

A NEW GENUS OF *Hemigobius* GENERIC GROUP GOBY BASED ON MORPHOLOGICAL AND MOLECULAR EVIDENCE, WITH DESCRIPTION OF A NEW SPECIES

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Key words: new genus, new species, brackish water, mangrove.

ABSTRACT

Wuhanlinigobius, a new genus of *Hemigobius* generic group would be established and assigned from *Mugilogobius polylepis* Wu and Ni, 1985. *Mugilogobius polylepis* has been regarded as belong to genus *Eugnathogobius* based on lacking head pores and representing longitudinal sensory papillae in previous taxonomic study. However, we compared the osteological features of *Mugilogobius polylepis* Wu and Ni, 1985 and *Eugnathogobius microps* Smith, 1931 as well as the molecular phylogenetic analysis based on the mtDNA ND5, Cyt-b genes and D-loop region. The molecular phylogenetic tree including 9 related *Hemigobius* generic group reveal that this new genus represents an independent clade which is well separate from other related *Hemigobius* generic group. The papillae pattern, osteological features and molecular evidence strongly conclude that *Eugnathogobius polylepis* should be a new genus of *Hemigobius* generic group, on the other hand, an additional new species of *Wuhanlinigobius* also will be described herein, and the diagnostic key of this new genus will be provided in this paper.

I. INTRODUCTION

Among the subfamily Gobiellinae of family Gobiidae, *Hemigobius* generic group defined herein consists of genera *Brachygobius*, *Caecogobius*, *Calamiana*, *Eugnathogobius*, *Hemigobius*, *Mugilogobius*, *Pandaka*, *Pseudogobiopsis*, *Pseudogobius*, *Redigobius*, *Stigmatogobius*, *Tamanka* and *Weberogobius*, which are related genera sharing the typical longitudinal

papillar patterns. Among the taxonomic studies of *Hemigobius* generic group, thought Larson consider that genus *Weberogobius* Koumans, 1953 [15] is synonym of genus *Mugilogobius* Smitt, 1900 [28], however, Miller consider genus *Weberogobius* is valid [20], in this study, we also consider that genus *Weberogobius* is a valid genus, genus *Weberogobius* can be easy distinguished from genus *Mugilogobius* by they have different vertebral count (11+15-16 vs. 10+16) as well as other their own features.

On the other hand, among the genus *Eugnathogobius* Smith, 1931, the genus *Eugnathogobius* was established based on *Eugnathogobius microps* Smith, 1931. According to mentions of Larson, genus *Eugnathogobius* consists of 9 nominal species [18], including *E. illotus* (Larson, 1999), *E. indicus* (Larson, 2009), *E. kabilia* (Herre, 1940), *E. microps* Smith, 1931, *E. mindora* (Herre, 1945), *E. polylepis* (Wu and Ni, 1985), *E. siamensis* (Fowler, 1934), *E. stictos* (Larson, 2009) and *E. variegatus* (Peters, 1868) [6, 8, 9, 16, 18, 24, 27, 32]. However, we consider the *E. siamensis* should belong to genus *Pseudogobiopsis* Koumans, 1935 [33], and *E. mindora*, *E. illotus* and *E. variegatus* should belong genus *Calamiana* Herre, 1945 [17] based on their different morphological features in head pores presented and medium size of mouth in adult male individual.

Mugilogobius polylepis Wu and Ni, 1985 was reported been a new species which is collected from southern China and has been considered that it should place to genus *Eugnathogobius* Smith, 1931 [18].

Eugnathogobius occurs in brackish water habitat of mangrove and estuary around the Indo-west Pacific, including southern China, Southeast Asia and Australia [18], the *E. polylepis* was distributed over in China and partial area of Southeast Asia [18, 33], furthermore, this species possess quite different exterior morphological features compare to type species, *E. microps*. Therefore, the further detailed comparison between the rather different species should be conducted to check the validity of their own generic status.

In this study, authors examine the *E. polylepis* specimens which are collected from Taiwan and southern China, and

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compare to the *E. microps* and other related *Hemigobius* generic group based on exterior morphological features, osteological features and molecular evidence, the specific features and molecular phylogenetic result reveals the *E. polylepis* should be a distinct new genus, the detail morphological comparison and molecular phylogeny of this new genus and other related *Hemigobius* generic group will be provided herein.

II. MATERIALS AND METHODS

1. Sample Collection

All the examined *Hemigobius* generic group species specimens collected from Taiwan, Palau, Malay Peninsula and China were collected by hand-net. Specimens tissues used for molecular analysis were preserved in 95% ethanol; specimens used for morphological studies were fixed in 10% formalin before being transferred into 70% ethanol for long-term preservation.

2. Morphological Studies

Morphometric methods follow Miller [21]; meristic methods follow Chen and Shao, Chen and Kottelat, Chen and Miller and Huang and Chen [3-5, 10]; osteological methods follow Murdy and Birdsong *et al.* [1, 22]. Terminology of cephalic sensory canals and free neuromast organs (sensory papillae) is from Wongrat and Miller [31], based on Sanzo [26]. All examined materials are deposited at the Institute of Marine Biology, National Taiwan Ocean University, Keelung, Taiwan (NTOU).

Meristic abbreviations are as follows: A, anal fin; C, caudal fin; D1 and D2, first and second dorsal fins, respectively; LR, longitudinal scale series; P, pectoral fin; PreD, predorsal scales; SDP, scale series from origin of first dorsal fin to upper pectoral origin; TR, transverse scale series from second dorsal to anal fin; VC, vertebral count. All fish lengths are standard length (SL).

3. Molecular Phylogenetic Analysis

The phylogenetic relationships are employed the mtDNA sequence of full length of Cytochrom b (Cyt b), D-loop and partial mitochondrial NADH dehydrogenase subunit 5 (ND5) in this study. All DNA extractions of the samples were using a kit (Roche, High Pure Product Preparation kit). Cyt b region were amplified by polymerase chain reaction (PCR) using following two primers: (GluF: 5'-TAACCAGGACTARTG RCTTG-3'; GproR: 5'-GTTARAATCTCYTTCTTTGA-3'); D-loop region were amplified by polymerase chain reaction (PCR) using following two primers: (GTHR: 5'-TCAGCGCC AGAGCGCCGKTCTTGTA-3'; PGL5: 5'-CTAGGGYCTA TCCTAACATCTTCA-3'); ND5 region were amplified by PCR using following two primers: (PgleuD1: 5'-AAAGGAT AACAGCTCATCCGTTGGTCT-3'; ND5MR: 5'-CCTATTT TKCGGATGTCYTG-3').

PCR was done in a MODEL 2700 or 9700 thermal cycler (Perkin-Elmer) and 30-40 cycles were carried out. The 50 μ L

reaction volume contained 33.5 μ L of sterile distilled water, 5 μ L of 10X PCR buffer (Takara), 4 μ L of dNTP (2.5 mM each), 3 μ L of MgCl₂ (2.5 mM each), 1 μ L of each primer, 0.5 μ L of 0.5 unit Ex *Taq* (Takara) and 2 μ L of template. The thermal cycler profile 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 was carried out for each run of PCR. The PCR products were run on a 1.0% L 03 agarose gel (Takara) and stained with ethidium bromide for band characterization under ultraviolet trans-illumination.

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 PCR using primers. All sequencing reactions were performed according to the manufacturer's instructions. Labeled fragments were analyzed using as ABI PRISM Model 377-64 DNA Automated sequencer (ABI).

Nucleotide sequence alignment was verified manually after running through BIOEDIT version 5.9 [7]. The analysis of aligned mutation sites were conducted using Molecular Evolutionary Genetics Analysis (MEGA) version 5.05 [18] for aligned mutation sites analysis.

The parsimony (MP) analysis was carried out using PAUP* version 4.0B10 [29] using heuristic search. Branch support was established via bootstrap analysis (2000 replications). For the Bayesian (BI) analysis, the best-fitting model for sequence evolution was determined for mtDNA D-loop and ND5 sequences using MrMODELTEST version 2.2 [23]. The BI analyses were performed using MrBayes 3.0 [25]. The posterior probabilities of each node were computed from remaining 75% of all sampled trees.

III. RESULTS

Molecular phylogenetic analysis

The aligned Cytb, D-loop and ND5 sequence consists of 56 haplotypes and from all 9 related genera of *Hemigobius* generic group as 21 species with 47 individuals, we choose *Rhinogobius changtinensis* Huang and Chen, 2007 [10] as out-group. The length of combined sequence of Cytb, ND5 and D-loop sequence is 2994-3137 in total (1141 bp in Cytb, 818-961 bp in D-loop, and 1035 bp in ND5). This alignment contain 2508 total number of mutations, and 1501 number of polymorphic (segregating) sites. The phylogenetic analysis using neighbour-joining (NJ), parsimony (MP) analysis and Bayesian analysis (BI) method provided. The phylogenetic tree was reconstructed by NJ analysis based on Kimura 2-parameter model. The phylogenetic tree reconstructed by BI analysis based on HKY 85+G model.

The result of MP analysis by heuristic search only one tree, and tree length 6589; the Consistency index (CI) being 0.4117, Retention index (RI) being 0.7449 and Homoplasy index (HI) being 0.5883.

The phylogenetic trees reconstructed by NJ, MP and BI methods shows that same grouping result. The phylogenetic trees congruently reveal that this new genus is an independent clade, and the node with high bootstrap value reach to 95 in MP tree, 76 in NJ tree and posterior probabilities as high as 100 in BI tree, the specific level of node between this new genus and other related genera are strongly supported.

In comparison with mitogenetic divergence of all examined *Hemigobius* generic group genera, the range of mitogenetic divergence of this new genus and other 8 genera are 18.1-24.0% for Cytb sequences; 22.6-37.5% for ND5 sequences; and 21.7-39.3% for D-loop sequences; and 22.5-32.2% for combined Cytb, ND5 and D-loop sequences based on K2P model.

Compare to other related *Hemigobius* generic group, previous studies reveal the genetic divergence between *Mugilogobius abei* (Jordan and Snyder, 1901) [13] (KF 128984) and *Pseudogobius javanicus* (Bleeker, 1856) [2] (KF 193873) are 23.6 % for combined Cytb, ND5 and D-loop sequences [11, 12], compare to our study (22.5-32.2%), the genetic divergence between this new genus and other 8 genera are almost higher, so the molecular evidence strongly support that *Eugnathogobius polylepis* should be a independent genus based on their differentiation of mtDNA sequence from this new genus and other related genera.

IV. SYSTEMATICS

Wuhanlinigobius new genus

Diagnosis

This new genus can be well distinguished from other related genus by the unique combination of following features: (1) fin rays: D2 I/7-8; A I/8-9; P 16-19; First dorsal fin low and rounded, spines never filamentous. (2) squamation: LR 47-59; TR 15-17; PreD 0-29 ; Body covered with small sized ctenoid scales; cheek, operculum, prepelvic region and pectoral base region naked. (3) specific coloration: Adult individual with thin red lines at their upper and lower lip in both sexes. Caudal fin membrane with distinct black margin, upper caudal fin base with a circle black spot. (4) Head lateral-line system: Head pores absent; cheek with typical longitudinal papilla pattern; Sensory papillae row *c* merely with single papilla and located on under the starting point of row *b*; row *s* with two row papillae; row *p* complete. (5) Generally description: Body elongate and lowed; mouth medium sized in both sexes.

Type species

Mugilogobius polylepis Wu and Ni, 1985

Osteology of type species

In jaws and suspensorium, the maxilla stout and short. The ectopterygoid stout and short, and triangle shaped. The palatine stout and short, and anterior tip with cartage. The metapterygoid rectangular form shaped. The posterior portion of premaxilla stout and short, upper portion elongated. The

posterior portion of dentary tall and tip squared. The anguloarticular with two tips and upper tip longer than lower one. The quadrate joins anguloarticular and ectopterygoid in anterior margin, joins symplectic in posterior margin, joins metapterygoid in upper margin, and joins preopercle in lower margin. The hyomandibula joins metapterygoid in anterior margin, and joins preopercle in posterior margin. The preopercle L shaped, anterior tip elongated and sharpened. The interopercle elongated and anterior tip sharpened.

In anterior vertebrae skeleton, the anterior vertebrae and associated pterygiophore formula is 3-12210. In the caudal skeleton, the hypurals 1&2, 3&4 close up each other respectively, caudal skeleton consists of hypurals 1&2, hypurals 3&4, hypurals 5, single epural and parhypural, the epural rectangular form shaped, the parhypural elongated and sharpened.

Etymology

The generic name, *Wuhanlinigobius*, is referred to the Chinese ichthyologist, "Prof. Wu, Han-lin" for recognizing his great contribution for the ichthyological research in China especially for gobioid fishes.

Remarks

Among the subfamily Gobionellinae of family Gobiidae, *Hemigobius* generic group consists of genera *Brachygobius*, *Caecogobius*, *Calamiana*, *Eugnathogobius*, *Hemigobius*, *Mugilogobius*, *Pandaka*, *Pseudogobiopsis*, *Pseudogobius*, *Redigobius*, *Stigmatogobius*, *Tamanka*, *Weberogobius* and this new genus.

Wuhanlinigobius polylepis can be well distinguished from *Eugnathogobius microps* and other *Hemigobius* generic group genera based on following morphological features:

Wuhanlinigobius can be well distinguished from genera *Calamiana*, *Hemigobius*, *Pseudogobius*, *Pseudogobiopsis*, *Redigobius* and *Stigmatogobius* by lacking head pores; and distinguished from *Caecogobius* by *Caecogobius* having greatly reduced eye; and genus *Wuhanlinigobius* can be distinguished from genera *Mugilogobius*, *Pandaka* and *Tamanka* by different sensory papillae row *c* (genus *Wuhanlinigobius* with single papillae *c*; genera *Mugilogobius* and *Tamanka* with papillae row *c* and *c1*; genus *Pandaka* with a row of papillae row *c*). *Wuhanlinigobius* can be well distinguished from genus *Brachygobius* by *Brachygobius* having distinctive dark banded color pattern and scale present on upper operclum region. *Wuhanlinigobius* can be well distinguished from genus *Weberogobius* by genus *Weberogobius* with vertebral count 11+15-16, and restricted in freshwater lake of Sulawesi, Indonesia; *Wuhanlinigobius* with vertebral count 10+16, and live in brackish water of mangrove habitat.

Further more, in comparison of *Eugnathogobius microps*, which is type species of genus *Eugnathogobius*, *Wuhanlinigobius* can be well distinguished based on following morphological features:

In exterior morphological features, this new species snout slightly prominent than the lower lip; mouth medium sized,

Table 1. Sampling localities, OTU codes and accession number of molecular sequence analysis of *Wuhanlinigobius* species and other *Hemigobius* generic group species and outgroup from Taiwan, Palau, China, Tailand and Malay Peninsula.

Code	Species	Locality	Accession number		
			Cytb	ND5	D-loop
BKAML1	<i>Brachygobius kabiliensis</i>	Matang mangrove, Malaysia	KF929308	KF955612	KF779949
CVAML1	<i>Calamiana variegata</i>	Matang mangrove, Malaysia	KF929307	KF958735	KF779947
CVAML2	<i>Calamiana variegata</i>	Matang mangrove, Malaysia	KF929307	KF958735	KF779948
HHOML1	<i>Hemigobius hoevenii</i>	Matang mangrove, Malaysia	KF851357	KC995183	KC995177
HMIML1	<i>Hemigobius mingi</i>	Matang mangrove, Malaysia	KF851358	KF851362	KF779934
MABPZ1	<i>Mugilogobius abei</i>	Estuary of Putzu River, Dongshi Township, Chiayi County, Taiwan	KF128984	KF128984	KF128984
MABHK1	<i>Mugilogobius abei</i>	Mangrove of Hong Kong, China	KF929327	KC995178	KC995172
MCAPZ1	<i>Mugilogobius cavifrons</i>	Estuary of Putzu River, Dongshi Township, Chiayi County, Taiwan	KF929321	JX133912	JX133902
MCAZA1	<i>Mugilogobius cavifrons</i>	Estuary of Zhuan River, Toucheng Township, Yilan County, Taiwan	KF929321	JX133912	JX133902
MCHHK1	<i>Mugilogobius chulae</i>	Mangrove of Hong Kong, China	KF929323	KC995180	KC995174
MCHPK1	<i>Mugilogobius chulae</i>	Sai Yuan, Phuket Island, Thailand	KF929319	KF958744	KF779962
MCHZA1	<i>Mugilogobius chulae</i>	Estuary of Zhuan River, Toucheng Township, Yilan County, Taiwan	KF929324	JX133911	JX133900
MMEPL1	<i>Mugilogobius mertoni</i>	Mangrove of Palau	KF929318	KF958743	KF779961
MMEZA1	<i>Mugilogobius mertoni</i>	Estuary of Zhuan River, Toucheng Township, Yilan County, Taiwan	KF929322	JX133914	JX133904
MMYHJ1	<i>Mugilogobius myxodermus</i>	Meizhou City, Hanjiang River basin, Guangdong Province, China	KF929326	KC995181	KC995175
MMYYL1	<i>Mugilogobius myxodermus</i>	Lower reach of Yangliao River, Xinwu Township, Taoyuan County, Taiwan	KF929325	JX133913	JX133901
MTIML1	<i>Mugilogobius tigrinus</i>	Matang mangrove, Malaysia	KF929316	KF958742	KF779958
MTIML2	<i>Mugilogobius tigrinus</i>	Matang mangrove, Malaysia	KF929317	KF958742	KF779959
PPYPL1	<i>Pandaka pygmaea</i>	Mangrove of Palau	KF929309	KF955613	KF779950
POLPK1	<i>Pseudogobioptis oligactis</i>	Tha Ruea, Phuket Island, Thailand	KF929301	KF958728	KF779940
PAVML1	<i>Pseudogobius avicennia</i>	Matang mangrove, Malaysia	KF929310	KF958736	KF779951
PAVML2	<i>Pseudogobius avicennia</i>	Matang mangrove, Malaysia	KF929310	KF958736	KF779952
PFUML1	<i>Pseudogobius fulvicaudus</i>	Matang mangrove, Malaysia	KF929330	KF958747	KF779965
PFUML2	<i>Pseudogobius fulvicaudus</i>	Matang mangrove, Malaysia	KF929331	KF958748	KF779966
PGAPK1	<i>Pseudogobius gastrospilos</i>	Cherngtalay, Phuket Island, Thailand	KF929313	KF958739	KF779955
PGAPL1	<i>Pseudogobius gastrospilos</i>	Mangrove of Palau	KF929311	KF958737	KF779953
PGAZA1	<i>Pseudogobius gastrospilos</i>	Estuary of Zhuan River, Toucheng Township, Yilan County, Taiwan	KF929312	KF958738	KF779954
PJAKM1	<i>Pseudogobius javanicus</i>	Mangrove of Liehyu Island, Kinmen County, Taiwan	KF193873	KF193873	KF193873
PJAPK1	<i>Pseudogobius javanicus</i>	Sai Yuan, Phuket Island, Thailand	KF929320	KC995182	KC995176
PMAKM1	<i>Pseudogobius masago</i>	Estuary of Jinsha River, Kinmen Island, Taiwan	KF929314	KF958740	KF779956
PMAPZ1	<i>Pseudogobius masago</i>	Estuary of Putzu River, Dongshi Township, Chiayi County, Taiwan	KF929315	KF958741	KF779957
PTAZA1	<i>Pseudogobius taijiangensis</i>	Estuary of Zhuan River, Toucheng Township, Yilan County, Taiwan	KF929328	KF958745	KF779963
PTAHM1	<i>Pseudogobius taijiangensis</i>	Estuary of Jioulung River, Haimen Island, Fujian Province, China	KF929329	KF958746	KF779964
RBIGL1	<i>Redigobius bikolanus</i>	Estuary of Shuangsi River, Gongliao District, New Taipei City, Taiwan	KF929302	KF958730	KF779942
RBIZA1	<i>Redigobius bikolanus</i>	Estuary of Zhuan River, Toucheng Township, Yilan County, Taiwan	KF929302	KF958729	KF779941
WPOGD1	<i>Wuhanlinigobius polylepis</i>	Estuary of Danshui River, Guandu, Taipei City, Taiwan	KF929303	KF958731	KF779943
WPOGD2	<i>Wuhanlinigobius polylepis</i>	Estuary of Danshui River, Guandu, Taipei City, Taiwan	KF929304	KF958732	KF779944
WPOHK1	<i>Wuhanlinigobius polylepis</i>	Mangrove of Hong Kong, China	KF929305	KF958733	KF779945
WPOHM1	<i>Wuhanlinigobius polylepis</i>	Estuary of Jioulung River, Haimen Island, Fujian Province, China	KF929306	KF958734	KF779946
WMAML1	<i>Wuhanlinigobius malayensis</i> n. sp.	Matang mangrove, Malaysia	KF929331	KF958749	KF779967
RCHCT1	<i>Rhinogobius changtinensis</i>	Hanjiang River basin, Changtin County, Fujian Province, China	KF929300	KF958727	KF779939

maxillary extending to the vertical of anterior margin of pupil in male; body covered with small sized ctenoid scales and with more longitudinal scale series 47-50. The *E. microps* mouth medium sized, maxillary beyond the posterior margin of orbit and extending to preoperculum region in adult male; body covered with big sized ctenoid scales and with fewer longitudinal scale series 23-27 (original description see

Larson, 2009).

In osteological features, compare to *E. microps* (original drawing see Fig. 11 in Larson, 2009), this new genus can be well distinguished from *E. microps* based on jaws and suspensorium system. In this new genus, the maxilla and palatine stout and short. The ectopterygoid stout and short, and triangle shaped. The metapterygoid rectangular form shaped.

Table 2. Morphometric measurements of two *Wuhanlinigobius* species from Taiwan, China and Malay Peninsula.

Characters	<i>Wuhanlinigobius polylepis</i>		<i>Wuhanlinigobius malayensis</i> n. sp.	
	Male	Female	Male	Female
n	6	6	6	3
Percent standard length (%)				
Head length	26.7 – 28.1 (27.5)	25.7 – 26.4 (26.0)	24.8 – 25.4 (25.2)	24.7 – 25.1 (24.9)
Predorsal length	37.3 – 38.6 (37.9)	36.0 – 37.8 (36.8)	36.6 – 37.9 (37.2)	36.5 – 39.5 (37.6)
Snout to 2nd dorsal origin	58.1 – 58.7 (58.4)	57.3 – 57.8 (57.6)	56.5 – 58.7 (57.6)	58.3 – 60.0 (59.3)
Snout to anus	53.3 – 55.1 (54.2)	53.7 – 54.8 (54.2)	53.6 – 54.6 (54.2)	54.0 – 56.6 (55.7)
Snout to anal fin origin	56.4 – 58.2 (57.2)	59.2 – 60.8 (60.1)	56.9 – 59.2 (58.3)	57.3 – 61.6 (60.0)
Prepelvic length	26.3 – 27.9 (26.9)	27.6 – 28.6 (28.2)	26.0 – 28.7 (27.6)	26.7 – 29.0 (28.1)
Caudal peduncle length	27.2 – 28.8 (27.9)	26.0 – 28.7 (27.0)	26.7 – 28.0 (27.0)	25.9 – 27.5 (26.9)
Caudal peduncle depth	10.8 – 12.4 (11.7)	11.7 – 12.4 (12.0)	12.4 – 13.9 (13.2)	12.7 – 13.4 (13.0)
1st dorsal fin base	10.4 – 10.9 (10.6)	9.8 – 11.4 (10.8)	9.3 – 10.2 (9.8)	8.1 – 9.8 (8.7)
2nd dorsal fin base	18.0 – 19.7 (18.6)	18.0 – 20.1 (18.9)	15.2 – 17.1 (16.2)	13.0 – 14.5 (13.9)
Anal fin base	14.8 – 16.6 (15.8)	14.2 – 17.1 (16.1)	15.5 – 16.0 (15.8)	14.1 – 15.0 (14.6)
Caudal fin length	19.7 – 20.1 (19.9)	19.1 – 19.5 (19.3)	18.8 – 22.0 (20.3)	19.4 – 21.6 (20.3)
Pectoral fin length	20.6 – 22.1 (21.2)	19.3 – 19.7 (19.6)	19.0 – 22.0 (20.3)	19.6 – 20.0 (19.8)
Pelvic fin length	13.4 – 15.2 (14.0)	12.6 – 14.4 (13.3)	13.3 – 14.2 (13.7)	14.1 – 14.6 (14.3)
Body depth at pelvic fin origin	16.3 – 17.3 (16.7)	14.8 – 15.2 (15.0)	16.0 – 18.7 (17.2)	16.4 – 18.0 (17.3)
Body depth at anal fin origin	14.2 – 15.4 (14.7)	14.1 – 15.0 (14.6)	14.8 – 15.8 (15.3)	15.0 – 17.3 (16.0)
Body width at anal fin origin	9.5 – 10.8 (10.1)	9.2 – 10.3 (9.8)	10.6 – 12.5 (11.5)	11.3 – 11.4 (11.4)
Pelvic fin origin to anus	24.7 – 26.5 (25.4)	27.5 – 27.8 (27.7)	27.2 – 29.2 (27.9)	28.2 – 29.1 (28.6)
Percent head length (%)				
Snout length	32.3 – 35.7 (33.5)	30.9 – 32.3 (31.6)	27.4 – 30.6 (28.8)	29.5 – 32.7 (30.6)
Eye diameter	23.4 – 28.3 (25.4)	23.4 – 26.9 (24.7)	25.1 – 27.7 (26.2)	23.4 – 28.1 (26.3)
Cheek depth	25.9 – 28.8 (27.7)	22.7 – 25.0 (23.6)	21.1 – 23.3 (22.0)	18.2 – 21.9 (19.9)
Postorbital length	48.2 – 50.1 (49.4)	47.7 – 51.1 (49.0)	53.5 – 55.9 (54.7)	50.8 – 53.1 (52.2)
Head width in maximum	74.0 – 74.5 (74.3)	70.5 – 76.2 (74.2)	72.3 – 79.5 (76.0)	69.1 – 77.1 (74.2)
Head width in upper gill	41.8 – 49.1 (44.7)	46.2 – 46.8 (46.6)	47.8 – 54.9 (53.5)	50.8 – 51.6 (51.1)
Bony interorbital width	19.5 – 19.7 (19.6)	19.4 – 21.3 (20.3)	19.9 – 23.3 (21.3)	17.5 – 19.6 (18.6)
Fleshy interorbital width	35.5 – 38.7 (37.4)	35.1 – 38.7 (36.7)	36.1 – 39.5 (37.7)	35.2 – 37.5 (36.1)
Lower jaw length	47.9 – 51.4 (49.8)	43.9 – 46.2 (44.8)	40.4 – 45.7 (43.1)	33.8 – 35.9 (34.4)

The posterior portion of premaxilla stout and short, upper portion elongated. The posterior portion of dentary tall and tip squared. The preopercle L shaped, anterior tip elongated and sharpened. The interopercle elongated and anterior tip sharpened. In *E. microps*, the maxilla and palatine slender and long. The ectopterygoid long. The metapterygoid triangular form shaped. The posterior portion of premaxilla slender and very long, the tip sharpened, upper portion short. The posterior portion of dentary low and tip sharpened. The preopercle L shaped, anterior tip short and truncated.

In head lateral-line system, compare to *E. microps* (original drawing see Fig. 12 in Larson, 2009), this new genus can be well distinguished from *E. microps* based on following features: this new genus row *c* located on under the starting point of row *b*, and *E. microps* row *c* located on under the center of row *b*. This new genus with row *d* elongate and forming a straight line on upper lip margin; *E. microps* with row *d* forming a Y-shaped on upper lip margin.

Wuhanlinigobius polylepis (Wu and Ni, 1985)

(Tables 2, 3, Figs. 1a, 2a, 3a, 3b)

Mugilogobius polylepis Wu and Ni, 1985: 95 (Zhonggang, Fengjian, Shanghai, China); Wu and Zhong, 2008: 500 [32, 33].

Calamiana sp. nov. 2: Larson, 2001: 61 [17].

Calamiana polylepis: Larson *et al.*, 2008: 141 [19].

Eugnathogobius polylepis: Larson, 2009: 143 [18].

Material examined

Holotype of *Mugilogobius polylepis*, SFC S-0001, 31.2 mm SL, Zhonggang, Fengxian, Shanghai, 1985.

Other material

China: NTOUP 2012-04-132, 4 specimens, 17.1-21.1 mm SL, estuary of Jioulung River, mangrove of Haimen Island, Fujian Province, coll. S. P. Huang, 6 March, 2012. NTOUP

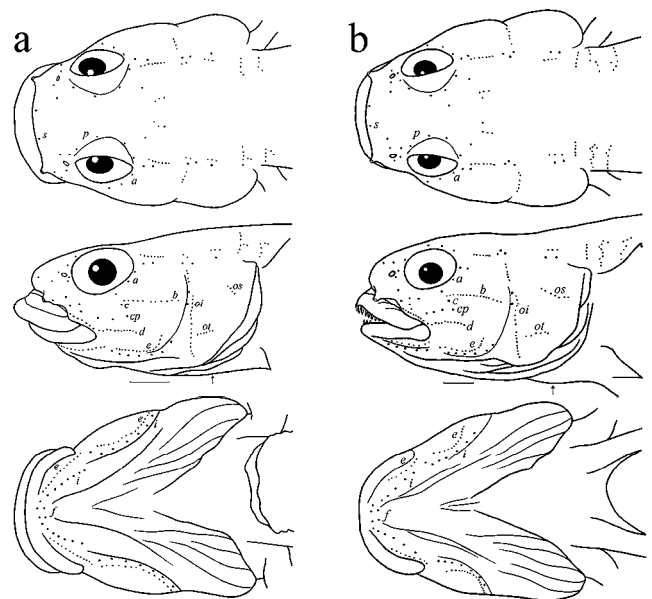
Table 3. Frequency distribution of meristic features of the two *Wuhanlinigobius* species from Taiwan, China and Malay Peninsula.

	D1		D2			A			P				
	VI	x	I/7	I/8	x	I/8	I/9	x	16	17	18	19	x
<i>W. polylepis</i>	20	6.0	–	20	8.0	19	1	8.6	2	16	19	1	17.5
<i>W. malayensis</i> n. sp.	15	6.0	2	13	7.9	9	6	8.4	–	–	12	10	18.5

	LR										TR			
	47	48	49	50	~	56	57	58	59	x	15	16	17	x
<i>W. polylepis</i>	4	18	8	8	–	–	–	–	–	48.5	7	13	–	15.7
<i>W. malayensis</i> n. sp.	–	–	–	–	–	6	7	5	2	57.2	–	10	5	16.3

	PreD																					
	0	~	6	7	8	9	10	11	~	19	20	21	22	23	24	25	26	27	28	29	x	
<i>W. polylepis</i>	11	–	3	–	2	1	1	1	–	1	–	–	–	–	–	–	–	–	–	–	–	4.2
<i>W. malayensis</i> n. sp.	–	–	–	–	–	–	–	–	–	–	–	–	–	2	1	3	5	–	1	1	1	25.5

	SDP					VC	
	11	12	13	14	x	26	x
<i>W. polylepis</i>	7	8	5	–	11.9	7	26.0
<i>W. malayensis</i> n. sp.	–	–	12	3	13.2	10	26.0

**Fig. 1.** The specimen photographs of two *Wuhanlinigobius* species, a, *Wuhanlinigobius polylepis*, NTOUP 2012-04-130, male, 31.0 mm SL; b, *Wuhanlinigobius malayensis* n. sp., NTOUP 2012-05-151, holotype, male, 31.3 mm SL.**Fig. 2.** Head lateral-line system of two *Wuhanlinigobius* species, a, *Wuhanlinigobius polylepis*, NTOUP 2012-04-133, male, 22.7 mm SL; b, *Wuhanlinigobius malayensis* n. sp., NTOUP 2012-05-151, male, 31.3 mm SL. Bar = 1 mm. Drawing by Shih-Pin Huang.

2012-04-133, 4 specimens, 19.7-22.7 mm SL, mangrove of Hong Kong, coll. I-S. Chen, 22 November, 2011.

Taiwan: NTOUP 201204-130, 3 specimens, 26.6-31.0 mm SL, estuary of Danshui River, Guandu, Taipei City, coll. M. T. Zhou and S. P. Huang, 1 April, 2012. NTOUP 2012-04-131, 17 specimens, 14.7-29.4 mm SL, estuary of Putzu River, Dongshi Township, Chiayi County, coll. I-S. Chen, December, 1995. NTOUP 2012-10-158, 5 specimens, 14.2-20.6 mm SL, mangrove of Kinmen Island, coll. I-S. Chen, 5 October, 2012.

Diagnosis

Wuhanlinigobius polylepis can be well distinguished by the

unique combination of following features: (1) fin rays: D2 I/8; A I/8-9 (modally 8); P 16-19 (modally 18); First dorsal fin low and rounded, spines never filamentous. (2) squamation: LR 47-50 (modally 48); TR 15-16 (modally 16); PreD 0-19 (modally 0); Body covered with small sized ctenoid scales. Predorsal region covered with cycloid scales in female, and usually naked in male. Cheek, Operculum, prepelvic region

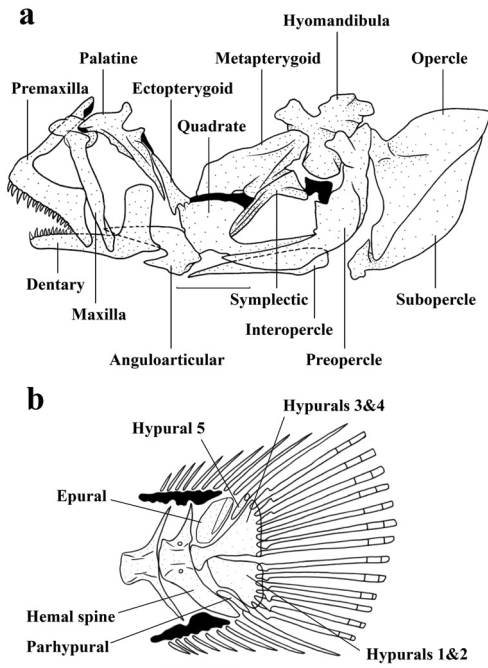


Fig. 3. Osteological features of *Wuhanlinigobius polylepis*, a, lateral view of jaws and suspensorium; b, caudal skeleton, NTOUP 2012-04-131, male, 23.6 mm SL. Bar = 1 mm. Drawing by Shih-Pin Huang.

and pectoral base region naked. (3) specific coloration: Cheek and preoperculum region with three horizontal blackish brown stripes. Adult individual with thin red lines at their upper and lower lip in both sexes. Caudal fin membrane yellow and with distinct black margin in adult male, caudal fin membrane with 3-5 vertical black line in both sexes.

Description

Body elongate and lowed, subcylindrical anteriorly and compressed posteriorly. Head medium sized. Snout slightly prominent than the lower lip. Eye rather large. Mouth medium sized, male slightly bigger than female, maxillary extending to the vertical of anterior margin of pupil in male, but maxillary reach the vertical of anterior margin of orbit in female. Anterior nasal as short tube, posterior nasal as round hole. Gill-opening extending ventrally forward the middle vertical line of operculum. VC 10 + 16 = 26 (in 7).

Fins

D1 VI; D2 I/8; A I/8-9 (modally 8); P 16-19 (modally 18). First dorsal fin low and rounded, spines never filamentous; second to fourth spines always longest, the spine is slightly longer in male than female, and can not extending to anterior edge of second dorsal when pressed in both sexes. Anal fin inserted below first branched rays of second dorsal fin. Pelvic fin medium sized and rounded. Caudal fin rounded.

Scales

LR 47-50 (modally 48); TR 15-16 (modally 16); PreD 0-19

(modally 0); SDP 11-13 (modally 12). Body covered with small sized ctenoid scales. Predorsal region covered with cycloid scales in female, and usually naked in male. Belly covered with smaller cycloid scales. Cheek, operculum, pre-pelvic region and pectoral base region naked.

Head lateral-line system

Head canals- Head pores absent.

Sensory papillae- Cheek with typical longitudinal papilla pattern. Row *a* hort, about half of orbit diameter. Row *b* with densely-set papillae, and equal to orbit diameter. Row *c* merely with single papilla and located on under the starting point of Row *b*. Row *cp* short, about half of orbit diameter. Row *d* long, about equal to orbit diameter. Row *s* with two row papillae. Row *p* complete. Opercular rows with rows *os*, *oi* and *ot*. Rows *oi* and *ot* slightly closed. Rows *f* with a pair of single papillae.

Coloration in life

Head and trunk with pale yellowish brown or pale yellow background. Body side and neck with many irregular brown bars. Belly pale yellowish white. Cheek and preoperculum region with three horizontal blackish brown stripes. Adult with thin red lines at their upper and lower lips in both sexes. Pectoral fin base with a horizontal brown bar. Upper caudal fin base with a big sized circle black spot, and merely smaller spot in female. First and second dorsal fin membranes gray and with yellow margin. Anal fin membrane gray and with white margin. Pectoral and pelvic fin membranes grayish white. Caudal fin membrane yellow and with distinct black margin in adult male, and caudal fin membrane grayish white and with indistinct black margin in female, caudal fin membrane with 3-5 vertical black line in both sexes.

Distribution. This species originally found from Shanghai, and it also found from Fujian, Guangdong Province in China and also Kinmen, Charyi counties in Taiwan.

***Wuhanlinigobius malayensis* new species**

(Tables 2, 3, Figs. 1b, 2b)

Material examined:

Holotype.-NTOUP 2012-05-151, 31.3 mm SL, male, Matang mangrove, Malaysia, coll. I-S. Chen and S. P. Huang, 20 April, 2011.

Paratypes.-NTOUP 2011-05-012, 8 specimens, 20.9-30.5 mm SL, Matang mangrove, Malaysia, coll. I-S. Chen and S. P. Huang, 20 April, 2011. NTOUP 2011-05-022, 8 specimens, 17.9-31.1 mm SL, Matang mangrove, Malaysia, coll. I-S. Chen and S. P. Huang, 21 April, 2011. NTOUP 2011-05-027, 7 specimens, 13.0-25.0 mm SL, Sungai Haji Dorani, Malaysia, coll. S. P. Huang and H. M. Huang, 22 April, 2011.

Diagnosis

Wuhanlinigobius malayensis n. sp. is well distinguished

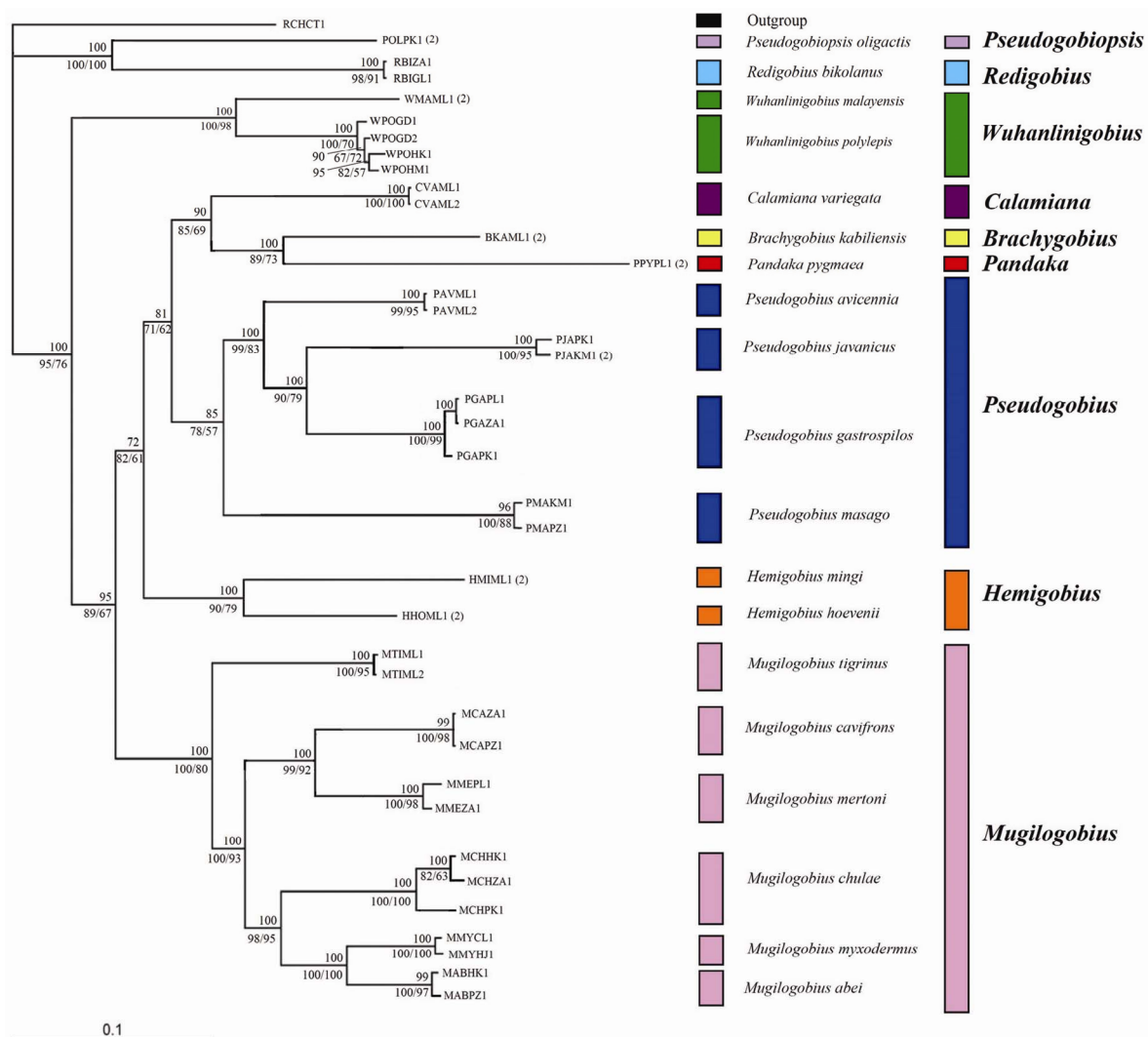


Fig. 4. Molecular phylogenetic tree of new genus and other *Hemigobius* generic group genera from Taiwan, Palau, Malay Peninsula and southern China based on combined Cytb, D-loop and partial ND5 sequence reconstructed by Bayesian analysis method based on the Kimura HKY 85+G model (values above the branch: posterior probability). The similar topology for bootstrap consensus tree by maximum parsimony method (anterior value) and neighbour-joining method (posterior value) list only the bootstrap (value below the branch: bootstrap number, 2000 replications).

from other congeners by the unique combinations of the following features: (1) fin rays: D2 I/7-8 (modally 8), A I/8-9 (modally 8), P 18-19 (modally 18), and first dorsal fin never filamentous; (2) squamation: lateral body with small ctenoid scales, longitudinal scale rows 56-59 (modally 57), predorsal scales 23-29 (modally 26); (3) specific coloration: Caudal fin creamy yellow or pale yellow, with broad black submarginal edge in male, upper caudal fin base with a large rounded black spot in male.

Description

Body elongate, subcylindrical anteriorly and compressed posteriorly. Head large. Upper lip more prominent than lower lip. Eyes rather large. Mouth small, maxillary extending to the vertical of center of pupil in male, and extending to the

vertical of anterior margin of pupil in female. Anterior nasal as short tube, posterior nasal as round hole. Gill-opening restricted, ventrally extending to the two third of vertical line of operculum. VC 10 + 16 = 26 (in 10).

Fins

D1 VI; D2 I/7-8 (modally 8); A I/8-9 (modally 8); P 18-19 (modally 18). First dorsal fin spines never filamentous, second to fourth spines longest. Anal fin inserted below spine of second dorsal fin. Pelvic fin large and rounded. Rear margin of caudal fin rounded.

Scales

LR 56-59 (modally 57); TR 16-17 (modally 16); PreD 23-29 (modally 26); SDP 13-14 (modally 13). Body covered

with small ctenoid scales. Predorsal region with small cycloid scales. Belly with smaller cycloid scales, prepelvic fin region naked. Cheek and opercle naked.

Head lateral-line system

Head canals- Head pores absent.

Sensory papillae- Row *a* short, about half of eye diameter. Row *b* with densely-set papillae, and longer than eye diameter, starting from vertical of rear margin of pupil. Row *c* merely single papillae. Row *cp* about two-third orbit diameter, and starting from vertical of anterior margin of pupil, extending to rear margin of orbit. Row *d* longer than eye diameter. Row *s* with two row papillae. Row *p* completed. Opercular rows with rows *os*, *oi* and *ot*. Rows *oi* and *ot* well separated. Rows *f* with a pair of single papillae.

Coloration in life

Head and body generally creamy yellow or pale yellowish brown, upper half of lateral trunk with many irregular vertical blackish brown short bars or spots. Lateral scales with blackish brown margin. Belly creamy white. Cheek and opercle with numerous black spots. Pectoral fin membrane pale gray or grayish white, base with some black spots. First dorsal fin and second dorsal fin grayish white, with pale yellow margin in adult male, but merely grayish white in female. Anal fin membrane gray, with white margin. Pelvic fin gray in male, pale gray in female. Caudal fin creamy yellow or pale yellow, with broad black submarginal edge in male, and with unapparent gray margin in female, upper caudal fin base with a large rounded black spot in male, and with an unapparent smaller gray spot in female.

Habitat

Wuhanlinigobius malayensis n. sp. can be found in the shallow tidal pools of the mangrove region of Malay Peninsula, a muddy intertidal habitat.

Distribution. This species found from Matang, and Sungai Haji Dorani, Malay peninsula and also found in Singapore.

Etymology

The Latin specific name, "*malayensis*" referring to this new species distributed in the brackish water habitat of Malay Peninsula region.

Remarks

Wuhanlinigobius malayensis n. sp. and *Wuhanlinigobius polylepis* with similar sensory papilla and small size of body scales, both species caudal fin with broad black submarginal edge, upper caudal fin base with a large rounded black spot in male. However, *W. malayensis* can be distinguished from *W. polylepis* by the following features: (1) more longitudinal scale series 56-59 vs. 47-50. (2) more predorsal scale series 23-29 vs. 0-19, and (3) *W. malayensis* with same predorsal scales distribution region in both sexes, the *W. polylepis* male

usually naked in predorsal region.

A diagnostic key to genus *Wuhanlinigobius*

- 1a. longitudinal scale series 47-50; predorsal scales series 0-19; predorsal region covered with cycloid scales in female, and usually naked in male; caudal fin membrane with 3-5 vertical black line in both sexes
.....*Wuhanlinigobius polylepis*
- 1b. longitudinal scale series 56-59; predorsal scales series 23-29; predorsal region covered with cycloid scales in both sexes; caudal fin membrane without vertical black line*Wuhanlinigobius malayensis* n. sp.

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