 15 February 2003; NTOUP 2010–11–543, 5 specimens, 33.3–57.1 mm SL, from the Zhuoshui River, Jiji Township, Nantou County, Taiwan Province, China, 26 December 2003, collected by C.-W. Wang; NTOUP 2007–12–198, 2 specimens, 42.3–50.0 mm SL, Kaoping River, Ligang Township, Pingtung County, Taiwan Province, China, 30 December 2007, collected by S.-P. Huang; NTOUP 2007–12–199, 2 specimens, 37.1–39.4 mm SL, from the Zhuoshui River, Xiluo Township, Yunlin County, Taiwan Province, China, 28 December 2007, collected by S.-P. Huang; NTOUP 2009–10–112, 1 specimen, 44.4 mm SL, from the Kaoping River, Shanlin District, Kaohsiung City, Taiwan Province, China, 6 August 2009, collected by S.-P. Huang.

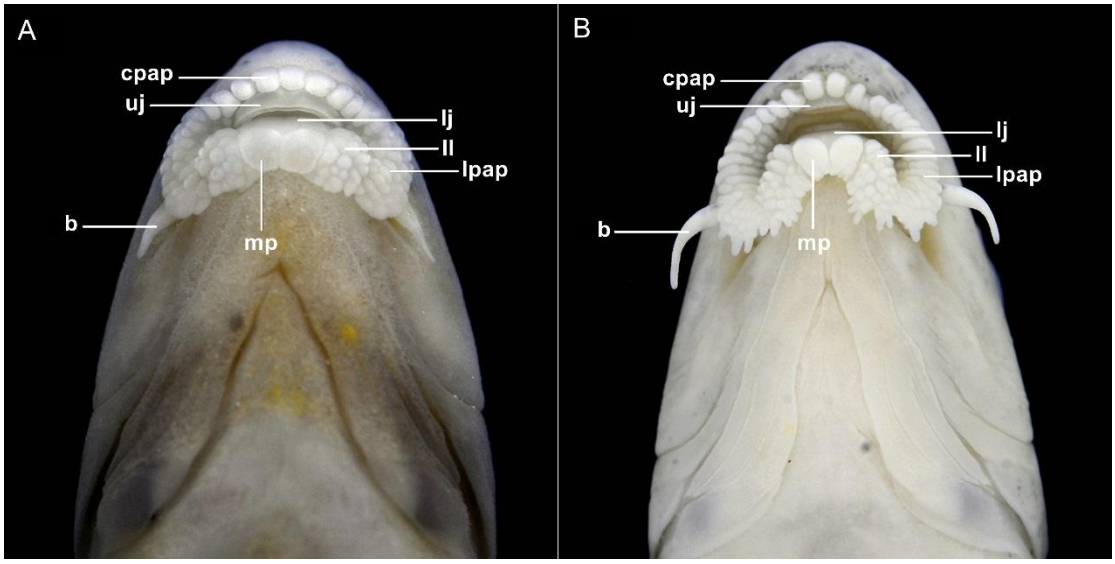
Microphysogobio fukiensis

ASIZB 220659, 1 specimen, 54.9 mm SL, from the stream of Mayangxi River, Minjiang River Basin, Changjian Village (N 27°37'27.43", E 117°40'10.35"), 13 April 2021, collected by Z.-X. Sun, R. Zhang, Q.-J. Chen and R. Xi (for lip papillae illustration, Figure 1H; for photograph, Figure S1B in Supplementary Marterials).

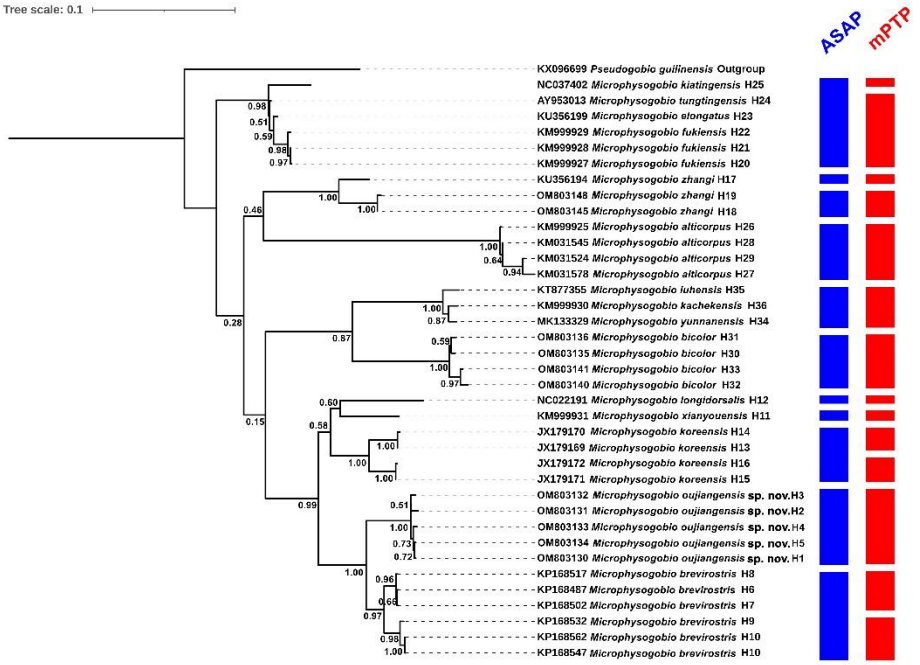
Microphysogobio zhangi

ASIZB 220677, 1 specimen, 60.7 mm SL, from the Yanshanhe River, Xinjiang River, middle Yangtze River Basin, Yongping Township (N28°13'10.09", E117°47'7.25"), Yanshan County, Shangrao City, Jiangxi Province, China, 12 April 2021, collected by

Z.-X. Sun and R. Zhang (for lip papillae illustration, Figure 1I).



Supplementary Figure S1 Diagram of lip papillae in *Microphysogobio*.
A: *Microphysogobio oujiangensis* **sp. nov.**, holotype, ASIZB 220814, 64.8 mm SL. B: *M. fukiensis*, ASIZB 220659, 54.9 mm SL. cpap=central portion of anterior papillae; lpap= lateral portion of anterior papillae; uj=upper jaw; lj=lower jaw; ll=lateral lobe; mp=medial pad; b=barbel.



Supplementary Figure S2 Molecular phylogenetic tree of *Microphysogobio oujiangensis* sp. nov. and congeners based on cyt *b* sequence reconstructed by maximum-likelihood analysis; bootstrap values are shown on nodes and species delimitation results are shown on right.

Supplementary Table S1 Codes, sampling localities, haplotypes, and accession numbers of *Microphysogobio* species and outgroup for molecular analyses.

Code	Species	Locality	Drainage	Haplotype	Accession no.	Source
ASIZB 220826	*M. oujiangensis* **sp. nov.**	Jinyun County, Zhejiang Prov.	River of Panxi, Oujiang River Basin	H1	OM803130	This Study
ASIZB 220827	*M. oujiangensis* **sp. nov.**	Jinyun County, Zhejiang Prov.	R. Panxi, Oujiang River Basin	H2	OM803131	This Study
ASIZB 220828	*M. oujiangensis* **sp. nov.**	Jinyun County, Zhejiang Prov.	R. Panxi, Oujiang River Basin	H3	OM803132	This Study
ASIZB 220829	*M. oujiangensis* **sp. nov.**	Jinyun County, Zhejiang Prov.	R. Panxi, Oujiang River Basin	H4	OM803133	This Study
ASIZB 220830	*M. oujiangensis* **sp. nov.**	Jinyun County, Zhejiang Prov.	R. Panxi, Oujiang River Basin	H5	OM803134	This Study
MBDH01	*M. brevirostris*	Taiwan Prov.	R. Dahan, Tamshui River Basin	H6	KP168487	Chang *et al*., 2016
MBGL01	*M. brevirostris*	Taiwan Prov.	R. Keelung, Tamshui River Basin	H7	KP168502	Chang *et al*., 2016
MBSD01	*M. brevirostris*	Taiwan Prov.	R. Shindian, Tamshui River Basin	H8	KP168517	Chang *et al*., 2016
MBHL01	*M. brevirostris*	Taiwan Prov.	Houlung River Basin	H9	KP168532	Chang *et al*., 2016
MBFC01	*M. brevirostris*	Taiwan Prov.	Fengshan River Basin	H10	KP168547	Chang *et al*., 2016
MBTC01	*M. brevirostris*	Taiwan Prov.	Touchien River Basin	H10	KP168562	Chang *et al*., 2016
MXIML1	*M. xianyouensis*	Xianyou County, Fujian Prov.	Mulanxi River Basin	H11	KM999931	Huang *et al*., 2016
CBM-ZF-11551	*M. longidorsalis*	Korea (Aquarium)	-------	H12	NC022191	Tang *et al*., 2011
-------	*M. koreensis*	Korea	-------	H13	JX179169	Kim *et al*. (Unpublished)
-------	*M. koreensis*	Korea	-------	H14	JX179170	Kim *et al*. (Unpublished)
-------	*M. koreensis*	Korea	-------	H15	JX179171	Kim *et al*.

-------	*M. koreensis*	Korea	-------	H16	JX179172	(Unpublished) Kim *et al*. (Unpublished)
MZHGL1	*M. zhangi*	Guilin City, Guangxi Zhuang Aut. Reg.	R. Lijiang, Pearl River Basin	H17	KU356194	Huang *et al*., 2017
ASIZB 220682	*M. zhangi*	Yanshan County, Jiangxi Prov.	R. Xinjiang, middle Yangtze River Basin	H18	OM803145	Sun & Zhao, 2022
ASIZB 220715	*M. zhangi*	Wuyuan County, Jiangxi Prov.	R. Raohe, middle Yangtze River Basin	H19	OM803148	Sun & Zhao, 2022
MFUMJ1	*M. fukiensis*	Shaowu City, Fujian Province	R. Futunxi, Minjiang River Basin	H20	KM999927	Huang *et al*., 2016
MFUMJ2	*M. fukiensis*	Shaowu City, Fujian Province	R. Futunxi, Minjiang River Basin	H21	KM999928	Huang *et al*., 2016
MFUMJ3	*M. fukiensis*	Xinquan Town, Fujian Province	R. Tingjiang, Hanjiang River Basin	H22	KM999929	Huang *et al*., 2016
MELQZ1	*M. elongatus*	Quanzhou County, Guangxi Zhuang Aut. Reg.	R. Xiangjiang, middle Yangtze River Basin	H23	KU356199	Huang *et al*., 2017
-------	*M. tungtingensis*	-------	Middle Yangtze River Basin	H24	AY953013	Yang *et al*., 2006
20170925BB05	*M. kiatingensis*	Chengdu City, Sichuan Prov.	Upper Yangtze River Basin	H25	NC037402	Zou *et al*., 2018
MALKP1	*M. alticorpus*	Pingtung County, Taiwan Prov.	Kaoping River Basin	H26	KM999925	Huang *et al*., 2016
MATD01	*M. alticorpus*	Nantou County, Taiwan Prov.	Tadu River Basin	H27	KM031578	Jean *et al*., 2014
MAKP01	*M. alticorpus*	Kaohsiung City, Taiwan Prov.	Kaoping River Basin	H28	KM031545	Jean *et al*., 2014
MABC01	*M. alticorpus*	Chiayi County, Taiwan Prov.	Bazhang River Basin	H29	KM031524	Jean *et al*., 2014
ASIZB 220619	*M. bicolor*	Yanshan County, Jiangxi Prov.	R. Xinjiang, Yangtze River Basin	H30	OM803135	Sun & Zhao, 2022
ASIZB 220620	*M. bicolor*	Yanshan County, Jiangxi Prov.	R. Xinjiang, Yangtze River Basin	H31	OM803136	Sun & Zhao, 2022
ASIZB 220630	*M. bicolor*	Wuyuan County, Jiangxi Prov.	R. Raohe, Yangtze River Basin	H32	OM803140	Sun & Zhao, 2022
ASIZB 220646	*M. bicolor*	Wuyuan County, Jiangxi Prov.	R. Raohe, Yangtze River Basin	H33	OM803141	Sun & Zhao, 2022
MYUVN1	*M. yunnanensis*	Dien Bien, Vietamn	R. Lixianjiang, Red River Basin	H34	MK133329	Huang *et al*., 2018
MLURJ1	*M. luhensis*	Luhe County, Guangdong Prov.	Rongjiang River Basin	H35	KT877355	Huang *et al*., 2018
MKAND1	*M. kachekensis*	Nankai Town, Hainan Prov.	Nandujiang River Baisn	H36	KM999930	Huang *et al*., 2016

Outgroup					
PG-YS01	*Pseudogobio guilinensis*	Unknown	Unknown	KX096699	Unpublished

References

Chang HY, Wang WK, Chen KN, Su JK, Hsin CY, Li J, et al. 2016. Phylogeography and genetic structure of the endemic cyprinid fish *Microphysogobio brevirostris* in northern Taiwan. *Biochemical Systematics and Ecology*, **65**: 176–184.

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. *Proceedings of the Biological Society of Washington*, **129**(1): 195–211.

Huang SP, Zhao YH, Chen IS, Shao KT. 2017. A new species of *Microphysogobio* (Cypriniformes: Cyprinidae) from Guangxi Province, Southern China. *Zoological Studies*, **56**: 8.

Huang SP, Chen IS, Zhao YH, Shao KT. 2018. Description of a new species of the gudgeon genus *Microphysogobio* mori 1934 (Cypriniformes: Cyprinidae) from Guangdong province, Southern China. *Zoological Studies*, **57**: 58.

Jean CT, Wu CY, Tsai KC, Wang WK, Hsu YY, Chang YM, et al. 2014. Population genetic structure in the endemic cyprinid fish *Microphysogobio alticorpus* in Taiwan: Evidence for a new phylogeographical area. *Biochemical Systematics and Ecology*, **57**: 108–116.

Sun ZX, Zhao YH. 2022. Revalidation and redescription of a gobionine species *Microphysogobio bicolor* (Nichols, 1930) (Teleostei: Cypriniformes) from the Yangtze River Basin, China. *Zootaxa*, **5092**(3): 361–377.

Tang K, Agnew M, Chen WJ, Hirt M, Raley M, Sado T, et al. 2011. Phylogeny of the gudgeons (Teleostei: Cyprinidae: Gobioninae). *Molecular Phylogenetics and Evolution*, **61**(1): 103–124.

Yang JQ, He SP, Freyhof J, Witte K, Liu HZ. 2006. The phylogenetic relationships of the Gobioninae (Teleostei: Cyprinidae) inferred from mitochondrial cytochrome *b* gene sequences. *Hydrobiologia*, **553**(1): 255–266.

Zou YC, Zhang JY, Zhang T, Xie M, Wu T. 2018. Sequence identification and phylogenetic analysis of the mitochondrial genome of *Microphysogobio kiatingensis* (Cypriniformes: Cyprinidae). *Mitochondrial DNA Part B*, **3**(1): 414–415.

Supplementary Table S2 Morphometric measurements of *Microphysogobio oujiangensis* sp. nov., *M. brevirostris*, and *M. xianyouensis*.

Characters	*Microphysogobio oujiangensis* **sp. nov.**(n=21)				*Microphysogobio brevirostris* (n=9)			*Microphysogobio xianyouensis* (*n*=11)			
	Holotype	All type specimens			Range	Mean	SD	Holotype	Holotype and Paratypes		
		Range	Mean	SD					Range	Mean	SD
Dorsal-fin rays	7	7	7		7	7		7	7	7	
Anal-fin rays	6	6	6		6	6		6	6	6	
Pectoral-fin rays	11	10–11	11		11–12	11		12	11–12	12	
Pelvic-fin rays	7	7	7		7	7		7	7	7	
Lateral line scales	37	36–38	37		38–39	38		36	35-36	36	
Scales above lateral line	3.5	3.5–4.5	4		4.5	4.5		4	4	4	
Scales below lateral line	2	2	2		2	2		2	2	2	
Pre-dorsal scales	10	8–10	9		11	11		10	10	10	
Circumpeduncular scales	12	12	12		12	12		12	12	12	
Standard Length (mm)	64.8	56.4–70.1	63.0		55.4–72.5	63.4		61.3	53.4–60.7	63.4	
In percentage of SL											
Body depth	22.2	19.8–24.3	21.7	1.2	17.6–22.4	20.9	2.0	15.7	15.7–16.8	16.2	–
Head length	22.6	21.4–23.7	22.3	0.5	22.7–24.7	23.5	0.7	21.4	20.9–22.3	21.7	–
Dorsal-fin length	23.1	21.2–23.5	22.1	0.7	20.0–23.7	22.4	1.2	23.5	23.4–24.5	23.9	–
Dorsal-fin base length	14.6	13.4–15.	14.3	0.4	13.2–15.6	14.8	0.7	14.6	13.2–14.	14.3	–

		1							7		
Pectoral-fin length	21.7	19.4–23.4	21.5	1.1	22.5–25.3	24.0	0.9	22.6	22.1–24.4	23.3	–
Pectoral-fin base length	5.5	5.1–6.2	5.7	0.4	5.5–6.6	6.0	0.4	–	–	–	–
Pelvic-fin length	16.9	15.4–18.3	17.0	0.7	17.1–20.2	18.5	0.9	18.0	17.8-20.1	18.9	–
Pelvic-fin base length	4.7	3.8–5.3	4.6	0.4	5.0–5.5	5.3	0.2	–	–	–	–
Anal-fin length	15.4	13.6–16.1	14.8	0.6	15.0–17.8	16.3	0.8	16.6	15.3–17.4	16.8	–
Anal-fin base length	7.7	6.8–8.2	7.6	0.4	7.9–9.5	8.8	0.6	9.2	8.4–9.2	9.0	–
Predorsal length	44.5	42.6–46.5	44.3	1.2	42.8–45.2	44.3	0.8	43.8	42.7–44.4	43.7	–
Caudal peduncle length	16.3	15.9–18.4	17.1	0.7	17.8–20.4	19.0	1.0	19.9	19.4–21.3	20.3	–
Caudal peduncle depth	8.8	8.5–10.0	9.1	0.4	9.6–11.7	10.4	0.8	9.0	8.4–9.0	8.8	–
Head Length (mm)	14.7	12.7–16.0	14.1		13.2–16.6	14.9		–	–	–	
In percentage of HL											
Head depth	58.8	58.1–64.4	60.6	1.8	60.5–65.2	63.1	1.5	62.5	60.0-62.5	61.4	–
Head width	65.7	62.9–71.1	67.6	2.6	60.3–69.6	65.9	3.0	64.0	63.5-65.5	64.4	–
Eye diameter	32.7	30.9–36.9	34.0	1.5	25.3–30.9	27.5	1.7	27.7	27.4-29.5	28.4	–
Interorbital width	21.3	21.3–27.6	24.3	1.6	29.9–32.6	31.3	1.1	28.9	28.4-30.2	29.1	–

Snout length	38.6	35.2–42.7	38.5	1.7	38.4–42.0	39.9	1.2	–	–	–	–
Anterior papillae length	60.1	56.0–66.4	60.9	3.1	57.5–68.4	64.1	3.6	–	–	–	–
Anterior papillae width	74.7	69.0–84.0	76.1	3.8	73.3–89.2	79.0	5.0	–	–	–	–
Central anterior papillae width	20.8	16.9–23.7	20.5	1.6	21.7–24.1	22.5	0.7	–	–	–	–
Upper jaw cutting edge width	38.6	35.2–42.7	38.5	1.7	38.4–42.0	39.9	1.2	–	–	–	–
Medial Pad width	39.0	38.5–43.8	40.8	1.6	41.0–47.3	44.4	2.4	–	–	–	–
Mouth depth	51.0	45.9–53.4	50.0	1.8	50.0–58.3	53.5	2.9	–	–	–	–
Mouth width	71.8	70.4–82.9	76.7	3.4	71.9–88.6	81.2	4.8	–	–	–	–
Barbel length	27.0	26.7–31.4	29.2	1.1	28.0–30.6	29.4	0.8	–	–	–	–

Supplementary Table S3 Genetic distances of cyt *b* gene among 15 species of *Microphysogobio*.

	Species	Intraspecific	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	*M. oujiangensis* **sp. nov.**	0.007															
2	*M. brevirostris*	0.015	0.052														
3	*M. xianyouensis*	n/c	0.094	0.083													
4	*M. longidorsalis*	n/c	0.101	0.097	0.081												
5	*M. koreensis*	0.025	0.091	0.079	0.075	0.082											
6	*M. zhangi*	0.030	0.132	0.123	0.130	0.134	0.122										
7	*M. fukiensis*	0.003	0.112	0.110	0.110	0.112	0.110	0.112									
8	*M. elongatus*	n/c	0.108	0.107	0.110	0.112	0.104	0.112	0.015								
9	*M. tungtingensis*	n/c	0.109	0.106	0.109	0.110	0.106	0.108	0.015	0.006							
10	*M. kiatingensis*	n/c	0.127	0.118	0.111	0.125	0.116	0.111	0.035	0.033	0.029						
11	*M. alticorpus*	0.015	0.157	0.160	0.150	0.146	0.148	0.145	0.136	0.139	0.137	0.150					
1	*M. bicolor*	0.012	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.12	0.13	0.14				

2			4	5	8	5	4	8	0	0	8	5	5				
13	*M. yunnanensis*	n/c	0.129	0.128	0.132	0.134	0.132	0.135	0.129	0.129	0.126	0.140	0.136	0.102			
14	*M. luhensis*	n/c	0.134	0.131	0.134	0.136	0.132	0.134	0.131	0.128	0.128	0.141	0.138	0.103	0.020		
15	*M. kachekensis*	n/c	0.131	0.131	0.130	0.135	0.133	0.134	0.131	0.129	0.126	0.137	0.142	0.101	0.012	0.021	
16	**Outgroup *P. guilinensis***	n/c	0.169	0.164	0.166	0.163	0.163	0.164	0.152	0.148	0.144	0.155	0.188	0.166	0.159	0.159	0.157