Zootaxa 5189 (1): 029–044
https://www.mapress.com/zt/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

https://doi.org/10.11646/zootaxa.5189.1.6
http://zoobank.org/urn:lsid:zoobank.org:pub:346ABFF6-8E8F-4936-AE5B-FA941233524D

A new freshwater gobiid species of *Rhinogobius* Gill, 1859 (Teleostei: Gobiidae) from northern Taiwan

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Abstract

A new freshwater rhinogoby has been collected and surveyed from northern Taiwan. The new species, *Rhinogobius yangminshanensis* n. sp. with fluvial life history can be well distinguished from other congeners by the following combination of features: (1) fin rays: second dorsal fin rays I/9; anal fin rays I/8; pectoral fin rays modally 16; (2) squamation: longitudinal scale series 28–30 (modally 29); perdorsal scales 9–10 (modally 9); (4) vertebral count 27; (5) rear edge of mouth: merely extending to vertical of anterior margin of pupil in male and (6) specific colouration: lateral side with 6–7 longitudinal rows of bright orange to orange red spots in male which general size about 1/2 of pupil diameter. Cheek and opercle with 24–35 orange spots in male. Branchiostegal membrane with many minute orange spots in male. Caudal fin with distally orange zone in male with about 3 vertical rows of orange or orange red spots. First dorsal fin with broad orange band on distally 1/3 area. A middle black spot in abterior first dorsal fin. Pectoral fin with two rows red orange spots in male. The phylogenetic comparisons have revealed that the great mitogenetic differences of *R. yangminshanensis* with all other congeneric species and sister species would be *R. rubromaculatus* in Taiwan. A diagnostic key to all valid species of *Rhinogobius* from Taiwan is also provided.

Key words: new goby, freshwater fish, *Rhinogobius*, fish fauna, Taiwan

Introduction

Gobioid fishes are the very important components of benthic freshwater fish fauna in East Asia. The freshwater goby, *Rhinogobius* Gill, 1859, is widely distributed on several islands of the Western Pacific including Japan (Akihito *et al.*, 1984, 1993, 2002; Masuda *et al.* 1989; Suzuki *et al.* 2011), Taiwan (Aonuma & Chen 1996; Chen & Shao 1996; Lee & Chang 1996; Chen *et al.* 1998; Chen & Fang 1999), Hainan (Wu & Ni 1985; Chen *et al.* 2002; Chen & Miller 2013), and Philippines (Herre 1927), and also continental Asia, in Russia, Korea, China, Vietnam, Laos, Cambodia, and Thailand (Chu & Wu 1965; Zheng & Wu 1985; Chen *et al.*, 1999a–c, Chen & Kottelat 2000, 2003, 2005; Chen & Fang 2006; Chen & Miller 2008; Huang & Chen 2007; Li & Zhong 2007; Li *et al.* 2007; Chen *et al.* 2008; Chen 2009; Wu *et al.* 2009).

The life history of *Rhinogobius* species comprises non-diadromous, landlocked, fluvial species species (Mizuno 1960; Mizuno & Goto 1987; Iguchi & Mizuno 1991; Akihito *et al.* 1984, 1993, 2002) as well as lake-river migratory species and lentic species (Takahashi & Okazaki 2002).

At present, the first author (ISC) estimates that there are at least over 85 species are known in East and Southeast Asia and some of them still need formal description (Chen & Kottelat 2003, 2005; Chen & Fang 2006; Chen *et al.* 2008; Yang *et al.* 2008; Chen & Miller 2013).

In Taiwan, except the derived brief transverse infraorbital papillae group: *Rhinogobius similis* Gill, 1859 (previously *Rhinogobius giurinus* (Rutter, 1897)), all remaining species are with typical longitudinal infraorbital papillae - *Rhinogobius brunneus* (Temminck & Schlegel, 1845) species complex including following 8 species: *Rhinogobius candidianus* (Regan, 1908), *Rhinogobius formosanus* Oshima, 1919, *Rhinogobius gigas* Aonuma & Chen, 1996, *Rhinogobius nantaiensis* Aonuma & Chen, 1996, *Rhinogobius rubromaculatus* Lee & Chang,

Accepted by Y.-T. Shao: 14 Jul. 2022; published: 23 Sept. 2022

1996; *Rhinogobius delicatus* Chen & Shao, 1996, *Rhinogobius henchuenensis* Chen & Shao, 1996, *Rhinogobius maculafascistus* Chen & Shao, 1996, and *Rhinogobius lanyuensis* Chen *et al.*, 1998.

During the recent inventory of freshwater fish fauna from northern Taiwan especially for the most river basins of the Yangminshan National Park, the undescribed fluvial species can be collected and recognized from the small tributaries of hill-streams. The description of this new species with fluvial life history which is rather similar to *Rhinogobius rubromaculatus* Lee & Chang is provided herein. The mitgogenetic survey of current species as well as other endemic species from Taiwan are also carried out for getting both mitogenetic recognition of new species and their phylogenetic insights around Taiwanese species. A diagnostic key to all valid species of *Rhinogobius* from Taiwan is also provided.

MATERIALS AND METHODS

Sample collection and morphological survey type specimens of the new goby were collected by hand-net and cast-net. The type specimens of current new species which collected from the Yangminshan National Park are actually under the official collection permit of the National Park during our processing the fish biodiversity survey project of all river basins of Yangminshan area approved by the National Park. Other collection which river basins are outside the National Park is no need any of collection permit.

All counts and measurements were made from specimens finally preserved in 70% ethanol. Morphometric methods follow Miller (1988) and meristic methods follow Akihito *et al.* (1984) and Chen & Shao (1996). Terminology of cephalic sensory canals and free neuromast organs (sensory papillae) was from Wongrat & Miller (1991), mainly based on Sanzo (1911). Meristic abbreviations were as follows: A = anal fin; C = caudal fin; D1 = first dorsal fin; D2 = second dorsal fin; LR = longitudinal scale rows; P = pectoral fin; PreD = predorsal scales; SDP = scale series from origin of first dorsal fin to upper pectoral fin origin; TR = transverse scale series from second dorsal to anal fins; V = pelvic fin; VC = vertebral count. All fish lengths were expressed by standard length (SL).

The type specimens and comparative materials of the remaining rhinogobies are deposited in the Biodiversity Research Center, Academia Sinica, Taipei (ASIZP); British Museum (Natural History), London (BMNH); California Academy of Science, San Francisco (CAS); National Museum of Marine Biology/Aquarium, Pingtung (NMMBP); and Pisces collection of National Taiwan Ocean University, Keelung (NTOUP). The all other comparative materials of congeneric species from Taiwan are listed in Appendix I.

The fresh specimens of eight Taiwanese endemic *Rhingobius* species including the new one used for molecular analysis were directly preserved in 95% ethanol when caught and transferred frozen after preservation to the laboratory. In this study, all nine species were used in the molecular analysis and phylogenetic studies. The *Tridenitger bifasciatus* was assigned as an outgroup of all member of *Rhinogobus* species.

All DNA extractions of the rhinogobies were done according to the general protocols of the phenol-chloroform method (Sambrook *et al.* 1989; Chen *et al.* 2002). The DNA fragments of about 1,300 bp, including the full length of D-loop region, were amplified by polymerase chain reaction (PCR) using primers based on the flanking region that were designed from the sequences of tRNA-PHE and 12S rRNA (Chen *et al.* 2002). PCR was done in a Model 9700 thermal cycler (Perkin-Elmer) and 30–40 cycles were carried out. The 25 μL reaction volume contained 14.4 μL of sterile distilled water, 2.5 μL of 10× PCR buffer (Takara), 2.0 μL of dNTP (2.5 mM each), 2.5 μL of each primer (5 μM), 0.1 μL of 0.5 unit Ex Taq (Takara) and 1.0 μL of template. The thermal cycle profile was as follows: denaturation at 94 ° C for 15 seconds, annealing at 50° C for 15 seconds and extension at 72 ° C for 60 seconds. A negative control without template DNA 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 (Boehringer Mannheim, High Pure PCR Product Purification kit), before undergoing direct cycle sequencing with dye-labeled terminators (ABI Big-Dye kit). The primers used were the same as those for PCR. All sequencing reactions were performed according to the manufacturers' instructions. Labeled fragments were analyzed using an ABI Model 377- 64 DNA sequencer (ABI). Nucleotide sequence alignment was done manually. The phylogenetic and molecular evolutionary analyses were conducted using MEGA11: Molecular Evolutionary Genetics Analysis version 11 (Tamura *et al.* 2021) by neighbor-joining (NJ) and maximum likelihood (ML) methods with 1,000 bootstrap replications.

SYSTEMATICS

Rhinogobius Gill, 1859

Rhinogobius yangminshanensis new species
(Figures. 1–5)

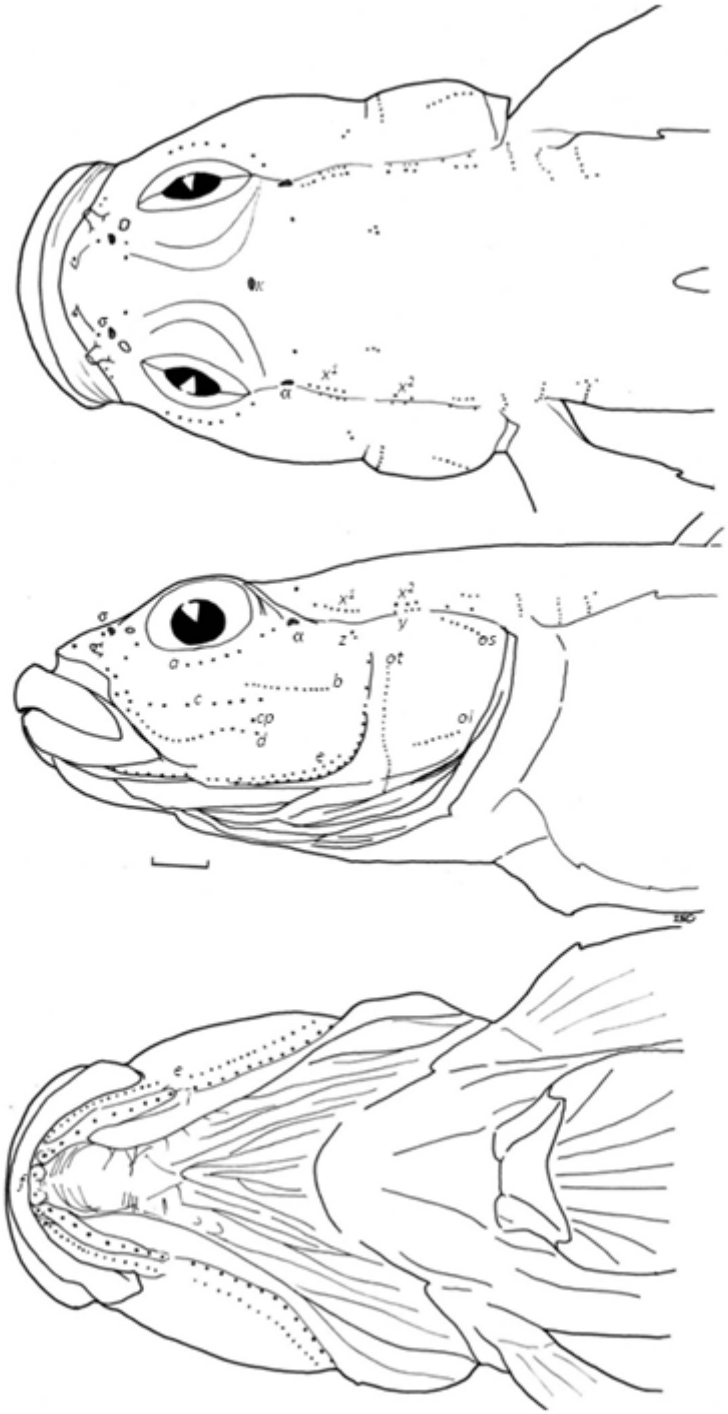

FIGURE. 1. Head lateral-line system of *Rhinogobius yangminshanensis*, male, holotype, NTOUP-2017-06-322, 27.6 mm SL, Tanshuei River basin, Taipei City. (Bar = 1 mm)

FIGURE 2. *Rhinogobius yangminshanenesis*, male (upper one), NTOUP-2017-06-325, 27.9 mm SL, paratype, Neishuanshi, Shihlin, Tanshuei River basin, Taipei City, Taiwan; female (lower one), NTOUP-2017-06-324, 28.3 mm SL, paratype, Nanhuangshi, Peitou, Tanshuei River basin, Taipei City, Taiwan.

Materials examined.

Holotype: NTOUP-2017-06-322, 27.6 mm SL, male, July 12, 2016, coll. I-S. Chen, Yunchuenliao, Nanhuangshi, Beitou, Tanshuei River basin, Taipei City, Taiwan, ROC.

Paratypes: NTOUP-2017-06-323, 8 specimens, 27.1–31.9 mm SL, March 5, 2014, coll. Y.W. Liu, Tsaolanshi, Chitu, Keelung River, Tanshuei River basin, Keelung City, Taiwan, ROC. NTOUP-2017-06-324, 5 specimens, 24.6–28.3 mm SL, July 12, 2016, coll. I-S. Chen, Yunchenliao, Nanhuangshi, Beitou, Tanshuei River basin, Taipei City, Taiwan, ROC. NTOUP-2017-06-325, 2 specimens, 26.2–27.9 mm SL, Aug 20, 2016, coll. I-S. Chen, Tianshiyuan, Shihlin, Tanshuei River basin, Taipei City, Taiwan, ROC. NTOUP-2017-06-326, 4 specimens, 24.8–29.6 mm SL, June 3, 2017, coll. I-S. Chen, small tributary of Tartunshi basin, Tanshuei, New Taipei City, Taiwan, ROC. NTOUP-2017-06-327, 4 specimens, 28.5–32.0 mm SL, June 4, 2017, coll. I-S. Chen, Chinshueishi, Jinshan, Huangshi basin, New Taipei City, Taiwan, ROC.

Diagnosis.

Rhinogobius yangminshanensis can be well distinguished from all other congeners by the unique combination of the following features: (1) fins: second dorsal fin rays I/9; anal fin rays I/8; pectoral fin rays modally 16; (2) squamation: longitudinal scale series 28–30 (modally 29); perdorsal scales 9–10 (modally 9); (4) vertebral count 27; (5) rear edge of mouth: merely extending to vertical of anterior margin of pupil in male and (6) specific colouration: lateral side of trunk with 6–7 longitudinal rows of bright orange to orange red spots in male which general size about 1/2 of pupil diameter. Snout with two parallel brownish gray stripes (orange one as posterior stripes in male). Cheek and opercle with 24–35 orange spots in male. Branchiostegal membrane with many minute orange spots in male. Caudal fin with distally orange zone in male with about 3 vertical rows of orange or orange red spots. First dorsal fin with broad orange band on distally 1/3 area. A middle black spot in front of the third spine of first dorsal fin in both sexes. Pectoral fin with two rows (totally 7–9 spots) of orange to red orange spots in male.

TABLE 1. Morphometry of *R. yangminshanensis* n. sp. and *R. rubromaculatus*

Type	*R. yangminshanensis*			*R. rubromaculatus*	
	H	H+P	P		
Sex	Male	Male(n=8)	Female(n=6)	Male (n=8)	Female (n=6)
Standard length	27.6	24.6-31.9	26.4-31.7	28.4-40.0	26.5-38.5
%in SL					
Head length	30.7	30.2-34.6(32.3)	29.4-32.7(31.3)	26.6-30.0(28.1)	26.0-29.3(28.0)
Predorsal length	41.0	38.9-41.6(40.3)	38.8-42.8(40.9)	37.9-42.2(39.9)	38.7-43.1(40.1)
Snout to 2nd dorsal origin	58.1	57.0-61.4(59.0)	56.3-59.8(58.7)	55.5-60.2(58.6)	55.3-61.9(59.1)
Snout to anus	62.5	61.5-63.7(62.6)	60.2-63.4(61.9)	55.2-57.7(56.6)	53.9-59.1(57.0)
Snout to anal fin origin	57.1	56.6-58.9(57.3)	55.7-60.1(57.8)	60.8-63.9(62.2)	61.3-65.1(63.6)
Prepelvic length	30.9	30.9-35.5(32.4)	29.8-33.3(30.9)	29.3-33.4(31.1)	29.3-33.5(30.9)
Caudal peduncle length	22.9	20.4-24.8(22.7)	22.2-25.9(23.4)	20.3-25.4(22.8)	22.6-24.9(23.4)
Caudal peduncle depth	12.9	12.6-13.4(13.0)	12.2-13.3(12.8)	12.6-14.6(13.5)	10.8-13.3(12.0)
First dorsal fin base	19.1	17.1-19.5(18.5)	15.4-18.8(17.1)	13.0-18.4(15.1)	13.3-16.4(14.4)
Second dorsal fin base	24.0	22.5-26.7(23.9)	21.2-24.2(22.9)	19.0-25.3(21.7)	19.3-22.0(20.0)
Anal fin base	19.3	17.5-22.1(19.3)	17.2-20.1(19.1)	14.9-18.2(1.6)	15.2-19.2(17.1)
Caudal fin length	27.5	27.4-31.9(28.5)	25.6-29.7(27.4)	21.4-26.7(23.8)	22.9-25.0(23.7)
Pectoral fin length	25.9	23.5-27.7(26.6)	22.2-26.9(24.3)	20.7-24.3(22.3)	20.6-25.1(23.4)
Pelvic fin length	17.4	15.8-18.9(17.8)	16.6-19.8(18.5)	14.2-17.8(15.3)	13.9-18.0(16.4)
Body depth of pelvic fin origin	17.1	15.8-18.3(17.1)	15.2-18.2(16.7)	14.6-18.2(16.7)	14.9-17.8(16.6)
Body depth of anal fin origin	15.6	15.2-17.4(16.0)	14.7-17.4(16.1)	14.4-18.1(16.7)	15.7-18.0(16.6)
Body width of anal fin origin	12.6	10.0-12.6(11.8)	11.5-13.0(12.2)	10.1-14.4(12.8)	12.1-13.5(13.0)
Pelvic fin origin to anus	27.4	27.4-29.3(28.3)	26.0-31.1(28.7)	26.6-31.7(28.3)	24.4-30.4(27.6)
%in HL					
Snout length	32.9	28.2-33.1(32.9)	27.2-31.8(29.8)	25.8-32.2(30.4)	28.4-30.6(29.4)
Eye diameter	21.8	20.2-24.6(22.4)	22.2-25.1(23.4)	15.7-22.5(18.9)	15.6-21.8(18.3)
postorbital length	49.8	47.4-56.5(52.8)	50.4-55.6(52.8)	47.1-57.1(52.3)	51.0-58.1(54.8)
Cheek depth	30.4	27.1-33.1(30.7)	24.6-29.8(27.2)	27.0-37.0(31.9)	24.9-34.5(30.1)
Head width in upper gill opening	50.8	44.6-53.6(49.5)	45.4-54.5(49.1)	46.5-55.3(50.4)	47.0-55.0(51.2)
Head width in maximum	64.7	55.8-64.4(61.6)	56.9-70.3(62.1)	53.7-76.8(69.1)	61.9-75.3(69.2)
Fleshy interorbital width	23.7	19.7-25.8(22.3)	26.0-30.2(27.7)	20.2-24.3(22.1)	21.4-24.9(23.2)
Bony interorbital width	10.3	7.7-11.7(9.4)	7.4-10.9(9.7)	5.7-8.3(6.7)	6.2-7.4(6.8)
Lower jaw length	39.5	35.0-41.6(38.7)	29.0-37.3(33.1)	27.6-33.0(30.3)	25.1-35.4(29.3)

Description.

Body proportions in Table 1. Body cylindrical anteriorly, compressed posteriorly. Head rather large, somewhat depressed in male. Eye large, dorsolateral. Snout pointed. Cheek somewhat fleshy in male. Lips thick. Mouth oblique, rear edge extending near or just to vertical of anterior margin of eye in male, but not reaching vertical in female. Both jaws with 3–4 rows of conical teeth, outer jaws enlarged. Tongue margin rounded. Anterior nostril in short tube and posterior nostril round. Gill opening small, extending ventrally to vertical of rear margin of cheek. Vertebral count 10 + 17 = 27 (examined in 10 larger specimens).

Fins.—D1 VI, D2 I/8–9 (modally I/9); A I/8; P 15–17 (modally 16); V I/5+I/5 (distribution frequency in Table 2). D1 rounded, 3rd and 4th rays slightly longer, with rear tip while depressed extending beyond D2 origin in male. Origin of A inserted below second branched rays of D2. Rear tips of D2 and A fin rays extending near or beyond

procurrent rays of C in male. P moderate large and oblong, rear tip near reaching vertical line through anus in male. V small and rounded, spinous rays with somewhat pointed membrane lobe. C elliptical, rear edge rounded.

Scales.—Body with moderately large ctenoid scales, anterior region of predorsal area naked; posterior dorsal area and belly cycloid. LR 29–32 (modally 30); TR 10–12 (modally 11); PreD 10–14 (modally 12); and SDP 7–8 (modally 8) (distribution frequency in Table 2). Head and prepelvic region naked. Anterior edge of midpredorsal squamation extending beyond the vertical of midline of opercle.

Head lateral-line system.—(Figure 1)

Canals: Nasal extension of anterior oculoscapular canal with terminal pore σ located in between anterior and posterior nostrils. Anterior interorbital sections of oculoscapular canal with paired pore λ. A single pore κ in near rear of interorbital region in lacking both with paired pore λ and pore ω. Lateral section of anterior oculoscapular canal with terminal pore α. No posterior oculoscapular canal. No preopercular canal.

Sensory papillae: Row *a* extending beyond vertical midline of orbit. Row *b* length about equal to eye diameter. Rows *c*, *d* longer. A single *cp* papilla. Row *f* paired. Anterior edge of row *oi* well separated to lower region of row *ot*.

TABLE 2. Distribution frequency of fin-ray counts of *Rhinogobius yangminshanensis* with other congerenic species from Taiwan

	D2					A						P										
	I/7	8	9	10	X	I/6	7	8	9	10	X	14	15	16	17	18	19	20	21	22	23	X
***R. yangminshanensis* n. sp.**		**1**	**17**		**9.0**			**18**			**8.0**		**7**	**29**								**15.8**
R. candidianus	3	16	1		7.9		5	41	1		7.9				6	9	5					18.0
R. delicatus	2	18	1		8.0		2	16	2	1	8.1				2	11	8					18.3
R. formosanus	1	13	1		8.0		2	13			7.8						3	9	6			20.0
R. gigas		15					1	14			7.9								5	7	2	21.7
R. henchuenensis	1	18	1		8.0		1	19			8.0					2	7	9	2			19.6
R. lanyuensis		15			8.0			15			8.0						1	9	5			20.3
R. macluafasciatus		16	4	1	8.3			19	2		8.1					1	10	10				19.4
R. nantaiensis		13	4		8.2		1	16			7.9					11	9					18.5
R. rubromaculatus	1	13	1		8.0	1	11	3			7.2	4	6	18	2							15.6
R. similis		20			8.0			20			8.0						11	9				19.5

Colouration while fresh.—(Figures 2–5)

Body creamy yellow to yellowish brown. Side of body without distinct gray or brown blotches or merely with very faint gray blotches; lateral body with 6–7 longitudinal rows of bright orange to orange red spots about 1/2 of pupil diameter in male; orange brown spots less than 1/4 of pupil diameter in female . Dorsal region of body with 5–6 indistinct gray blotches.

Head creamy yellow to yellowish brown. Cheek and opercle with 24–35 orange spots in male, with small brown spots 26–30 in female. Larger cheek spot about 1/3 of pupil diameter. Nape with many orange spots in male, with small brown spots in female. Snout on dorsal side with a pair of red to brown stripe united at snout tip. Snout on lateral side with two parallel brownish gray stripes (orange one as posterior stripes in male) both terminating toward margin of upper jaw; lower one bright orange with shiny narrow blue margin in male, but brownish gray in female. Lips and dorsal snout pale brown. Branchiostegal membrane with many minute orange spots in male, but spotless in female.

First dorsal fin pale brown with broad orange band on distally 1/3 area. A middle black spot in front of third spine of first dorsal fin in both sexes . Basal region of first dorsal fin with 5–7 orange spots in male, 6–8 in female. Second dorsal fin pale with orange band on distal 1/3 region, 2–3 longitudinal rows of orange spots in male; translucent with thin gray band, 3–4 longitudinal blackish brown spots in female. Anal fin orange with distal white to gray margin. Caudal fin surrounding with distally orange zone in male accompanying with 3 somewhat vertical rows of orange or orange red spots; with pale brown zone in female accompanying with 3–4 vertical rows of small brown spots. Pectoral fin with two rows (totally 7–9 spots) of orange to red orange spots which 3–4 larger orange

spots on anterior row in male; with two rows (totally 8–12 spots) of smaller brown spots in female. A bluish white band between the two rows of orange spots in male near the basal region of pectoral fin. Pelvic fin pale gray in male, whitish in female.

Etymology.—the specific name, *yangminshanensis*, is referred to the type locality of holotype which the tributary of Tanshuei River basin originating from the main area of Yangminshan National Park.

Distribtuion.—The new species can be found in several hill-streams of Tanshuei river basin and possibly following streams of small river basins from Gunshitanshi basin eastward along the northern coast to Marsushi basins, Shuanshi basins, in both Taipei, New Taipei cities and Taoyuan city.

FIGURE 3. *Rhinogobius rubromaculatus*, holotype, female (upper one), ASIZP-056640, 39.0 mm SL, Wushi basin, Taichung City; male (middle one), NTOUP-2017-06-321, 31.0 mm SL; female (lower one), NTOUP-2017-06-321, 30.8 mm SL, Wufong, Wushi basin, Taichung City.

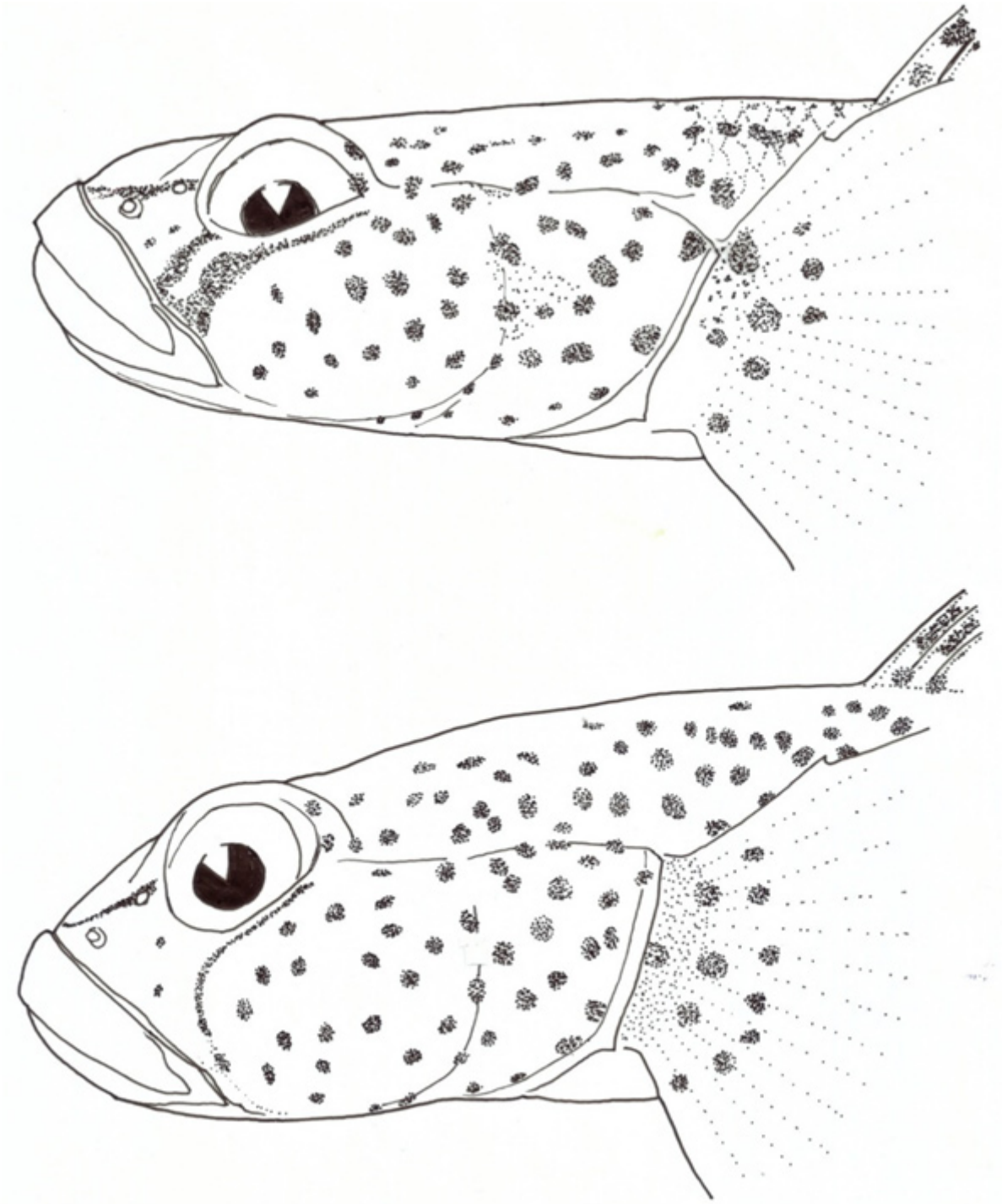

FIGURE 4. Head colour-pattern drawing of male specimens of *Rhinogobius yangminshanensis* (upper one), NTOUP-2017-06-325, 27.9 mm SL, the Tanshuei River basin, Taipei City; and *Rhinogobius rubromaculatus* (lower one), NTOUP-2017-06-321, 31.0 mm SL, the Wushi basin, Taichung City, Taiwan.

Phylogenetic insights of *Rhinogobius* species in Taiwan.

The complete mtDNA D-loop sequences of *Rhinogobius* species in Taiwan have been sequenced. The results of mitogenetic analysis clearly support the great mitogenetic distinction of new species *R. yangminshanensis* from closely related *R. rubromaculatus*. However, the differentiated, mitogenetic distance is about 3–4 times more than interspecific distances among all recognized, endemic species like *R. candidianus, R. henchuensis, R. delicatus, R.*

gigas as well as *R. formosanus* (Figure 6). Most of three branches are with rather high bootstrap support in both methods (62–99 above species level).

In intraspecific comparison of *R. yangminshanesis*, all 36 sequenced specimens provided 10 halotypes of mtDNA from those drainages of northern Taiwan.

The molecular phylogenetic studies have been analyzed and show Taiwanese species to be a monophyletic group both by neighbour-joining (NJ) and maximum parsimony (MP) methods. The molecular phylogenetic tree shows that the plesiomorphic state of *R. similis* can be seen here. All remaining species of Taiwanese member of *Rhinogobius* species with longitudinal infraorbital papillae could be considered as monophyletic group. Therefore, the detailed phylogenetic comparison of Chinese and Japanese species is still needed to carry out to fulfill the global evolutionary history of the great diversified group of freshwater rhinogobies.

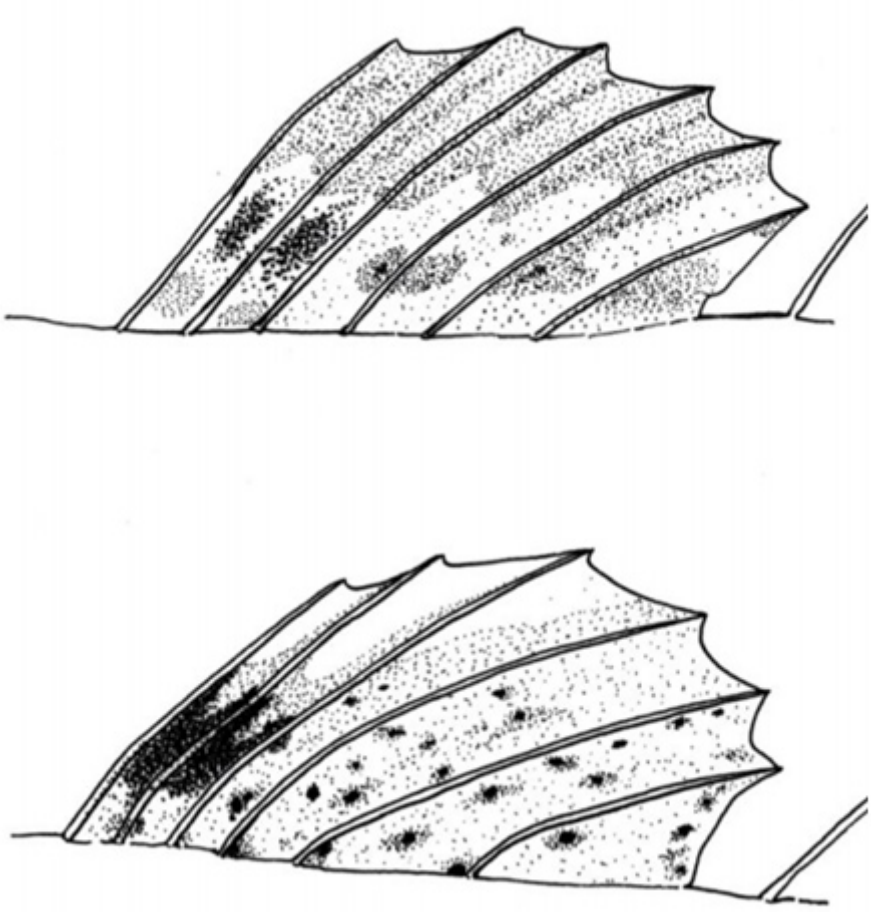

FIGURE 5. Pigmentation pattern on first dorsal fin of male specimens of *Rhinogobius yangminshanensis* (upper one), NTOUP-2017-06-325, 27.9 mm SL, the Tanshuei River basin, Taipei City; and *Rhinogobius rubromaculatus* (lower one) NTOUP-2017-06-321, 31.0 mm SL, the Wushi basin, Taichung City, Taiwan.

Remarks

The new species can be immediately separated from most congeners from Taiwan including typical longitudinal infraorbital sensory papillae group -*Rhinogobius candidianus* (Regan, 1908), *Rhinogobius formosanus* Oshima, 1919, *Rhinogobius gigas* Aonuma & Chen, 1996, *Rhinogobius nantaienesis* Aonuma & Chen, 1996, *Rhinogobius delicatus* Chen & Shao, 1996, *Rhinogobius maculafascistus* Chen & Shao, 1996, *and Rhinogobius lanyuensis* Chen, Miller & Fang, 1998 by high vertebral account modally 27 vs. 26 and lower count of pectoral fin rays modally 16 vs. more than17.

However, this new species is most similar to *Rhinogobius rubromaculatus* than any other congeneric species from Taiwan with higher account of vertebrae and lower account of pectoral fin base. Although Lee & Chang

(1996) reported typical fluvial species, *Rhinogobius rubromaculatus* may exist in the different basins from western Taiwan, our further unpublished data from both morphological re-examination and molecular survey can be found that there are at least not less than two allopatric species in the drainages of western Taiwan within the *Rhinogobius rubromaculatus* species complex (Chen, unpublished data).

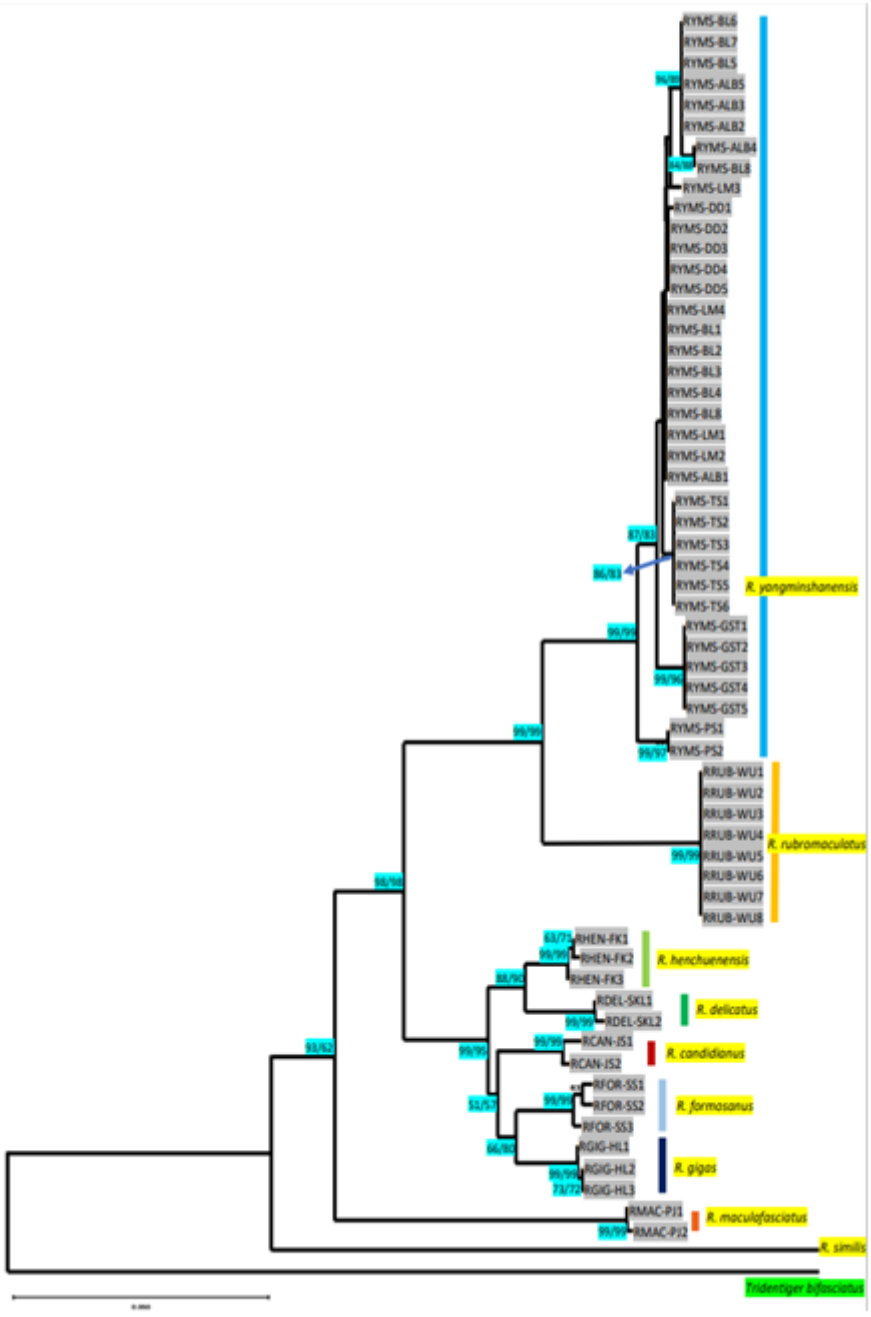

FIGURE 6. Molecular phylogenetic tree of 8 endemic *Rhinogobius* species of Taiwan which was reconstructed by the neighbor-joining (NJ) method and maximum likelihood (ML) inferred from complete mitochondrial D-loop sequences. The outgroup is *Tridentiger bifasciatus*. The values of bootstrap support more than 50% are shown in each branch shown as the values of above (NJ) / below (ML).

TABLE 3. Distribution frequency of scale and vertebral counts of *Rhinogobius yangminshanensis* with other congerenic species from Taiwan

	LR													TR						
	28	29	30	31	32	33	34	35	36	37	38	39	X	9	10	11	12	13	14	X
***R. yangminshanensis* n. sp.**		**4**	**16**	**12**	**4**								**30.4**		**2**	**14**	**2**			**11.0**
R. candidianus							2	7	8	4	2		35.9				9	7	4	12.8
R. delicatus					3	7	7	3	1				33.6			11	8	2		11.8
R. formosanus					2	8	5						33.2			9	5	1		11.5
R. gigas									1	4	8	2	37.7				7	7		12.5
R. henchuenensis							5	8	6	1			35.2			8	9	3		11.8
R. lanyuensis						2	9	4					34.1			2	12	1		11.9
R. macluafasciatus			4	9	8								31.2		15	6				10.3
R. nantaiensis						3	6	8	3				34.6			4	13	3		12.0
R. rubromaculatus	1	20	9										29.3		12	3				10.2
R. similis		3	8	7	2								30.4	3	17					9.9

	PreD															
	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	X
***R. yangminshanensis* n. sp.**						**1**	**4**	**7**	**5**	**1**						**12.1**
R. candidianus					1	2	1	1	2	1	4	4	2	1	1	14.4
R. delicatus						2	4	8	5	1						12.0
R. formosanus					3	5	5	1								10.5
R. gigas	1	2	3		1	3	2			2		1				9.5
R. henchuenensis								1	4	10	4	1				14.0
R. lanyuensis									3	6	2	2	2			14.6
R. macluafasciatus				1	6	8	4	2								10.0
R. nantaiensis									6	6	4	1	2			14.3
R. rubromaculatus						1	8	6								11.3
R. similis							4	5	8	2						12.4

	SDP											Vert		
	6	7	8	9	10	11	12	13	14	15	X	26	27	X
***R. yangminshanensis* n. sp.**		**1**	**17**								**8.0**		**10**	**27**
R. candidianus						1	7	9	2	1	12.8	20		26
R. delicatus				1	14	4	2				10.3	21		26
R. formosanus					4	7	4				11.0	15		26
R. gigas					3	5	5	1			11.3	14		26
R. henchuenensis					11	9					10.5	20		26
R. lanyuensis					1	11	3				10.1	10		26
R. macluafasciatus	5	12	4								7.0	23		26
R. nantaiensis					4	12	4				11.0	20		26
R. rubromaculatus			14	1							8.1		15	27
R. similis	7	13									6.7	20		26

Based on the holotype of *Rhinogobius rubromaculatus* assigned from the Wushi basin by the authors, the morphological data from true *R. rubromaculatus* can be compared morphologically as the followings. *Rhinogobius yangminshanensis* can be well distinguished from *Rhinogobius rubromaculatus* by the following morphological features: (1) second dorsal fin rays modally I/9 vs. I/8; (2) anal fin rays modally I/8 vs. I/7; (3) rear edge of mouth merely extending to vertical of anterior margin of pupil in male vs. extending to middle vertical of eye in male; (4) two parallel thick gray stripes (or orange stripe as posterior one in male) on lateral side of snout vs. merely one narrow orange stripe turning vertically; (5) conspicuous vertical bluish white band present in pectoral fin of male vs. absent in male; (6) larger one of cheek orange spots about equal to 1/3 of pupil diameter in male vs. less than 1/5 of pupil diameter in male; (7) distally orange band present on about upper 1/3 region of first dorsal fin in male vs. no such orange band on distal region as translucent or pale white in male.

Diagnostic key to all nominal species of *Rhinogobius* species from Taiwan

1a Infraorbital papillae row *a* and *c* with transverse extension; predorsal region with large ctenoid scales; pelvic fin large and elliptical . . . . . *R. similis* Gill, 1859
1b Infraorbital papillae row *a* and *c* as typically linear, longitudinal pattern; predorsal region naked or merely with small cycloid scales; pelvic fin small and rounded 2
2a no preopercular canal 3
2b preopercular canal present 4
3a second dorsal fin modally I/9; anal fin modally I/8; rear edge of mouth extending merely to vertical of anterior margin of pupil in male; snout with two broad, lateral stripes (posterior one orange in male) . . . . . ***R. yangminshanensis* n. sp.**
3b second dorsal fin modally I/8; anal fin modally I/7; rear edge of mouth extending to middle vertical of eye in male; snout with one lateral orange or brownish red curve turning vertically . . . . . *R. rubromaculatus* Lee & Chang, 1996
4a scale rows between origin of first dorsal fin and pectoral fin 6–8; abdomen median naked before anus . . . . . *R. maculafasciatus* Chen & Shao, 1996
4b scale rows between origin of first dorsal fin and pectoral fin 9–15; abdomen median with small cycloid scales before anus . 5
5a second dorsal fin and caudal fin pale 6
5b second dorsal fin and caudal fin with some rows of spots 7
6a row *b* with 7–8 papillae; lateral body uniformly unmarked . . . . . *R. candidianus* (Regan, 1908)
6b row *b* with 12–13 papillae; lateral midline of trunk with 5–6 deep gray bloches . . . . . *R. nantaiensis* Aonuma & Chen, 1996
7a Cheek with brownish red wavy lines . . . . . *R. formosanus* Oshima, 1919
7b Cheek with orange or brown, blackish brown spots 8
8a Lateral midline of trunk with vertical deep brown or grayish brown blotches; caudal fin base with a blackish brown curves . 9
8b Lateral midline of trunk in lacking conspicuous blotches; caudal fin with two well separate deep brown bars 10
9a pectoral fin rays 21–23; longitudinal scale rows 36–39; prepelvic area naked . . . . . *R. gigas* Aonuma & Chen, 1996
9b pectoral fin rays 19–21; longitudinal scale rows 32–35; prepelvic area with small cycloid scales . . . . . *R. lanyuensis* Chen, Miller & Fang, 1998
10a pectoral fin rays modally 18; median fin black; cheek with 100–120 very tiny black spots . . . . . *R. delicatus* Chen & Shao, 1996
10b pectoral fin rays always 19–20; median fin pale brown; cheek with 15–30 red or brownish red small spots . . . . . *R. henchuenensis* Chen & Shao, 1996

Acknowledgements

The authors wish to thank the research grant support from the Headquarter of Yangminshan National Park, Taiwan for aquatic biodiversity survey of those the drainages around the National Park. They wish to thank Prof. S.C. Lee for his very helpful research information of *Rhinogobius rubromaculatus*. ISC is very grateful to the grant support for freshwater fish breeding and conservation from the Agriculture Council, Taipei, Taiwan.

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APPENDIX I. Comparative materials of *Rhinogobius* species of Taiwan

***Rhinogobius candidianus* (Regan, 1908)**

Syntypes.—BMNH 1908.5.27.29–33, 5 specimens, 54.7–58.4 mm SL, coll. T. Regan, Lake Candidius, Nantou County, Taiwan.

Other material.—ASIZP-057221, 4 specimens, 57.5–78.4 mm SL, July 20, 1989, coll. I-S. Chen, Tachia River basin, Taichung City, Taiwan. ASIZP-057222, 2 specimens, 42.1–42.2 mm SL, June 10, 1990, coll. I-S. Chen, Marsushi basin, New Taipei City, Taiwan. ASIZP-057223, 2 specimens, 43.2–44.0 mm SL, Aug. 12, 1993, coll. I-S. Chen, Peishihshi, Tanshuei River basin, New Taipei City, NMMBP-00304, 5 specimens, 41.0–44.9 mm SL, Spt. 4, 1993, coll. I-S. Chen, Wulaokunshi basin, Ilan County, Taiwan. NMMBP-00305, 4 specimens, 34.6–47.5 mm SL, Feb. 16, 1994, coll. I-S. Chen, Keelung River, Tanshuei River basin, New Taipei City, Taiwan.

***Rhinogobius delicatus* Chen & Shao, 1996**

Holotype.—ASIZP-057227, 64.9 mm SL, Dec. 29, 1993, coll. I-S. Chen, Fuli, small tributary of Shokuluanshi basin, Hualien County, Taiwan.

Paratypes.—ASIZP-057228, 36.0–63.1 mm SL, Spt. 14, 1993, coll. I-S. Chen, Shinwulushi, Peinandarshi basin, Taitung County, Taiwan. ASIZP-057229, 6 specimens, 48.5–62.7 mm SL, Dec. 12, 1994, Tonghe, Marwukushi basin, Taitung County, Taiwan.

***Rhinogobius formosanus* Oshima, 1919**

ASIZP-057231, 9 specimens, 42.3–48.4 mm SL, July 10, 1990, coll. I-S. Chen, Marsushi basin, New Taipei City, Taiwan. ASIZP-057232, 4 specimens, 44.2–48.7 mm SL, July 5, 1990, coll. I-S. Chen, Peishihshi, Tanshuei River basin, New Taipei City, Taiwan. NMMBP-00306, 48.0 mm SL, Aug. 25, 1993, coll. I-S. Chen, Peishihshi, Tanshuei River basin, New Taipei City, Taiwan. NMMBP-00307, 45.2 mm SL, Spt. 8, 1993, coll. I-S. Chen, Wulaokunshi basin, Ilan County, Taiwan.

***Rhinogobius gigas* Aonuma & Chen, 1996**

Holotype.—ASIZP-057224, 80.5 mm SL, Dec. 12, 1993, coll. I-S. Chen, Shinwulushi, Peinandarshi basin, Taitung County, Taiwan.

Paratypes.—ASIZP-057225, 3 specimens, 39.9–57.4 mm SL, April 3, 1993, coll. I-S. Chen, Sanjanshi basin, Hualian County, Taiwan. ASIZP-057226, 8 specimens, 51.7–67.4 mm SL, Spt. 8, 1993, coll. I-S. Chen, Nanaushi, Ilan County, Taiwan. NMMBP-00302, 3 specimens, 27.6–48.9 mm SL, Dec. 21, 1993, coll. I-S. Chen, Kinglunshi basin, Taitung County, Taiwan.

***Rhinogobius henchuenensis* Chen & Shao, 1996**

Holotype.—ASIZP-057241, 37.0 mm SL, Oct. 21, 1993, coll. I-S. Chen, Fongkongshi basin, Pingtung County, Taiwan.

Paratypes.—ASIZP-057242, 10 specimens, 31.3–44.5 mm SL, data as holotype. ASIZP-057243, 9 specimens, 28.0–38.0 mm SL, May 15, 1994, coll. I-S. Chen, Fongkongshi basin, Taitung County, Taiwan.

***Rhinogobius lanyuensis* Chen, Miller & Fang, 1998**

Holotype.—ASIZP-057811, 66.9 mm SL, Aug. 25, 1995, coll. I-S. Chen, Dongchingshi basin, Lanyu Island, Taitung County, Taiwan.

Paratypes.—ASIZP-057812, 45.6 mm SL, June 12, 1993, coll. J.P. Chen, Yeyushi basin, Lanyu Island, Taitung County, Taiwan. ASIZP-057813, 5 specimens, 48.2–55.6 mm SL, other data same as holotype. NMMBP-00470, 8 specimens, 41.0–64.9 mm SL, other data same as holotype.

***Rhinogobius maculafascistus* Chen & Shao, 1996**

Holotype.—ASIZP-057233, 44.0 mm SL, March 7, 1993, coll. Y.H. Chen, Chinlunku, Maulin, Jokoshi, Kaopingshi basin, Pingtung County, Taiwan.

Paratypes.—ASIZP-057234, 4 specimens, 34.6–42.8 mm SL, Nov. 6, 1993, coll. I-S. Chen, Yujing, Tzengwenshi basin, Tainan County, Taiwan. ASIZP-057235, 15 specimens, 30.1–50.0 mm SL, Jan. 14, 1994, coll. I-S. Chen,

Darpu, Tzengwhenshi basin, Tainan County, Taiwan. ASIZP-057236, 33.2 mm SL, April 3, 1993, coll. I-S. Chen, Yujing, Tzengwenshi basin, Tainan County, Taiwan.

Rhinogobius nantaienesis **Aonuma & Chen, 1996**

Holotype.—ASIZP-057233, 44.2 mm SL, March 26, 1993, coll. I-S. Chen, Ailiaopeishi, Kaopingshi basin, Pingtung County, Taiwan.

Paratypes.—ASIZP-057238, 3 specimens, 22.4–29.4 mm SL, Jan. 26, 1994, coll. I-S. Chen, Nantsishanshi, Kaopingshi basin, Kaohsiung City, Taiwan. ASIZP-057239, 2 specimens, 26.9–40.3 mm SL, 26.9–40.3 mm SL, other data same as holotype.

Others.—NTOUP-2015-05-301, 10 specimens, 33.9–53.1 mm SL, Spt. 24, 1994, coll. I-S. Chen, Laononshi, Kaopingshi basin, Pingutng County, Taiwan.

Rhinogobius rubromaculatus **Lee & Chang, 1996**

Holotype.—ASIZP-056640, 39.0 mm SL, Nov. 30, 1991, coll. C.S. Tzeng, Wushi basin (= Tadushi basin), Taichung City, Taiwan.

Others.—NTOUP-2017-06-321, 15 specimens, 16.6–31.0 mm SL, Aug. 16, 2016, coll. I-S. Chen, Wufong, Fongkushi, Wushi basin, Taichung City, Taiwan.

NTOUP-2008-06-298, 14 specimens, 26.5–40.0 mm SL, Dec. 16, 2007, coll.W.C. Jan *et al.*, Puli, Wushi basin, Nantou County, Taiwan.

Rhinogobius similis **Gill, 1859**

Holotype of *Gobius giurinus* Rutter, 1897 (= junior synonym of *Rhingobius similis* Gill, 1859). CAS-104990, 60.0 mm SL, coll. A.M. Fielde, Swatow, Hanjiang basin, Kwangtong Province, China.

Others.—ASIZP-057219, 3 specimens, 62.8–69.8 mm SL, June 20, 1990, coll. I-S. Chen, Peishihshi, Tanshuei River basin, New Taipei City, Taiwan. ASIZP-057220, 4 specimens, 31.6–47.6 mm SL, April 3, 1993, coll. I-S. Chen, Liyu Lake, Hualien County, Taiwan. NMMBP-00303, 6 specimens, 38.6–56.9 mm SL, Feb. 20, 1994, coll. I-S. Chen, Chusan, Chinshueishi, Joshueishi basin, Nantou County, Taiwan. NMMBP-00309, 5 specimens, 42.7–53.2 mm SL, Jan. 15, 1994, coll. I-S. Chen, Tzengwenshi basin, Charyi County, Taiwan.