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Redescription and validity of *Formosania gilberti* Oshima, 1919 (Cypriniformes: Balitoridae), an endemic hillstream loach from Taiwan

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Abstract

For over a century, only one species of *Formosania* has been recognised in Taiwanese waters. However, through the use of mitogenomic analyses and the identification of morphological differences, an endemic balitorid loach was revalidated and redescribed based on specimens herein of northern Taiwan. *Formosania gilberti* Oshima, 1919 was previously mistreated as a junior synonym of *F. lacustris* (Steindachner, 1908), but the two can be clearly differentiated by their vertebral counts (26+11 vs. 26+12) and body marking patterns (intensely mottled with vermicular markings vs. cloud-like blotches or brown and blotchless). Furthermore, mitogenomic analyses also showed high intra-specific diversity among *Formosania* loaches in Taiwan, which not only supports *F. gilberti* as a valid species but also hints the presence of a species complex distributed in western Taiwan.

Key words: *Formosania*, Balitoridae, taxonomy, redescription, *Formosania gilberti*

Introduction

The rheophilic fishes of the teleostean family Balitoridae, commonly known as hillstream loaches, often inhabit the fast-running upper stream creeks or riffles with high dissolved oxygen rates from East Asia to the subcontinental regions of India (Li 2015). In Taiwan, there are currently three genera with six valid, endemic species of balitorid loaches recorded, *viz. Hemimyzon formosanus* (Boulenger, 1894), *Formosania lacustris* (Steindachner, 1908b), *Sinogastromyzon puliensis* Liang, 1974, *Hemimyzon taitungensis* Tzeng & Shen, 1982, *Sinogastromyzon nantaiensis* Chen, Han & Fang, 2002, *Hemimyzon sheni* Chen & Fang, 2009, and *Hemimyzon yushanensis* Chen, Harefa, Chang & Han, 2022 (Shen 1993; Chen & Fang 1999; Chen & Chang 2005).

The balitorid genus *Formosania* was erected by Oshima (1919), and was then recommended as the senior synonym of *Crossostoma* Sauvage, 1878 (a junior homonym of *Crossostoma* Morris & Lycett, 1851 in Gastropoda) by Novák *et al.* (2006). There are currently 10 nominal, valid species of *Formosania* described, *viz. F. davidi* (Sauvage, 1878), *F. lacustris*, *F. fascicauda* (Nichols, 1926), *F. stigmata* (Nichols, 1926), *F. tinkhami* (Herre, 1934), *F. paucisquama* (Zheng, 1981), *F. chenyiyui* (Zheng, 1991), *F. fasciolata* (Wang, Fan & Chen, 2006), *F. galericula* (Zhang & Wang, 2011), and *F. immaculata* Sun, Zhou & Yang, 2023. In Taiwan's history, a total of three *Formosania* species were described: *viz. F. lacustris* from Lake Candidius (Zhuoshuei River basin), *F. gilberti* Oshima, 1919 from Tamusui River near Shinten (Tamshui River basin), and *F. tengi* (Watanabe, 1983) from Kaoshiung County (Kaoping River basin). However, *Formosania gilberti* and *F. tengi* were later designated as junior synonyms of *F. lacustris* by Myers (1929) and Tzeng *et al.* (1990), respectively. As a result, the balitorid genus *Formosania*

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in Taiwan now only includes one nominal species, *F. lacustris*, which has a wide documented distribution from northern to central Taiwan (Tzeng & Shen 1982; Watanabe 1983; Novák *et al.* 2006).

During a comprehensive survey of the river systems in Taiwan over several years, we observed subtle differences in the morphology and coloration of different populations of *Formosania lacustris* collected. These discrepancies were also noted by Chen and Chang (2005) in their synopsis of Taiwanese balitorid loaches, which raised our suspicion that the aforementioned morphological differences represent specific or subspecific diagnostic characters of possible cryptic species of *Formosania* that have been overlooked due to inaccurate taxonomical treatments or impetuous genetic analyses. After conducting mitogenomic analyses and detailed examinations of morphological and osteological characters, we found very conspicuous evidences that eventually lead to the revalidation of *Formosania gilberti* Oshima, 1919, whom was initially mistreated as a junior synonym of *F. lacustris* by Myers (1929) as aforementioned. In this study, we redescribe and revalidate *Formosania gilberti* with supportive genomic and morphological evidences, and their comparisons with those of the topotypical *F. lacustris* specimens. Furthermore, a new distributional record of *Formosania lacustris* from Tzengwen River basin of southwestern Taiwan is also firstly reported herein, and comments upon Taiwanese *Formosania* will also be addressed in discussions.

Materials and Methods

Fish collections. Specimens of *Formosania* loaches examined in this study were obtained from major river systems throughout northern and central Taiwan (Fig. 1) by direct capture or electrofishing with a 12V portable battery pack. Fin clips were sampled from the specimens collected and preserved in 95% EtOH, while the whole fish was fixed in 10% formalin for a few days after photo record of the fresh colorations, then transferred to 70% EtOH for long-term preservation and future examination.

Measurements and counts. Methods for morphometry measurements generally follow Kottelat (1984) and Yi *et al.* (2014) (see schematic diagram Fig. 2 for all measurement categories used); all measurements were taken directly (point to point), and lengths were measured with digital callipers to the nearest of 0.01 mm. Methods for meristic counts follow Kottelat (2001), except for vertebral counts following Randall *et al.* (2022). All counts were made from the left side of specimens. The terms "origin" and "insertion" refer to the anterior and posterior ends of fin bases for both paired and unpaired fins, respectively, and lateral-line scale counts were made upon the pored scales, including the pores on the caudal fin base. Fin ray counts were given as unbranched rays + branched rays. Barbel counts were only made upon clearly protruded barbels that were distinguishable from the rostral folds; total barbel counts were given as total counts of rostral barbels (including secondary rostral barbels) + maxillary barbels. Vertebral counts following Randall *et al.* (2022), given as abdominal vertebrae + caudal vertebrae, counts of abdominal vertebrae also include the Weberian apparatus (first rib-bearing vertebra is the fifth). All specimens were given an NTOUP accession number and deposited at the Pisces collection of NTOU. Abbreviations for meristic characters are shown as follows: D, dorsal fin rays; A, anal fin rays; P_1, pectoral fin rays; P_2, pelvic fin rays; LL, lateral line scales; V, vertebral counts.

Phylogenetic analyses. Nucleotide sequences of the protein-coding gene ATPase were employed as molecular markers for this research. Genomic DNA was extracted from fin clips using GeneMark™ kits following the manufacturer's methods. The targeted sequences were amplified with primer sets of BALI-86-LYSF (5'-TTGGCCTTTTAAGCCAAAGATTGGTG-3') and BALI-86-CO3AR (5'-ATCAACCATATGATATGCATGTGCTT-3'), a primer pair specifically designed for balitorid loaches. The targeted sequences were amplified using the polymerase chain reaction (PCR) technique (Miya & Nishida 1999). PCR reactions were performed with a MultigeneTM Optimax thermal cycler in reaction reagents composed of 25 µl AmpliqonTM Taq DNA polymerase 2x Master Mix RED, 21 µl ddH2O, 2 µl DNA template, and 1 µl primer (both H-strand and L-strand primers). PCR reactions start with an initial 5-minute, 50-second denaturation at 94℃, followed by 48 cycles of denaturation, annealing, and extension, with the temperature and time span of each reaction session determined by the primer employed in every run. The whole reaction concluded with a final 7 minutes of extension at 72℃, followed by storing the sample at 4℃ indefinitely until the reaction ended. All reaction efficiencies were confirmed by electrophoresis of the PCR product on 1% agarose gel. Successful products were purified with commercial purification kits (Roche™) following the manufacturer's methods. Purified PCR products were once again electrophoresis-checked for purification efficiency before sequencing. Samples were then delivered and sequenced at the Core Facilities of IBMS, Academia Sinica (Nangang District, Taipei City, Taiwan)

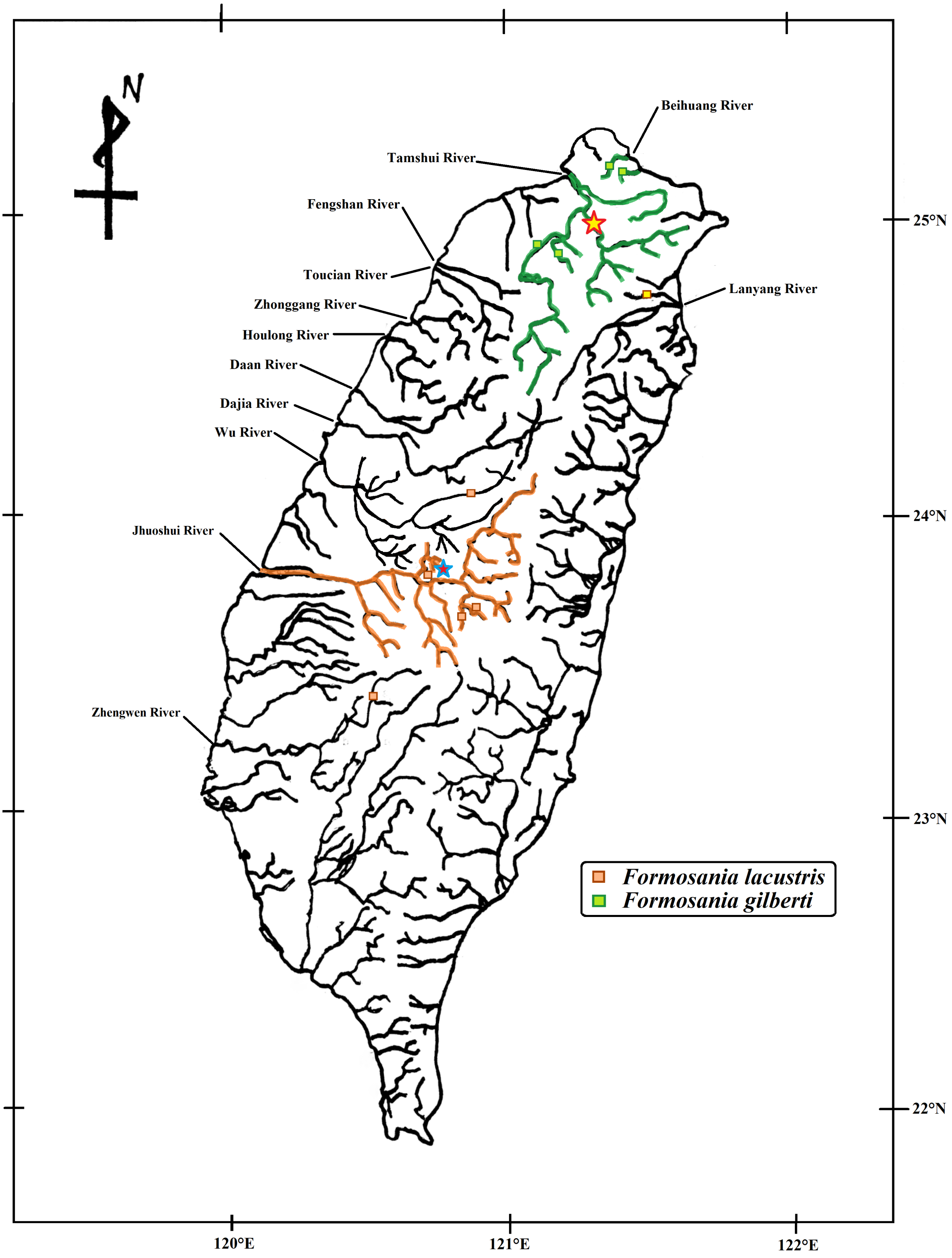

FIGURE 1. Collection sites for the *Formosania* loaches of Taiwan. River systems were highlighted with green and orange to show distribution of *F. gilberti* and *F. lacustris*, respectively. The red star outlined with yellow marks the type locality of *F. gilberti*, and red star outlined with blue marks Lake Candidius (Sun Moon Lake), the type locality for *F. lacustris*.

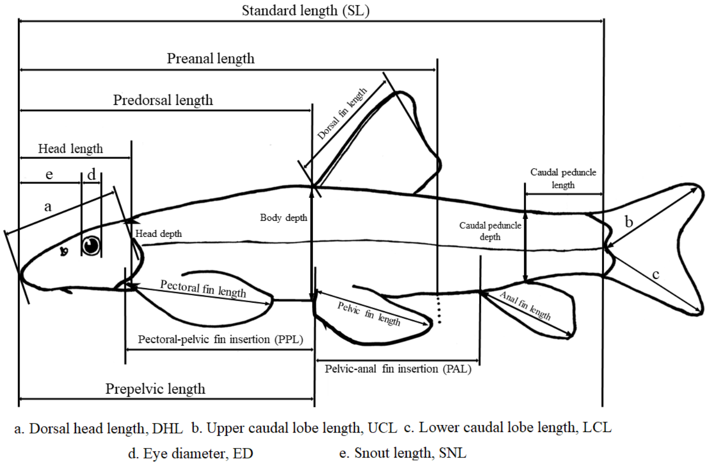

FIGURE 2. Schematic diagram of a generalized *Formosania* loach, showing various morphometric methods employed in this study.

FIGURE 3. *In situ* photo of the two *Formosania* species from Taiwan. A. *Formosania gilberti*, NTOUP2023-05-018, male, 68.80 mm SL; B. *Formosania lacustris*, NTOUP2022-11-005, female, 75.49 mm SL. Markings on the lateral side of trunk slightly faded due to stress caused by bright lighting.

FIGURE 4. Dorsal, lateral and ventral view of *Formosania gilberti* collected from Tamshui River basin. NTOUP2023-05-018, female, 100.35 mm SL.

with an ABI Model 3700 DNA sequencer using the Sanger dideoxynucleotide sequencing method. The ATPase sequences of a total of 12 *Formosania* individuals were selected and sequenced, and 2 *Vanmanenia* species were selected as outgroups (Saitoh *et al.* 2011; Chen & Li 2016; Liang *et al.* 2016; Chu *et al.* 2021) (Table. 1). The sequencing results were firstly manually edited and assembled by BioEdit Sequence Alignment Editor v.7.2.5 (Hall 1999), then aligned with ClustalW Multiple Alignment v.1.4 (Thompson *et al.* 1994). Genetic distances were calculated using MEGA 11 v 11.0.11 (Tamura *et al.* 2021), with variances estimated with the bootstrap method (Felsenstein 1985) under 1000 bootstrap replicates and the *p*-distance model as a substitution model (include transitions and transversions), and rates among sites set as having invariant sites (I). Both neighbor-joining (NJ) (Saitou & Nei

1987) and maximum likelihood (ML) methods were employed to reconstruct phylogenetic relationships; the best substitution model of GTR+I was first selected by MrModeltest v.2 (Nylander 2004) under Akaike's Information Criterion, then the ML and NJ tree topology generated by MEGA. Reliability of both tree topologies was tested with the bootstrap method under 1000 bootstrap replicates. Tajima's D test was also employed to test sequence neutrality (Tajima 1989).

TABLE 1. All OTUs employed within the molecular phylogenetic analyses.

Code	Species	River system	Locality	Genbank ID	Length (bp)	Source
BH1	*Formosania gilberti*	Chingshui brook, Beihuang River	Jinshan District, New Taipei City, Taiwan	-	841	This study
BH2	*Formosania gilberti*	Chingshui brook, Beihuang River	Jinshan District, New Taipei City, Taiwan	-	841	This study
BH3	*Formosania gilberti*	Toucian brook, Masu River	Wanli District, New Taipei City, Taiwan	-	841	This study
BH4	*Formosania gilberti*	Toucian brook, Masu River	Wanli District, New Taipei City, Taiwan	-	841	This study
TM1	*Formosania gilberti*	Dahan brook, Tamshui River	Daxi District, Taoyuan City, Taiwan	-	841	This study
TM2	*Formosania gilberti*	Dahan brook, Tamshui River	Daxi District, Taoyuan City, Taiwan	-	841	This study
TM3	*Formosania gilberti*	Dahan brook, Tamshui River	Daxi District, Taoyuan City, Taiwan	-	841	This study
TM4	*Formosania gilberti*	Dahan brook, Tamshui River	Daxi District, Taoyuan City, Taiwan	-	841	This study
TM5	*Formosania gilberti*	Sanxia brook, Tamshui River	Sanxia District, New Taipei City, Taiwan	-	841	This study
TM6	*Formosania gilberti*	Sanxia brook, Tamshui River	Sanxia District, New Taipei City, Taiwan	-	841	This study
TM7	*Formosania gilberti*	Dabao brook, Tamshui River	Sanxia District, New Taipei City, Taiwan	-	841	This study
TM8	*Formosania gilberti*	Dabao brook, Tamshui River	Sanxia District, New Taipei City, Taiwan	-	841	This study
ZG1	*Formosania lacustris*	Jhonggang River	Nanzuang Township, Miaoli County, Taiwan	-	841	This study
ZG2	*Formosania lacustris*	Dahu brook, Jhonggang River	Beipu Township, Miaoli County, Taiwan	-	841	This study
ZG3	*Formosania lacustris*	Nanzhuang brook, Jhonggang River	Nanzuang Township, Miaoli County, Taiwan	-	841	This study
WU1	*Formosania lacustris*	Beigang brook, Wu River	Renai Township, Nantou County, Taiwan	-	841	This study
WU2	*Formosania lacustris*	Beigang brook, Wu River	Renai Township, Nantou County, Taiwan	-	841	This study
JS1	*Formosania lacustris*	Shuili brook, Jhuoshui River	Shuili Township, Nantou County, Taiwan	-	841	This study

......continued on the next page

TABLE 1. (Continued)

Code	Species	River system	Locality	Genbank ID	Length (bp)	Source
JS2	*Formosania lacustris*	Heshe brook, Jhuoshui River	Xinyi Township, Nantou County, Taiwan	-	841	This study
JS3	*Formosania lacustris*	Baxiang brook, Jhuoshui River	Xinyi Township, Nantou County, Taiwan	-	841	This study
ZW1	*Formosania lacustris*	Zhengwen River, southwestern Taiwan	Alishan Township, Jiayi County, Taiwan	-	841	This study
ZW2	*Formosania lacustris*	Zhengwen River, southwestern Taiwan	Alishan Township, Jiayi County, Taiwan	-	841	This study
ZW3	*Formosania lacustris*	Zhengwen River, southwestern Taiwan	Alishan Township, Jiayi County, Taiwan	-	841	This study
-	*Formosania lacustris*	Nei Shuong River, Taipei, Taiwan	Shihlin District, Taipei City, Taiwan	AP010774	842	Saitoh *et al.* 2011
-	*Formosania galericula*	Feiyun River	Wuyanling National Nature Reserve, China	MZ447813	842	Chu *et al.* 2021
-	*Vanmanenia stenosoma*	Lianhuazhen River	Zhejiang Province, China	KX786161	841	Chen & Li 2016
-	*Vanmanenia pingchowensis*	Lishui River	Zhangjiajie, Hunan Province, China	KP005457	842	Liang *et al.* 2016

Comparative materials. *Formosania lacustris*: NTOUP2022-11-004, 5 (53.33–69.91 mm SL); Tfuya bridge, Zhengwen River, Jiayi County, Taiwan; coll. Hsien-En Li *et al.*, 4 Nov. 2022; NTOUP2022-11-005, 4 (59.99–82.42 mm SL); Baxiang Brook, Jhuoshui River, Nantou County, Taiwan; coll. Hsien-En Li *et al.*, 5 Nov. 2022; NTOUP2022-07-026, 5 (59.24–68.46 mm SL); Beigang Brook, Wu River, Nantou County, Taiwan; coll. Ming-Fong Yeh *et al.*, 26 Jul. 2022.

Systematics

Formosania **Oshima, 1919**

Formosania **Oshima, 1919: 194.**

Formosania gilberti **Oshima, 1919**

(吉氏纓口鰍)
(Figs. 5A; 6–8; 10A; 11A; 12A)

Formosania gilberti Oshima, 1919: 194 (Shintian, Taiwan).
Crossostoma lacustre (*non* Steindachner): Chen 1980: 103; Chen & Tang 2000: 466.

Materials Examined

NTOUP2022-05-030, 7 (45.24–83.78 mm SL); Chingshui brook, Beihuang River, Jinshan District, New Taipei City, Taiwan; coll. I-Shiung Chen *et al.*, 30 May. 2022; NTOUP2023-05-018, 8 (67.08–100.35 mm SL); Dabao brook, Tamshui River, Sanxia District, New Taipei City, Taiwan; coll. Hsien-En Li, I-Lang, Cheng & Hue Man Tran, 18 May. 2023.

FIGURE 5. A juvenile of *Formosania gilberti* (uncatalogued, approximately 1.5 mm SL) in its natural habitat in Yuanwangkeng creek, an upstream tributary of Shuang River, Gongliao County, Taiwan.

FIGURE 6. *Formosania gilberti* (uncatalogued, approximately 80 mm SL) in its natural habitat in Lujiaokeng creek, an upstream tributary of Beihuang River.

FIGURE 7. *Formosania lacustris* collected from Jhuoshui River basin. NTOUP2022-11-005, female, 63.20 mm SL.

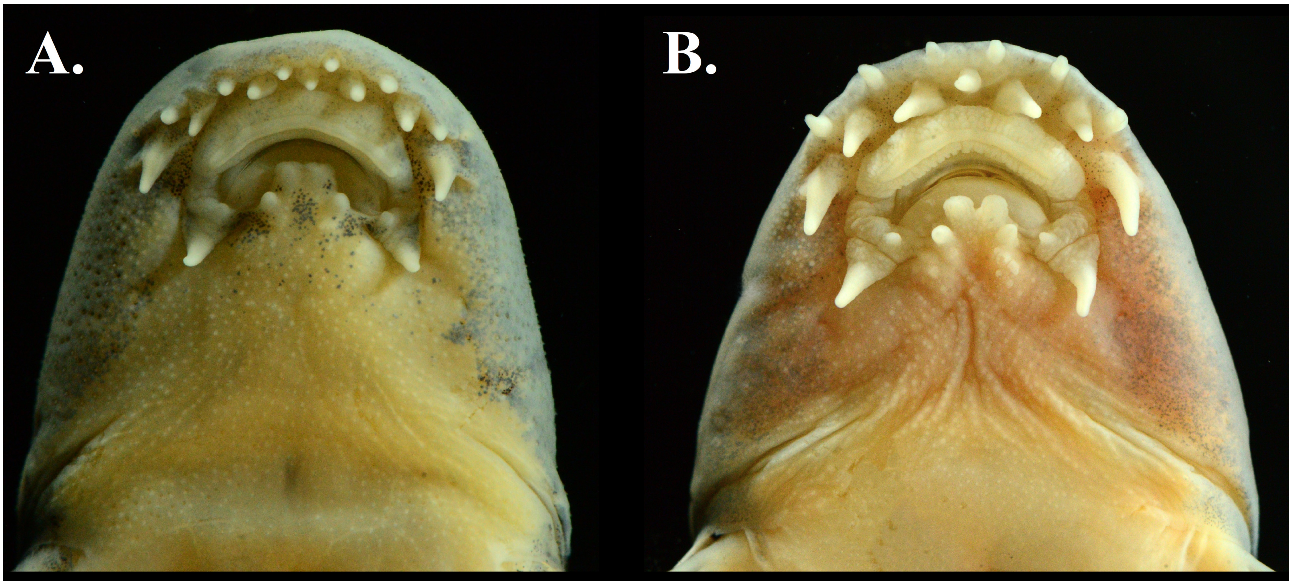

FIGURE 8. Ventral side of head of the two *Formosania* loaches from Taiwan. A. *Formosania gilberti*, NTOUP2023-05-018, male, 68.80 mm SL; B. *Formosania lacustris* NTOUP2022-11-005, female, 75.49 mm SL.

Diagnosis

Formosania gilberti can be well separated from its congeners by the following unique combination of characters (mode shown in brackets): D 3+8; A 2+5; P_1 1+14–15 (14); P_2 1+7–8 (8); LL 89–96 (89); barbels 9–13(13)+2; V 26+11=37.

Body ground color dark green, intensely mottled with cloud-like blotches, without lateral streak or vague when fresh; all fins yellowish orange, pectoral fins lined with 3 to 4 rows of large black spots; barbels orange when fresh.

FIGURE 9. Radiographs of the two *Formosania* loaches from Taiwan. A. *Formosania gilberti*, NTOUP2023-05-018, male, 69.99 mm SL; B. *Formosania lacustris* NTOUP2022-11-005, female, 75.49 mm SL. The Weberian apparatus was fused by 4 axial skeleton elements and is located anterior to the abdominal vertebrae series.

Redescriptions

Body proportions as shown in Table 2, frequencies of meristic counts in Table 3. Body slightly elongated, almost semicylindrical anteriorly and laterally compressed posteriorly behind dorsal fin base. Head depressed, bluntly triangular in dorsal view. Eye positioned dorso-laterally, with interorbital width significantly greater than eye diameter (18.13 vs. 43.56). Anterior and posterior nostrils separated with a small flap. Mouth inferior, opening curved; upper lip thick, pad like, with slightly wrinkled surface and a thin, smooth additional pad located along edge; median lower lip edge with two pairs of papillose protrusion. Total barbels 9 (3), 10 (2), 11 (3), 12 (1), or 13 (6)+2; rostral fold with specialized short, papilla-like secondary rostrum barbels located upon (sometimes indistinguishable with the rostrum folds); rostral groove narrow; maxillary barbels 2, located beside gape, a trapezoid-shaped pad present at bases of maxillary barbels, with a small, pointy papillose protrusion; all barbels no longer than half of eye diameter. Ventral side of head with numerous, tiny wart-like protrusions lined along branchiostegal region. Gill slits short, upper end not extending above eye and lower end extending forward to ventral side of head. Operculum slightly elongated, flexible, posterior tip extending beyond pectoral fin insertions. Body covered in numerous delicate, pointy tubercles along dorsal and ventral sides of head, trunk, and fin membranes, size of tubercles without significant differences between sexes. Trunk of female individuals appearing bulkier than male. Female genital papillae plumpy, blunt with wrinkled surface posteriorly and a short median notch. Vertebrates 4 (Weberian apparatus)+22 (abdominal vertebrae)+11 (caudal vertebrae)=37 total vertebrae (15).

TABLE 2. Morphometric percentages of the two *Formosania* species examined herein.

	***Formosania gilberti* Oshima, 1919**		***Formosania lacustris* (Steindachner, 1908b)**	
Sex	♂	♀	♂	♀
n	6	9	10	4
SL (mm)	46.00–75.67	45.24–100.35	45.24–100.35	59.99–82.42
Percent in SL (%)				
Head length (HL)	19.19 (17.65–20.30)	18.76 (16.41–20.40)	20.25 (18.45–21.20)	20.32 (19.46–21.51)
Dorsal head length	22.51 (21.70–23.22)	21.58 (19.94–22.80)	22.78 (22.25–23.66)	20.98 (19.58–22.58)
Body depth	17.43 (14.63–19.32)	16.60 (15.01–18.06)	19.46 (17.92–20.14)	16.40 (15.55–17.81)
CPL	13.82 (12.93–14.19)	13.09 (12.24–14.75)	14.43 (14.08–15.02)	14.48 (13.45–15.18)
CPD	11.46 (10.78–12.70)	11.26 (9.84–12.15)	11.98 (11.23–12.61)	11.83 (11.23–12.38)
Predorsal length	49.94 (47.95–52.56)	49.84 (48.02–51.35)	51.24 (49.90–52.62)	51.34 (49.66–52.50)
Prepelvic length	52.47 (50.93–53.73)	53.03 (51.38–54.54)	52.43 (51.48–53.85)	52.04 (50.93–54.30)
Preanal length	67.76 (65.70–69.21)	69.67 (66.84–71.29)	68.67 (65.74–70.39)	67.42 (63.84–67.98)
PPL	33.87 (32.23–35.75)	35.47 (32.45–38.66)	32.75 (31.43–33.68)	33.83 (31.25–35.57)
PAL	27.13 (24.88–29.22)	27.74 (25.46–36.44)	27.00 (25.70–28.07)	27.19 (26.69–27.62)
Dorsal fin length	19.73 (19.14–20.42)	19.92 (18.11–21.29)	19.60 (17.03–21.19)	19.44 (16.39–21.06)
Anal fin length	17.89 (17.24–19.51)	17.64 (16.67–18.25)	15.76 (14.39–16.32)	16.23 (14.46–18.68)
Anal fin base	7.01 (6.51–7.50)	7.11 (6.07–7.71)	7.76 (7.61–8.06)	6.89 (6.51–7.33)
Pectoral fin length	25.01 (24.73–25.17)	24.61 (23.57–25.38)	26.28 (26.11–26.40)	26.42 (25.89–26.96)
Length between pectoral fins	16.63 (15.63–17.57)	16.25 (15.37–17.07)	16.65 (16.13–17.59)	16.73 (16.39–17.55)
Pelvic fin length	20.17 (19.16–20.81)	20.06 (19.04–20.81)	20.76 (20.07–21.57)	21.91 (21.43–22.25)
Length between pelvic fins	12.69 (11.11–13.82)	13.24 (12.44–14.13)	13.63 (12.40–14.16)	12.86 (12.66–13.16)
Upper caudal lobe length	22.53 (20.47–25.30)	21.33 (18.59–22.96)	22.40 (21.37–23.38)	21.86 (21.25–22.47)
Lower caudal lobe length	22.93 (20.67–25.70)	21.88 (19.84–25.18)	22.59 (21.52–23.54)	23.25 (22.69–24.32)
Percent in CPL (%)				
CPD	82.97 (78.67–89.50)	86.20 (76.59–93.85)	83.11 (74.81–87.65)	81.74 (79.37–83.51)
Percent in HL (%)				
Head width	89.56 (81.69–96.32)	92.54 (85.12–102.61)	85.45 (78.32–96.65)	89.55 (82.74–95.35)
Head depth	60.12 (52.89–65.82)	60.34 (51.71–71.16)	60.74 (52.54–72.11)	58.84 (55.45–63.05)
Snout length	60.18 (53.94–66.14)	61.72 (57.99–67.56)	62.79 (56.22–67.20)	60.62 (57.41–66.22)
Interorbital width	43.93 (32.66–49.92)	43.32 (38.59–48.15)	46.27 (41.56–53.69)	41.48 (39.23–44.08)
Eye diameter	18.53 (15.79–19.52)	17.85 (32.66–49.92)	17.37 (15.36–22.95)	16.26 (14.30–18.53)
Mouth width	27.49 (24.84–31.01)	29.53 (26.02–34.35)	21.23 (19.19–24.62)	22.46 (18.61–25.44)

TABLE 3. Meristic count frequencies of the two *Formosania* species examined herein.

Species	D		A		P_1			P_2		
	iii, 8	Av.	ii, 5	Av.	i, 14	i, 15	Av.	i, 7	i, 8	Av.
Formosania gilberti	15	8	15	8	12	3	14	3	12	8
Formosania lacustris	14	8	14	8	12	2	14	4	10	8

Species	LL														
	82	85	89	90	91	92	93	-	96	97	98	99	100	-	102	Av.
F. gilberti	-	-	5	3	2	2	1	-	3	-	-	-	-	-	-	97
F. lacustris	1	1	-	1	-	1	-	-	2	2	2	2	2	-	1	102

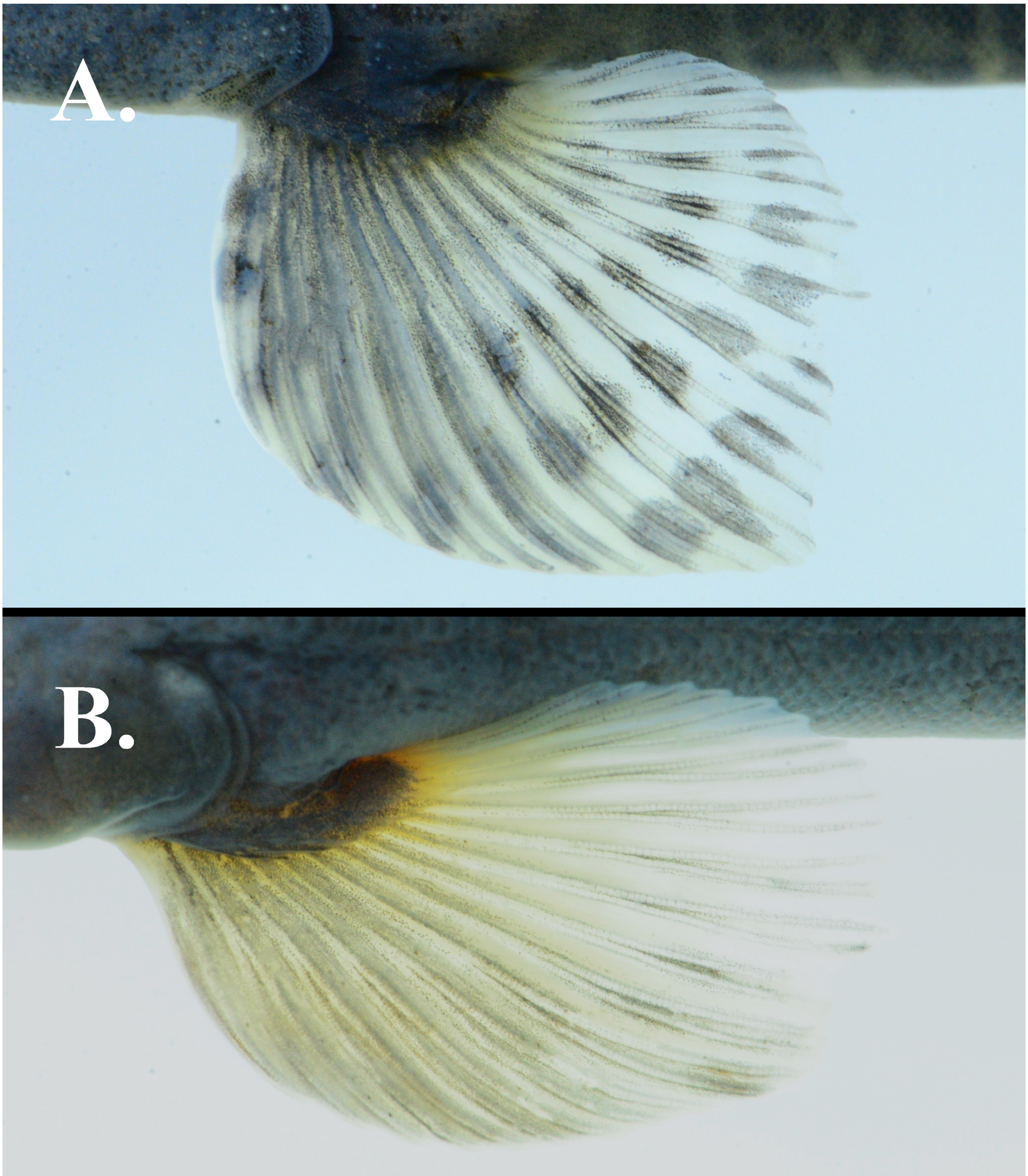

FIGURE 10. Left pectoral fin markings of the two *Formosania* loaches from Taiwan. A. *Formosania gilberti*, NTOUP2023-05-018, male, 69.99 mm SL; B. *Formosania lacustris* NTOUP2022-11-005, female, 75.49 mm SL.

Fins. Dorsal fin with 3 unbranched rays and 8 branched rays (15); anal fin with 2 unbranched rays and 5 branched rays (15); pectoral fins with 1 unbranched ray and 14 (12) or 15 (3) branched rays; pelvic fins with 1 unbranched ray and 7 (3) or 8 (12) branched rays. Dorsal fin triangular, with a slightly curved posterior edge; length of longest unbranched ray greater that body depth; fin origin situated before pelvic fin insertions. Posterior tip of anal fin almost reaching posterior end of caudal peduncle. Paired fins inserted horizontally; pectoral fins large, fin insertion point close to gill slit; pelvic fins separated, not fusing medially, fin ray tips extending posteriorly, covering anus when depressed; pelvic fin bases with a short, bud-like granule. Caudal fin slightly forked, with lower lobe slightly longer than upper lobe.

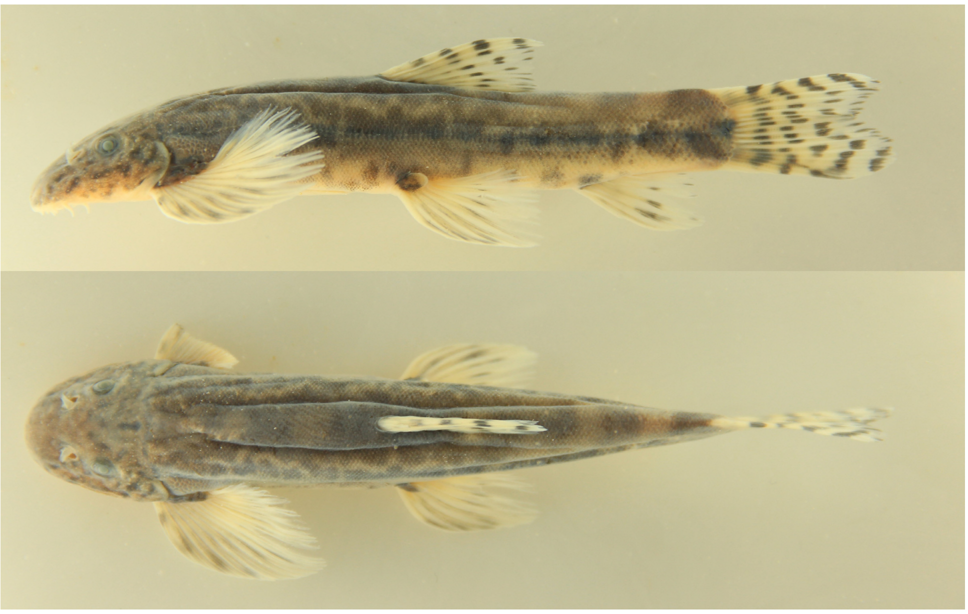

FIGURE 11. *Formosania lacustris* collected from Tzengwen River basin. NTOUP2022-11-006, female, 69.91 mm SL.

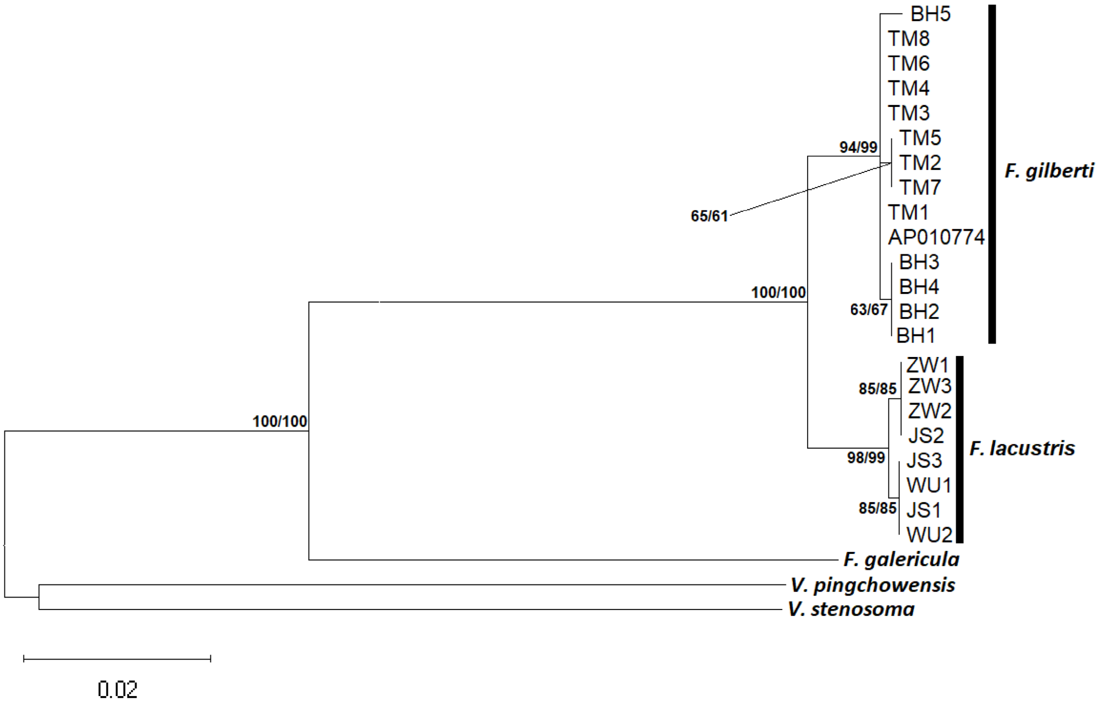

FIGURE 12. Molecular phylogenetic tree constructed by ATP8&6 sequences of 34 selected *Formosania* samples and 11 Cobitoidei species. Values at left and right sides of the slash at every node represents bootstrap values of neighbour-joining (NJ) and maximum likelihood (ML), respectively, and only bootstrap values that were above 75 were shown. For descriptions of every samples see Table 3.

Squamation. Pored lateral line scales 89 (5), 90 (3), 91 (2), 92 (2), 93 (1), or 96 (3). Body completely covered in cycloid scales except naked at head and pectoral fin bases. Thoracic region loosely covered with thin circular cycloid scales, scaled region extends anteriorly until lower edge of gill slit. Body scales elliptical, slightly rounded at anterior end. Lateral line scales with a tiny tubular pore at posterior end except for those upon caudal fin base, which possess a small posterior notch.

TABLE 4.

	F. galericula	*V. pingchowensis*	*V. stenosoma*	BH1	BH2	BH3	BH4	BH5	TM1	TM2	TM3	TM4	TM5	TM6	TM7	TM8	AP010774	JS1	JS2	JS3
F. galericula																				
V. pingchowensis	0.12723																			
V. stenosoma	0.12961	0.12366																		
BH1	0.08323	0.13436	0.14863																	
BH2	0.08323	0.13436	0.14863	0.00000																
BH3	0.08323	0.13436	0.14863	0.00000	0.00000															
BH4	0.08323	0.13436	0.14863	0.00000	0.00000	0.00000														
BH5	0.08323	0.13436	0.14982	0.00357	0.00357	0.00357	0.00357													
TM1	0.08205	0.13436	0.14982	0.00119	0.00119	0.00119	0.00119	0.00238												
TM2	0.08323	0.13555	0.15101	0.00238	0.00238	0.00238	0.00238	0.00357	0.00119											
TM3	0.08205	0.13436	0.14982	0.00119	0.00119	0.00119	0.00119	0.00238	0.00000	0.00119										
TM4	0.08205	0.13436	0.14982	0.00119	0.00119	0.00119	0.00119	0.00238	0.00000	0.00119	0.00000									
TM5	0.08323	0.13555	0.15101	0.00238	0.00238	0.00238	0.00238	0.00357	0.00119	0.00000	0.00119	0.00119								
TM6	0.08205	0.13436	0.14982	0.00119	0.00119	0.00119	0.00119	0.00238	0.00000	0.00119	0.00000	0.00000	0.00119							
TM7	0.08323	0.13555	0.15101	0.00238	0.00238	0.00238	0.00238	0.00357	0.00119	0.00000	0.00119	0.00119	0.00000	0.00119						
TM8	0.08205	0.13436	0.14982	0.00119	0.00119	0.00119	0.00119	0.00238	0.00000	0.00119	0.00000	0.00000	0.00119	0.00000	0.00119					
AP010774	0.08205	0.13436	0.14982	0.00119	0.00119	0.00119	0.00119	0.00238	0.00000	0.00119	0.00000	0.00000	0.00119	0.00000	0.00119	0.00000				
JS1	0.08086	0.13436	0.14744	0.01784	0.01784	0.01784	0.01784	0.01902	0.01665	0.01784	0.01665	0.01665	0.01784	0.01665	0.01784	0.01665	0.01665			
JS2	0.08323	0.13436	0.14744	0.01784	0.01784	0.01784	0.01784	0.01902	0.01665	0.01784	0.01665	0.01665	0.01784	0.01665	0.01784	0.01665	0.01665	0.00238		
JS3	0.08086	0.13436	0.14744	0.01784	0.01784	0.01784	0.01784	0.01902	0.01665	0.01784	0.01665	0.01665	0.01784	0.01665	0.01784	0.01665	0.01665	0.00000	0.00238	
WU1	0.08086	0.13436	0.14744	0.01784	0.01784	0.01784	0.01784	0.01902	0.01665	0.01784	0.01665	0.01665	0.01784	0.01665	0.01784	0.01665	0.01665	0.00000	0.00238	0.00000
WU2	0.08086	0.13436	0.14744	0.01784	0.01784	0.01784	0.01784	0.01902	0.01665	0.01784	0.01665	0.01665	0.01784	0.01665	0.01784	0.01665	0.01665	0.00000	0.00238	0.00000
ZW1	0.08323	0.13436	0.14744	0.01784	0.01784	0.01784	0.01784	0.01902	0.01665	0.01784	0.01665	0.01665	0.01784	0.01665	0.01784	0.01665	0.01665	0.00238	0.00000	0.00238
ZW2	0.08323	0.13436	0.14744	0.01784	0.01784	0.01784	0.01784	0.01902	0.01665	0.01784	0.01665	0.01665	0.01784	0.01665	0.01784	0.01665	0.01665	0.00238	0.00000	0.00238
ZW3	0.08323	0.13436	0.14744	0.01784	0.01784	0.01784	0.01784	0.01902	0.01665	0.01784	0.01665	0.01665	0.01784	0.01665	0.01784	0.01665	0.01665	0.00238	0.00000	0.00238

TABLE 4. (Continued)

	WU1	WU2	ZW1	ZW2	ZW3
F. galericula					
V. pingchowensis					
V. stenosoma					
BH1					
BH2					
BH3					
BH4					
BH5					
TM1					
TM2					
TM3					
TM4					
TM5					
TM6					
TM7					
TM8					
AP010774					
JS1					
JS2					
JS3					
WU1					
WU2	0.00000				
ZW1	0.00238	0.00238			
ZW2	0.00238	0.00238	0.00000		
ZW3	0.00238	0.00238	0.00000	0.00000	

Coloration in life. Juvenile transparent, with weakly pigmented anterior part of body and saddle like blotches on posterior end. All fins transparent, dorsal fins with a row of black median stripes and caudal fin with a black transverse band (Fig. 6). For adult individuals, body with light brown background color with an orangish shade; dorsal side of body with numerous saddle-like blotches; head and lateral sides of body intensely covered in vermicular markings (Fig. 5A; 8).

Coloration when fresh. Coloration and markings similar to those in life, but gaps between vermicular markings becoming mottled and darkened in color, thus blending with markings, resulting in less-distinguishable margins of blotches; vermicular markings extending downwards but not completely covering ventral side of head and body, which is greyish white, and ventral side of body behind pelvic fin, weakly pigmented around anus and anal fin base. Rostral barbels bright orange while maxillary barbels only slightly orangish. Corners of mouth with small area pigmented. All fin ray orange, lined with rows of large black spot with dorsal fin lined with 3–4 rows, paired fins with 2–4 rows and anal fin with 2–3 rows. Upper and lower lobes of caudal fin with 5–7 rows of transverse black bands, with fin between lined with 3–4 rows of spots along fin rays (Fig. 7).

Coloration in preservatives. Body overall greyish, all orange or dark green shades faded. All blotches and markings remained, not fading away.

Distribution

Formosania gilberti can be found in river systems of northern Taiwan, including Tamshui River, Beihuang River, and some other relatively small independent streams of northeastern Taiwan like ShuangRiver or Masu River.

Morphological comparisons

Compared with its Taiwanese relative *Formosania lacustris*, *Formosania gilberti* can be immediately distinguished from the former by meristic count differences of having lesser lateral line scale counts (89–96, x=97 *vs.* 82–102, x=102) and vertebral counts (22+11 *vs.* 22+12) (Fig. 9). *Formosania gilberti* can be further distinguished from *F. lacustris* by morphometric differences of having a smaller head length (18.98% in SL *vs.* 20.29% in SL), shorter predorsal length (49.89% in SL *vs.* 51.29% in SL), and larger eye (18.19% in HL *vs.* 16.81% in HL). Furthermore, *Formosania gilberti* also has shorter barbels and rostral folds than *F. lacustris*. In coloration patterns, *Formosania gilberti* can be distinguished from *F. lacustris* in having mottled, vermicular markings on sides (vs. cloud-like or brownish and blotchless with a thin longitudinal lateral streak) and paired fins lined with rows of large black spots (vs. blotchless or only weakly dark-shaded) (Fig. 10).

Notes on a new *Formosania* record from southwestern Taiwan

Our recent field collections gave a new distributional record for *Formosania lacustris* from the Tzengwen River basin (Fig. 11) as no previous researches or documents had recorded this fish before (Han & Fang 1997). A total of five individuals were collected, possessing 13+2 barbels, 3+8 dorsal fin elements, 2+5 anal fin elements, 1+14–15 pectoral fin elements, 1+8 pelvic fin elements, 96–99 pored lateral line scales and 22+11 total vertebrae counts. The overall coloration of the *F. lacustris* specimens collected from Tzengwen River showed a uniformly brownish body with a lateral streak and vague, cloud-like blotches dorsally, dorsal side of head with darkish brown markings and paired fins weakly spotted.

The habitat where the samples were collected is located in an upstream environment with rubbles and rocky substrates. However, the habitat was not very stable, as small upstream tributaries tend to dry out during dry seasons in Taiwan; the population hence has become a rather rare one and thus requires further, consistent monitoring of them.

Mitogenetic comparisons

Mitogenomic analyses of ATPase sequences, based on 24 collected and sequenced Taiwanese *Formosania* individuals and three selected OTUs, showed similar tree topology using NJ and ML methods (Fig. 12). The 24 Taiwanese *Formosania* species sequenced in this study were placed in two different, well-bootstrap-supported (bootstrap values of NJ and ML both 100) clades: the first clade contained individuals from Beihuang River (BH1–4) and Tamshui River (TM1–8); these were identified as *F. gilberti*; the other clade contained individuals from Jhuoshui River (JS1–3), Zhengwen River (ZW1–3), and Wu River (WU1 & WU2); these were identified as *F. lacustris*. Furthermore, the NCBI-downloaded OTU of AP01077 was also placed within the clade of *F. gilberti*.

From the genetic distances of the *Formosania* sequences analysed in this study, the genetic distances among *F. gilberti* were estimated to be 0.0000–0.0036 (avg. 0.0013), and the genetic distances among *F. lacustris* were estimated to be 0.0000–0.0024 (avg. 0.0014). The genetic distances between *F. gilberti* and *F. lacustris* were estimated to be 0.0167–0.019 (avg. 0.0174).

Discussions

The Taiwan-endemic species *Formosania gilberti* was firstly treated as a junior synonym of *F. lacustris* by Myers (1929). The publication also claimed that Oshima (1919) had overlooked the descriptions of Steindachner (1908a; 1908b). However, there was no further comparison nor description to support such treatment, and this problem had since crept its way into subsequent studies (Chen 1980; Tzeng & Shen 1982; Watanabe 1983; Chen & Tang 2000; Novák *et al.* 2006; Teng 2009), concealed as an unresolved fault. In this study, based on the examination of topotypical specimens and related species, the differences in previously described morphological examinations, including the detection of subtle features and osteological characters, and mitogenomic analyses, showing well-supported separation and genomic distances, indicate *Formosania gilberti* Oshima, 1919 should be treated as a valid, separated species and therefore should no longer be considered a junior synonym of *Formosania lacustris* (Steindachner, 1908b).

Based on the dichotomous diagnostic key in many studies (Chen 1980; Chen & Tang 2000; Teng 2009; Zhang & Wang 2011; Sun *et al.* 2023), *Formosania lacustris* was always initially differentiated from its congeners based on the caudal peduncle depth being greater than the caudal peduncle length. However, our morphometric results show that neither *F. lacustris* nor *F. gilberti*, the latter of which was formerly mistreated as a junior synonym of *F. lacustris*, possess these characters. On the contrary, their caudal peduncle depth was shorter than its length (84.91% in CPL). This character was not mentioned in the species descriptions of *Homaloptera formosanum* (=*Formosania lacustris*) or *F. gilberti* by Steindachner (1908a) and Oshima (1919), respectively. Furthermore, no types were examined in the aforementioned studies. Therefore, we suggest that this character should not be used in any further diagnostic key to differentiate the species, as the measurement value may vary due to different methods employed.

In our other collections, *Formosania* loaches from the western and northeastern Lanyang River basins showed unique marking patterns. These patterns were distinct from the strongly mottled *F. gilberti* and the lightly mottled to almost blotchless, typical *F. lacustris*. We suspect that there may be more cryptic species concealed within these unique *Formosania* loaches. Therefore, we have excluded these individuals from our current studies, as further analysis and comparisons are needed to address this issue.

Finally, considering the endemism of *Formosania lacustris*, studies claimed that this species was also distributed in coastal river systems of southern China from the Min River (Minjiang) southwards to the Jiulong River (Jiulongjiang), including the Long River, Chiulu River, Mulan River, Fongtze River, Luoyang River, and Jin River (Jinjiang) (Chen 1980; Chen & Tang 2000; Wang *et al.* 2007; Teng 2009), and such treatments were also supported by mitogenomic analyses of D-loop and cytb sequences (Wang *et al.* 2007; Teng 2009).

However, preliminary examinations of our *Formosania* collections from the Jin River showed apparent morphological differences, including only one pair of lower lip protrusions with a frontal pad-like process and body markings that were neither vermicular like *F. gilberti* nor blotchless with a lateral streak like *F. lacustris* (unpublished data). These morphological differences may be subtle hints of a distinct species, and therefore we suggest a wider selection of genetic markers to confirm the true identity and phylogenetic relationships of *Formosania* species from China and Taiwan.

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