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RESEARCH PAPER

Two new species of *Tanichthys* (Teleostei: Cypriniformes) from China

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Abstract. *Tanichthys albiventris*, new species, from the River Jiangping in Dongxing City, Guangxi Province is distinguished from *Tanichthys albonubes* by the presence of a reddish-orange dorsal-fin margin (*vs.* white) and 9-10 (9 in mode) branched anal-fin rays (*vs.* 8 in mode). *Tanichthys flavianalis*, new species, from the River Jiuqu in Qionghai City, Hainan Province is distinguished from *T. albiventris* and *T. albonubes* by the presence of a golden anal-fin margin (*vs.* white) and 7 (rarely 6) branched dorsal-fin rays (*vs.* 6 in mode). In *T. albiventris*, *T. albonubes*, and *T. flavianalis* the black lateral stripe is located on the dorsal half of the flank, distinguishing them from *Tanichthys kuehnei* and *Tanichthys micagemmae*, in which it is mid-lateral. *Tanichthys thabacensis* is different from all other species of *Tanichthys* in the shape of the mouth and insertion of the anal fin; it is tentatively referred to as *Aphyocypris*.

Key words: Cyprinidae, Tanichthyidae, white cloud mountain minnow, cytochrome *b*, phylogeny, taxonomy

Introduction

The minnow genus *Tanichthys* Lin, 1932 are small freshwater fishes characterized by confluent narial openings, and the presence of cornified tubercles on the snout posterior to the premaxilla in adult males (Weitzman & Chan 1966, Freyhof & Herder 2001, Bohlen et al. 2019). This genus was placed either in the subfamily Leuciscinae (Chu 1935, Yang & Huang 1964), the subfamily Danioninae (Chen 1998, Nelson et al. 2016), or a sister group to the subfamily Acheilognathinae (Liao et al. 2011) under the family Cyprinidae. However, some molecular phylogenetic studies have elevated the family Cyprinidae to the superfamily Cyprinoidea, in which a new family Tanichthyidae was proposed to include *Tanichthys* (Chen & Mayden 2009, Mayden & Chen 2010).

Tanichthys comprises four species, including *Tanichthys albonubes* Lin, 1932 (white cloud mountain minnow), *Tanichthys kuehnei* Bohlen, Dvořák, Thang

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& Šlechtová, 2019, *Tanichthys micagemmae* Freyhof & Herder, 2001, and *Tanichthys thacbaensis* Nguyen & Ngô, 2001. Unlike the other three species restricted to specific river basins with a narrow geographic distribution in northern and central Vietnam, *T. albonubes* has a wide distribution ranging across several different basins (the River Pearl and several small coastal rivers) in southern China (including Guangdong, Guangxi, Hainan and Hong Kong) and northern Vietnam (Quang Ninh Province) (Weitzman & Chan 1966, Pan 1991, Chen 1998, Kottelat 2001, Yi et al. 2004, Chan & Chen 2009, Li & Li 2011, Zhao et al. 2018). Recent molecular studies indicated that each of the wild populations of *T. albonubes* was monophyletic with significant genetic differentiation among them (Luo et al. 2015, Zhao et al. 2018). They were further considered as different cryptic species (Li et al. 2020). Although several studies made comparisons based on limited morphological characters or colour patterns among some populations from China, and reported variation among different populations (Weitzman & Chan 1966, Yi et al. 2004, Chan & Chen 2009, Li & Li 2011, Li et al. 2020), none of them provided clear diagnostic characters for different populations/cryptic species. It is apparent that the taxonomy of the *T. albonubes* species group remains unresolved.

Based on detailed examination of specimens of *Tanichthys* collected from six localities in southern China, and coupled with molecular analysis using the cytochrome *b* (cyt*b*) gene, we found two new species, and describe them in the present paper (Table 1, Fig. 1).

Material and Methods

Sampling

All specimens were collected using hand-nets. Freshly caught fish was euthanized with eugenol. Specimens used for morphological studies were initially fixed in 8% formalin for 5-7 days, then transferred to 70% ethanol for permanent preservation. A few specimens were preserved in 95% ethanol and stored in a –20°C freezer for DNA extraction. The specimens examined in this study are deposited in the Department of Oceanography, National Sun Yat-sen University, Kaohsiung (DOS) and Shanghai Natural History Museum, Shanghai (SNHM).

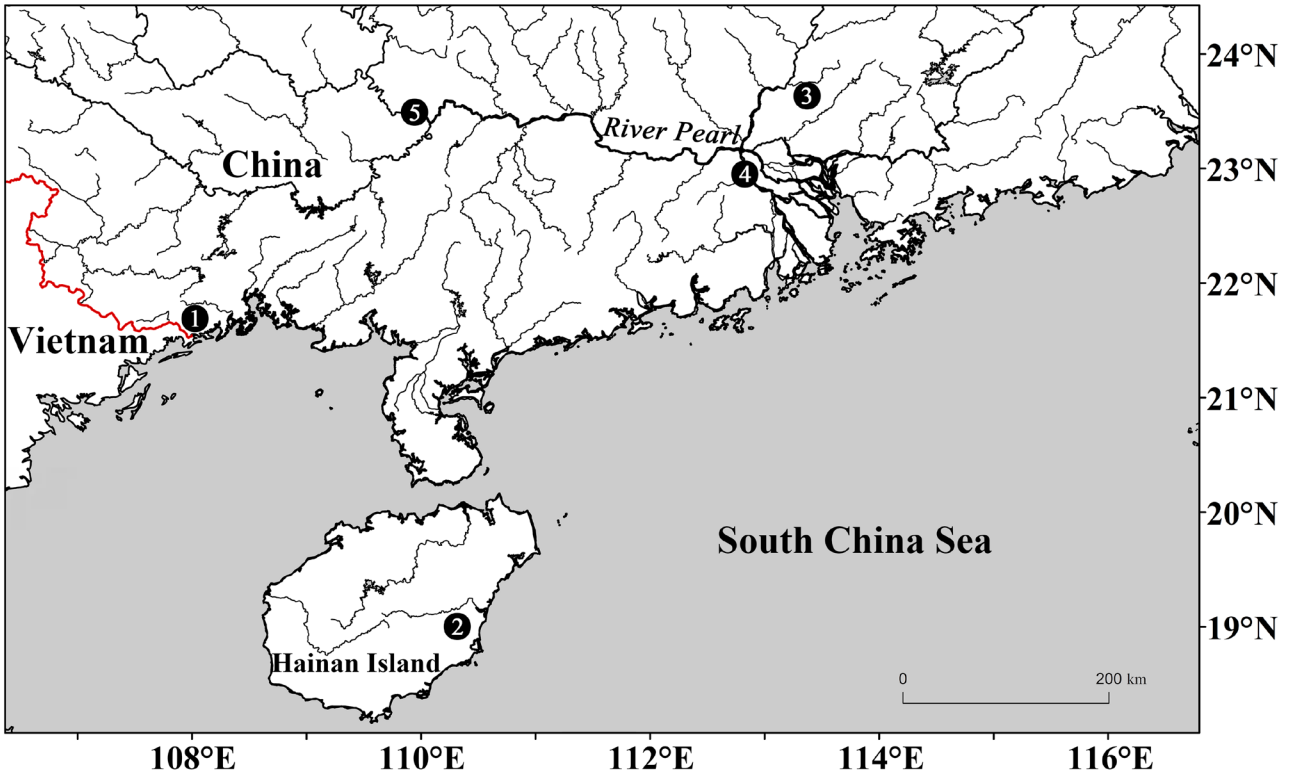

Fig. 1. Map of sampling localities. ① for both type locality, JP, and HZ of *Tanichthys albiventris* due to the short distance; ② for type locality, QH, of *T. flavianalis*; ③ for CH of *T. albonubes*; ④ for FS of *T. albonubes*; ⑤ for GP of *T. albonubes*. See Table 1 for site information.

Table 1. List of locality and GenBank accession numbers for cytochrome *b* sequence of six populations from China.

Code	Basin	Locality	GenBank accession number		
JP	River Jiangping	Guangxi: Dongxing: Jiangping Town	OK432527	OK432528	OK432529
HZ	River Huangzhu	Guangxi: Dongxing: Jiangping Town	OK432530	OK432531	OK432532
QH	River Jiuqu	Hainan: Qionghai: Huishan Town	OK432533	OK432534	OK432535
CH	River Pearl	Guangdong: Guangzhou: Conghua District	OK432536	OK432537	OK432538
FS	River Pearl	Guangdong: Foshan: Gaoming District	OK432539	OK432540	OK432541
GP	River Pearl	Guangxi: Guiping: Nanmu Town	OK432542	OK432543	OK432544

Morphological analyses

Measurements and counts generally follow Fang (1997). Head depth was taken at the posterior margin of the orbit. Body depth was taken at the origin of the pelvic fin. The transverse scale series was counted between the dorsal and anal fin origins; both of the small scales below the origin of the dorsal fin and above the origin of the anal fin were counted as 0.5. Pelvic- and pectoral-fin rays were counted on the left side. Only the simple dorsal- and anal-fin rays above the skin were counted. The last two ray elements separated at the same base in both dorsal and anal fins were counted as one.

Molecular analyses

DNA extraction from tissues was conducted using a modified phenol-chloroform protocol (Sambrook & Russell 2001). Mitochondrial cyt*b* of 18 specimens of *Tanichthys* (Table 1) was amplified by polymerase chain reaction (PCR) using the primer pair, cytbF 5′-TCG ACT AAT CAT AAA GAT ATC GGC AC-3′ and cyrbR 5′-TCG ACT AAT CAT AAA GAT ATC GGC AC-3′. PCR was conducted with an initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturing at 95 °C for 40 s, annealing at 50 °C for 40 s and extension at 72 °C for 1 min. A final extension was done at 72 °C for 8 min. PCR products were purified and sequenced by Sangon Biotech Co., Ltd. (Shanghai).

A total of 1,123 bp consistent cyt*b* sequences were obtained. For tree calculation the 18 newly amplified sequences were analysed with 21 *Tanichthys* sequences from GenBank (12 *T. albonubes* (KP893425, KP893448, KP893457, KP893464, KP893478, KP893490, KP893505, KP893514, MH918657, MH918658, MH918659 and MH918661), three *T. kuehnei* (MG952920, MH918664 and MH918663), and six *T. micagemmae* (HM224384, KX647170, KX647174, KX647179, KX647187 and MH918662)), and *Rhodeus ocellatus* (AB769519) was used as the outgroup for tree rooting. Cyt*b* sequences were aligned using ClustalW implemented in MEGA 6 (Tamura et al. 2011) and checked by eye. Maximum likelihood tree (ML) and Bayesian inference tree (BI) were reconstructed using MEGA 6 and MrBayes 3.2 (Ronquist & Huelsenbeck 2003), respectively. PartitionFinder 2.1.1 was used to select the optimal evolutionary models for phylogenetic analysis (Lanfear et al. 2017). The best model GTR + I + G was applied for both ML and BI reconstructions. The ML tree was run with 1,000 bootstrap replications (Felsenstein 1985). Bayesian inference was run with all parameters, except topology and branch length, and allowed to vary independently using the unlink command in MrBayes v3.2 (Ronquist & Huelsenbeck 2003). Analyses were conducted with sampling for six million generations when the split frequencies were below 0.01 (two concurrent simultaneous analyses, nruns = 2; three heated chains, nchains = 4; sample frequency 1,000; burnin = 25%). Trees were visualized in FigTree v.1.3.1 (http://tree.bio.ed.ac.uk/software/figtree). Genetic distances based on cyt*b* sequences were calculated using MEGA-X (Kumar et al. 2018, Stecher et al. 2020). Sequences generated in this study are available on GenBank and accession numbers of sequences for molecular analyses are provided in Table 1.

Results

Tanichthys albiventris Li F., Bohlen J. & Liao T.-Y., new species (Figs. 2A, 3A, 4; Tables 2-3)

Type series: Holotype: SNHM 10301, male, 27.0 mm SL; a small stream tributary of the River Jiangping, close to Nalou Village, Jiangping Town, Dongxing City, Guangxi Province, China (Code: JP); 24 December 2015.

Paratypes: SNHM 10302-10315, 14, 24.0-28.1 mm SL, same data as holotype.

Additional non-type material: SNHM 10401-10426, 26, 18.9-29.0 mm SL, same data as holotype. SNHM 10427-10437, 11, 22.0-30.9 mm SL, a small stream tributary of the River Huangzhu, close to Huangzhu Village, Jiangping Town, Dongxing City, Guangxi Province, China (Code: HZ); 26 March 2021.

Diagnosis: Tanichthys albiventris is distinguished from *T. albonubes* by the presence of more branched anal-fin rays (9-10 *vs.* 8 in mode), and the colour of the dorsal-fin margin (reddish-orange *vs.* white); from *T. kuehnei* and *T. micagemmae* by the presence of a black lateral stripe located on the dorsal half of the flank (*vs.* on middle of flank), and a reddish-orange dorsal-fin margin (*vs.* white); from *T. thacbaensis* by the origin of the anal fin, anterior to the base of the last dorsal-fin ray (*vs.* posterior), and mouth superior (*vs.* terminal).

Description: Morphometric and meristic data of holotype and paratypes as shown in Table 2. Body moderately compressed. Mouth large and oblique; corner of mouth extending to vertical of anterior margin of orbit; lower jaw projecting beyond upper jaw. Barbels absent. Anterior and posterior narial opening confluent, not separated by a skin wall. A row of five to eight small tubercles resembling teeth on lateral margin of upper jaw posterior to premaxilla, and a projecting row of two large conical tubercles laterally on anterior lower jaw present in both adult males and females, but more developed in adult males. Four small sharp tubercles present on the lower margin of the anterior premaxilla in adult males, but not observed in females (Fig. 2A).

Dorsal fin with 2 simple and 6 branched rays. Anal fin with 3 simple and 9-10 (mostly 9) branched rays. Pectoral-fin rays 12 (rarely 11), first and last one or two rays simple. Pelvic fin rays 7 (rarely 6), first ray simple. Principal caudal-fin rays 17 (rarely 16). Longitudinal scale series 32-35 (mean 33.3), including 30-33 (mean 32.1) on body and 1-2 on caudal-fin base. Lateral line scales absent. Transverse scale rows 8 (rarely 7). Predorsal scales 15-17 (mostly 15; mean 15.5). Circumpeduncular scales 12.

Colouration in life: Body colour mostly brownish. Lips slightly orange. A blueish lateral stripe along upper margin of a thin black stripe from eye to caudal-fin base. Pigmentation below lateral stripes

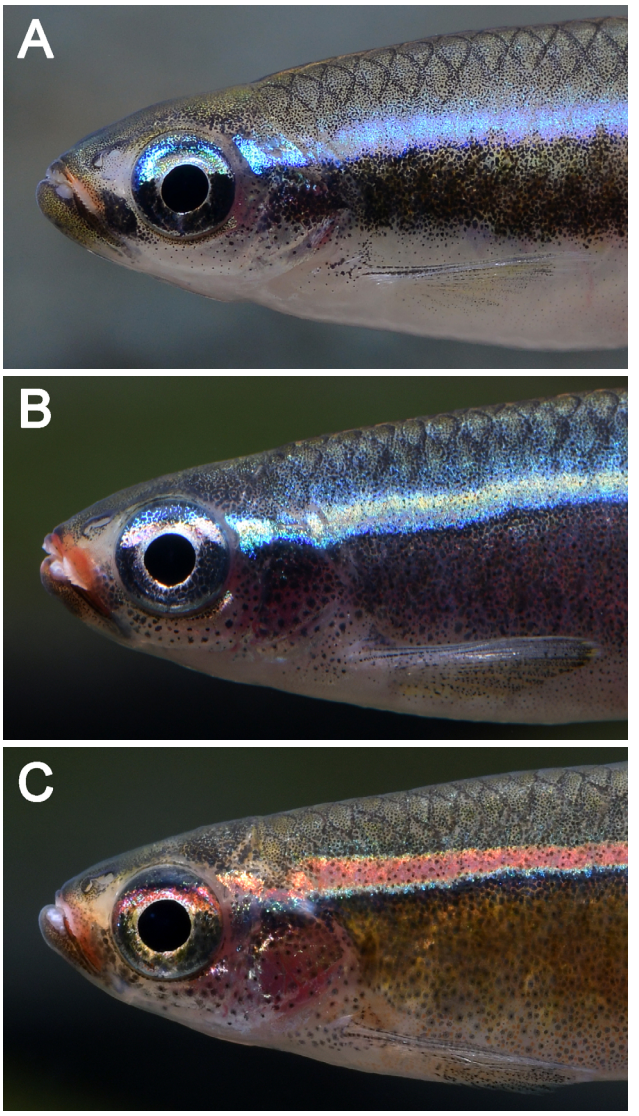

Fig. 2. Lateral view of head and anterior body of adult males in three species of *Tanichthys* from China, showing colour pattern and tubercles on snout. A) *T. albiventris*, male, from its type locality; B) *T. flavianalis*, male, from its type locality; C) *T. albonubes*, male, from Gaoming District, Foshan City, Guangdong Province, China. Specimens not preserved.

blackish with a definite lower outline (mixed with reddish on caudal peduncle), distinctly darker than above. Lower body white. Dorsal fin hyaline in base, red in middle, and margined with reddish-orange. Anal fin hyaline in base, yellow or reddish-orange in middle, followed by a slender black stripe and margined with white. Pelvic fin yellowish with white margin. Pectoral fin hyaline. Central caudal fin reddish-orange with a conspicuous black blotch in base (Figs. 2A, 3A).

Colour in preservative: An indistinct light lateral stripe, along with a slender black stripe, running from posterior margin of operculum to caudal-fin base. Black lateral stripe less than half of width of light lateral stripe, and more prominent than that

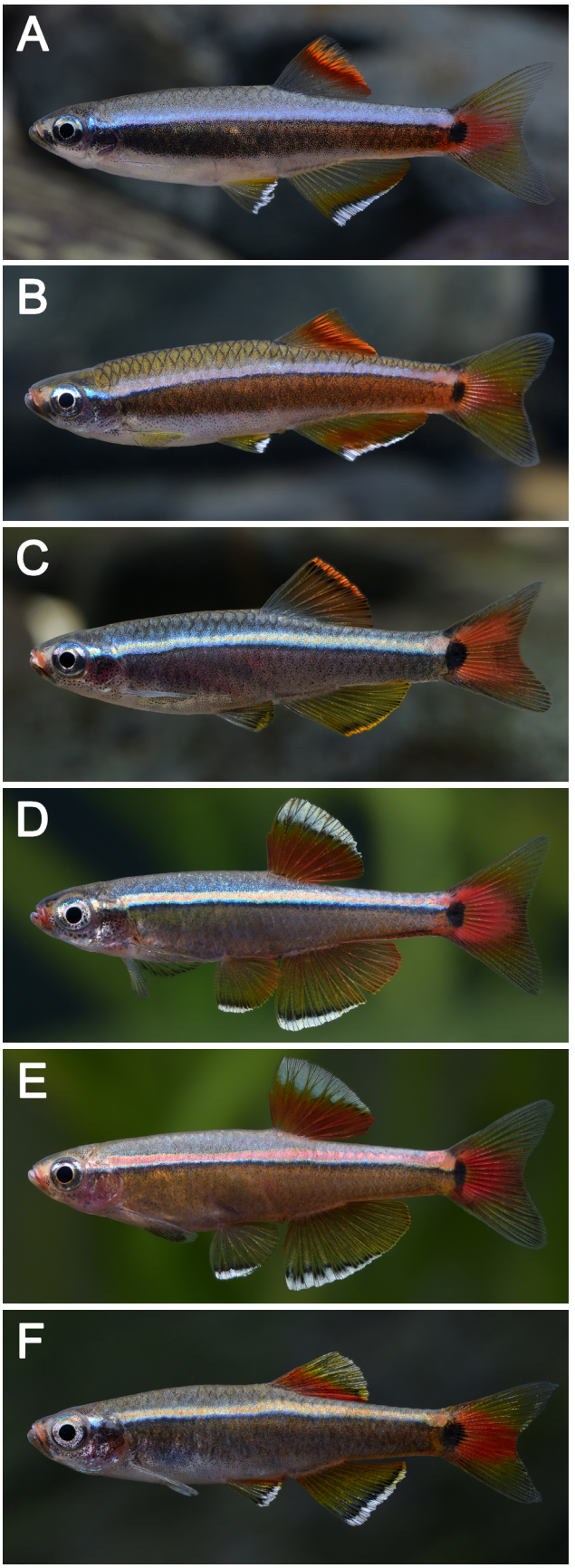

Fig. 3. Live specimens of *Tanichthys* from six localities in southern China. A) *T. albiventris*, male, from its type locality, JP; B) *T. albiventris*, male, from HZ; C) *T. flavianalis*, male, from its type locality, QH; D) *T. albonubes*, male, from CH; E) *T. albonubes*, male, from FS; F) *T. albonubes*, male, from GP. Specimens not preserved. See Table 1 for site information.

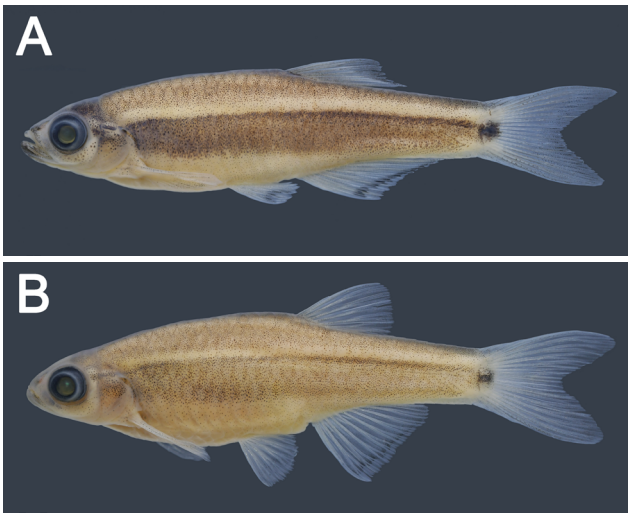

Fig. 4. *Tanichthys albiventris*, A) SNHM 10301, holotype, male, 27.0 mm SL; B) SNHM 10302, paratype, female, 28.1 mm SL.

in life. Anal and pelvic fins with black stripe near margin. Caudal fin base with a black blotch (Fig. 4).

Distribution and habitat: To date, known only from the upper reaches of the Rivers Jiangping and Huangzhu, in Jiangping Town, Dongxing City, Guangxi Province, China (Fig. 1). The type locality at the time of sampling was a small hill stream, about 2 m wide on average, located in the depths of the forest. Water depth varied from approximately 0.4 to 1 m. The bottom consisted of mud mixed with sand. Some aquatic plants (*Cryptocoryne crispatula* and *Blyxa* sp.) in the lower part of the habitat. Other fish species encountered were *Oryzias pectoralis*, *Macropodus hongkongensis* and *Rhinogobius* cf. *duospilus*.

Etymology: The specific name, *albiventris*, is constructed from the Latin words *albus*, meaning white, and *venter*, meaning belly, an adjective, alluding to the distinctive white belly. A suggested Chinese vernacular is 白腹唐鱼.

Tanichthys flavianalis Li F., Liao T.-Y. & Shen Z.-X., new species (Figs. 2B, 3C, 5; Tables 2-3)

Type series: Holotype: SNHM 10316, male, 21.8 mm SL; a small stream tributary to the River Jiuqu in Huishan Town, Qionghai City, Hainan Province, China (Code: QH); 9 June 2019.

Paratypes: SNHM 10317-10330, 14, 18.3-26.2 mm SL, same data as holotype.

Additional non-type material: SNHM 10438-10460, 23, 15.9-22.4 mm SL, same data as holotype.

Table 2. Meristic and morphometric data for holotypes and paratypes of two new species of *Tanichthys*. Numbers in parentheses in meristic data are number of examined specimens, and in morphometric data represent the mean number.

	T. albiventris		*T. flavianalis*	
	Holotype	Paratypes	Holotype	Paratypes
n	1	14	1	14
Standard length (mm)	27.0	24.0-28.1	21.8	18.3-26.2
Meristic data				
Branched dorsal-fin rays	6	6 (14)	7	6 (2), 7 (12)
Branched anal-fin rays	9	9 (11), 10 (4)	9	8 (1), 9 (12), 10 (1)
Pectoral-fin rays	12	11 (2), 12 (12)	12	11 (2), 12 (10), 13 (2)
Pelvic-fin rays	7	6 (1), 7 (13)	7	7 (13), 8 (1)
Principal caudal-fin rays	16	16 (2), 17 (12)	17	16 (4), 17 (10)
Scales in lateral series	35	32 (3), 33 (5), 34 (6)	33	31 (1), 32 (5), 33 (8),
Scales in transverse series	8	7 (2), 8 (12)	8	8 (14)
Circumpeduncular scales	12	12 (14)	12	12 (14)
Predorsal scales	15	15 (8), 16 (4), 17 (2)	16	15 (4), 16 (10)
Morphometric data (% standard length)				
Head length	23.8	22.2-24.2 (23.3)	24.9	24.5-26.3 (25.2)
Head depth	17.4	15.0-16.7 (15.9)	17.2	16.2-17.2 (16.7)
Snout length	4.3	4.1-4.8 (4.5)	4.7	4.0-5.4 (4.9)
Orbit diameter	8.9	7.9-8.6 (8.3)	9.0	8.3-9.3 (8.9)
Body depth	22.3	20.3-28.4 (24.6)	26.5	22.3-26.1 (25.2)
Predorsal length	58.6	57.6-61.3 (59.3)	57.7	57.6-59.1 (58.1)
Preanal length	60.3	59.7-63.1 (61.1)	60.3	59.3-61.5 (60.5)
Prepelvic length	46.2	44.9-48.7 (46.5)	46.0	45.3-48.2 (46.9)
Caudal peduncle length	27.9	24.9-28.8 (27.1)	25.9	26.3-28.3 (26.8)
Caudal peduncle depth	14.0	12.0-14.1 (13.3)	13.9	12.5-14.3 (13.4)
Dorsal-fin base length	8.3	6.3-8.8 (7.7)	10.1	8.4-11.7 (10.2)
Anal-fin base length	13.8	11.9-14.8 (13.2)	15.9	12.8-16.1 (14.4)

Diagnosis: Tanichthys flavianalis is distinguished from *T. albonubes* by the presence of more branched anal-fin rays (9-10, 9 in mode *vs.* 8 in mode), more branched dorsal-fin rays (7 in mode *vs.* 6 in mode), and a reddish-orange dorsal-fin margin (*vs.* white); from *T. albiventris* by the presence of more branched dorsal-fin rays (7 in mode *vs.* 6 in mode), and a golden anal-fin margin (*vs.* white); from *T. kuehnei* and from *T. micagemmae* by the presence of a black lateral stripe located on the dorsal half of the flank (*vs.* on middle of the flank), and a reddish-orange dorsal-fin margin (*vs.* white); from *T. thacbaensis* by the origin of the anal fin anterior to the base of the last dorsal-fin ray (*vs.* posterior), and mouth superior (*vs.* terminal).

Description: Morphometric and meristic data of holotype and paratypes as shown in Table 2. Body moderately compressed. Mouth large and oblique; corner of mouth extending to vertical of anterior margin of orbit; lower jaw projecting beyond upper jaw. Barbels absent. Anterior and posterior narial opening confluent, not separated by a skin wall. A row of six to nine small tubercles resembling teeth on the lateral margin of the upper jaw posterior to premaxilla, a projecting row of two or three large conical tubercles laterally on anterior lower jaw, and four to six small tubercles present on the lower margin of the anterior premaxilla in adult males, but not in females (Fig. 2B).

Dorsal fin with 2 simple and 7 branched rays (rarely 6). Anal fin with 3 simple and 9 branched rays (rarely 8 and 10). Pectoral-fin rays 11-13, first and last one or two rays simple. Pelvic-fin rays 7 (rarely 8), first ray simple. Principal caudal-fin rays

Table 3. Comparison of branched dorsal- (Br. D) and anal-fin rays (Br. A), and colouration of dorsal- and anal-fin margins among all species of *Tanichthys*. Numbers in parentheses are number of specimens examined.

Species	Locality	n	Br. D	Br. A	Dorsal-fin margin	Anal-fin margin	Source
T. albiventris	JP[a]	41[b]	6 (40), 7 (1)	9 (29), 10 (12)	reddish-orange	white	this study
	HZ	11	6 (10), 7 (1)	9 (8), 10 (2), 11 (1)	reddish-orange	white	this study
T. albonubes	[a]	1	7	8	green, transparent	green, transparent	Lin 1932
	[a]	62[c]	5 (1), 6 (59)	7 (4), 8 (55)	white	white	Weitzman & Chan 1966
	CH	34	6 (34)	7 (3), 8 (31)	white	white	this study
	FS	37	6 (37)	8 (25), 9 (12)	white/ yellowish-white	white	this study
	GP	31	6 (31)	8 (23), 9 (8)	white/ yellowish-white	white	this study
T. flavianalis	QH[a]	39[b]	6 (6), 7 (33)	8 (4), 9 (34), 10 (1)	reddish-orange	yellow	this study
T. kuehnei	[a]	11	6-7	9	greyish-white[d]	greyish-white[d]	Bohlen et al. 2019
T. micagemmae	[a]	21	6	8	white[d]	orange[d]	Freyhof & Herder 2001
T. thacbaensis	[a]	2	6-7	8	unknown	unknown	Nguyen & Ngô 2001

[a]type locality, [b]including type and non-type specimens from same locality, [c]not all specimens counted due to damage to some specimens, [d]according to figures of live specimens from original description.

17 (rarely 16). Longitudinal scale series 31-33 (mean 32.5), including 30-32 (mean 30.7) on body and 1-2 on caudal-fin base. Lateral line scales absent. Transverse scale rows 8. Predorsal scale rows 16 (rarely 15; mean 15.7). Circumpeduncular scales 12.

Colouration in life: Ground colour mostly brownish. Lips reddish. A broad blueish-white lateral stripe along upper margin of a thin blackish stripe from eye to caudal-fin base. Intensity of melanophores below lateral stripes similar to above. Dorsal fin mostly orange with a narrow reddish-orange margin. Anal fin mostly yellow with narrow golden margin. Margin of dorsal and anal fins more distinct in adult males than in females. Pelvic fin yellowish with yellow margin. Pectoral fin blackish. Centre of caudal fin red with a conspicuous black blotch at the base (Fig. 2B, 3C).

Colour in preservative: Ground colour lighter towards belly. A light lateral stripe, along with a slender black stripe, running from posterior margin of operculum to caudal-fin base. Black lateral stripe less than half of width of light lateral

Fig. 5. *Tanichthys flavianalis*, A) SNHM 10316, holotype, male, 21.8 mm SL; B) SNHM 10317, paratype, female, 21.9 mm SL.

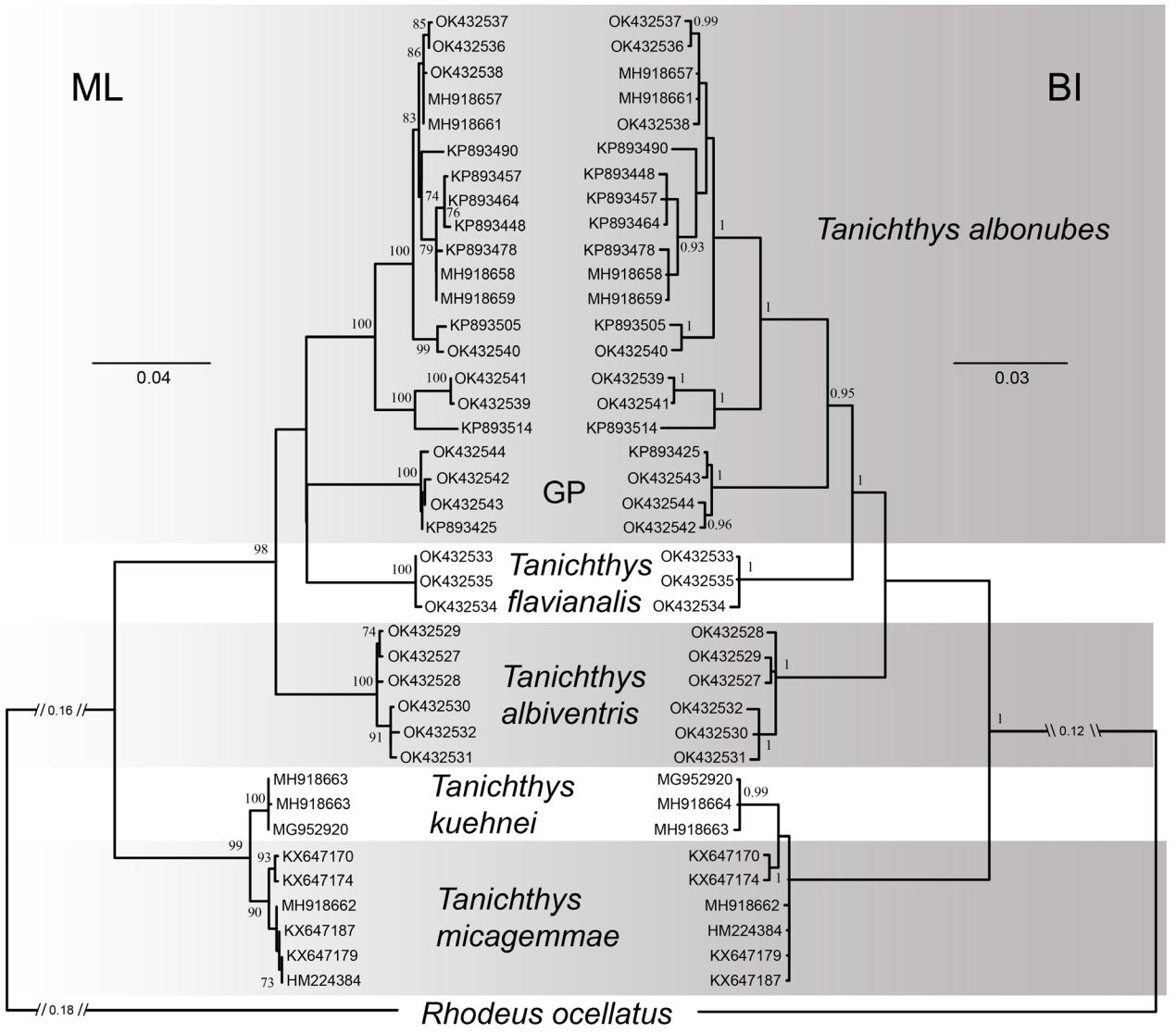

Fig. 6. Maximum likelihood (ML) and Bayesian inference (BI) trees based on cytochrome *b* sequences of five species of *Tanichthys*. Numbers near nodes are bootstrap values for ML analysis and posterior probability values for BI analysis; values lower than 70 and 95, respectively, are not shown. Refer to Table 1 for information of population GP.

Table 4. Meristic data for three populations of *Tanichthys albonubes*. Numbers in parentheses are number of examined specimens. All examined specimens are listed in comparative materials with symbol *.

Population	CH	FS	GP
n	15	15	15
Standard length (mm)	19.9-23.9	22.3-27.1	18.4-25.3
Branched dorsal-fin rays	6 (15)	6 (15)	6 (15)
Branched anal-fin rays	7 (1), 8 (14)	8 (10), 9 (5)	8 (12), 9 (3)
Pectoral-fin rays	11 (11), 12 (4)	11 (8), 12 (5), 13 (2)	10 (7), 11 (8)
Pelvic-fin rays	7 (15)	7 (14), 8 (1)	6 (1), 7(14)
Principal caudal-fin rays	17 (14), 18 (1)	16 (1), 17 (14)	15 (3), 16 (3), 17 (9)
Scales in lateral series	31 (5), 32 (8), 33 (2)	31 (4), 32 (9), 33 (2)	32 (6), 33 (6), 34 (3)
Scales in transverse series	8 (9), 9 (6)	7 (1), 8 (13), 9 (1)	8 (13), 9 (2)
Circumpeduncular scales	12 (15)	12 (15)	12 (15)
Predorsal scales	15 (6), 16 (2), 17 (7),	15 (3), 16 (9), 17 (2), 19 (1)	15 (6), 16 (8), 17 (1)

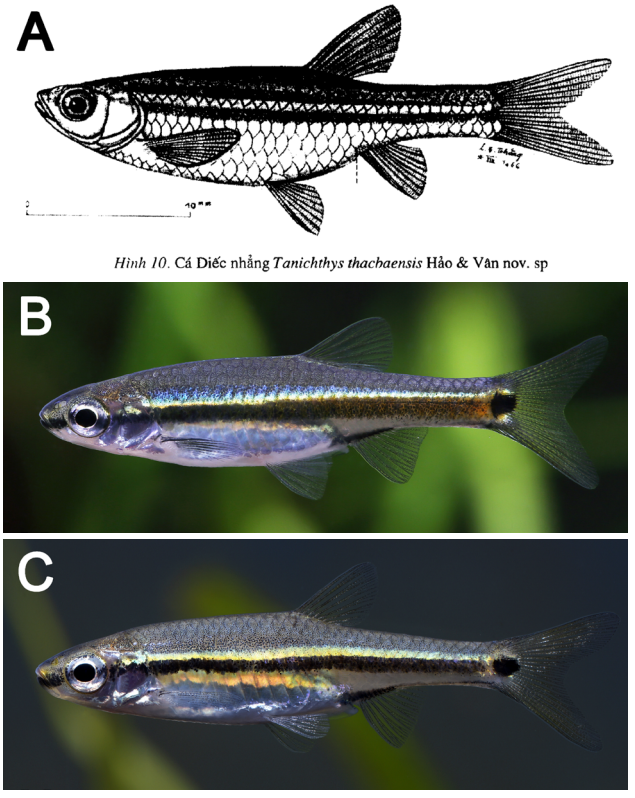

Fig. 7. A) Illustration of *Tanichthys thacbaensis* from Nguyen & Ngô (2001). B) male and C) female *Aphyocypris lini*, from Haifeng County, Guangdong Province, China; specimens not preserved.

stripe, and more prominent than in life. Dorsal, anal and pelvic fins with blackish stripe near margin. Caudal fin base with a black blotch (Fig. 5).

Distribution and habitat: To date, known only from the upper reach of the River Jiuqu, in Qionghai City, Hainan Province, China (Fig. 1). The type locality at the time of sampling was a small stream (about 1.5 m wide on average) with a mixed mud and gravel substrate. The depth was mostly less than 0.4 m. There was no aquatic vegetation, but a lot of leaf litter. Coexisting fishes were *O. pectoralis*, *Channa* cf. *gachua*, *M. hongkongensis*, *Rhinogobius wanchuangensis* and *Misgurnus anguillicaudatus*.

Etymology: The specific name, *flavianalis*, is constructed from the Latin words *flavus*, meaning yellow, and *analis*, meaning anal, an adjective, alluding to the diagnostic light yellow anal fin with golden margin. A suggested Chinese vernacular is 黄臀唐鱼.

Molecular analyses

BI and ML trees were reconstructed based on 1,123 bps. In both trees, the two new species were monophyletic respectively. The two formed a clade together with the third Chinese species, *T. albonubes*, while the Vietnamese species, *T. kuehnei* and *T. micagemmae*, together formed a second clade. However, the interrelationships among species varied. In the BI tree, *T. kuehnei* was a monophyletic group nested within *T. micagemmae* while in the ML tree, reciprocal monophyly of these two species was recovered. In the Chinese clade, *T. albiventris* was at the basal node in both ML and BI trees. *Tanichthys flavianalis* was the sister group of *T. albonubes* in the BI tree, but formed a monophyletic group with the GP population of *T. albonubes*, which was sister to the remaining *T. albonubes* in the ML tree (Fig. 6).

Inter-species genetic distances ranged from 1.49% (between *T. kuehnei* and *T. micagemmae*) to 10.52% (between *T. kuehnei* and *T. albonubes*). Genetic distances among *T. albiventris*, *T. albonubes* and *T. flavianalis* were from 6.42% (*T. albonubes vs. T. flavianalis*) to 7.02% (*T. albiventris vs. T. albonubes*). In *T. albonubes*, high genetic distance was found not only among different populations (6.53% between the GP population and the remaining *T. albonubes*), but also among different individuals of the FS population (up to 4.2% between OK432540 and OK432541), at approximately the inter-specific level compared to other species in China.

Discussion

Based on the data in the present study, the two new species can easily be distinguished from all other congeneric species, excluding *T. thacbaensis*, by the colour of the dorsal- and anal-fin margins and the number of branched dorsal- and anal-fin rays (Table 3; Figs. 2-3). *Tanichthys thacbaensis* is the only species of the genus for which colour photos are not available. It is unlikely that the opportunity to examine this species again will arise due to both the loss of all type specimens and the destruction of the type locality (Bohlen et al. 2019). However, based on the description and illustration provided in Nguyen & Ngô (2001), this species seems not to be a species of *Tanichthys*, but a species similar to *Aphyocypris lini* (Weitzman & Chan 1966) based on the position of the anal-fin origin posterior to the base of last dorsal-fin ray (*vs.* anterior in all other *Tanichthys*) and the lower jaw not projecting beyond the upper jaw (*vs.* projecting in all other *Tanichthys*). *Tanichthys thacbaensis* probably has no coloured stripes across its dorsal and anal fins as found in *A. lini* (*vs.* presence in all other *Tanichthys*), since Nguyen & Ngô (2001) described the colour pattern of *T. thacbaensis* in detail, including identification

of several stripes on the body and a large spot on caudal-fin base, but with no description of stripes on the dorsal and anal fins or shown on the illustration (Bohlen et al. 2019) (Fig. 7).

Colour pattern is an important character for species identification of *Tanichthys* (Freyhof & Herder 2001, Bohlen et al. 2019). In addition to differences in the colour of the dorsal- and anal-fin margins, the two closely related species of *Tanichthys* from Vietnam (*T. kuehnei* and *T. micagemmae*) can be easily distinguished from the three *Tanichthys* species from China (*T. albiventris*, *T. albonubes*, and *T. flavianalis*) by the position and width of the black lateral stripe (on the middle of the flank and of similar width as the white stripe along its upper margin *vs.* on the dorsal half of the flank and less than half width of the white stripe), and the colour of pigmentation below the lateral stripes (lighter than above *vs.* similar to or darker than above) (Lin 1932, Freyhof & Herder 2001, Bohlen et al. 2019).

The molecular phylogenetic analyses based on cytochrome *b* showed strong support for the monophyly of the two new species in both the BI and ML trees (Fig. 6), though the ML tree analysis was in disagreement with the BI tree analysis and morphological analysis for the GP population. In the ML tree, the GP population forms a monophyletic group with the *T. flavianalis* clade, and the GP + *T. flavianalis* clade is sister to the *T. albonubes* clade, including the CH and FS populations. The GP population was also considered as a cryptic species in Li et al. (2020). However, the GP population shares similar meristic characters and the above-mentioned colour features with the CH and FS populations of *T. albonubes* (Tables 3-4; Fig. 3), and the BI tree analysis also supported the monophyly of *T. albonubes* containing GP, CH and FS populations (Fig. 6). Based on morphological and molecular data, the GP population is still identified as *T. albonubes* in the present study. It is necessary to confirm the status of the GP population by using different morphological and molecular methods in the future.

Previous studies have also provided some morphological data for different populations of *Tanichthys* from China, but some of the data are distinctly inaccurate based on our examination of a large number of specimens collected from the same localities to those in previous studies. Previous data may have been obtained by an inaccurate counting method. The sampling locality of the lineage "DX" (from Dongxing City, Guangxi Province) in Zhao et al. (2018) and Li et al. (2020), which is the same as the type locality of *T. albiventris* (coded JP in the present study), was provided by the first author of this study via Dr. Hung-Du Lin who was the corresponding author of Zhao et al. (2018). Li et al. (2020) reported eight branched anal-fin rays for this population based on the examination of 30 specimens, whereas in the present study, the number of branched anal-fin rays of the JP population is 9-10 (mostly 9), and that of the HZ population of *T. albiventris* from an adjacent basin is 9-11 (mostly 9). All of the 52 specimens from the two localities in the present study have more than eight branched anal-fin rays (Table 3). Furthermore, Li & Li (2011) reported data for 15 specimens from Guiping City, Guangxi Province, including anal fin ii-10, dorsal fin iii-6, pectoral fin ii-9, etc. Li et al. (2020) based on 30 specimens of the same population provided the same number of branched anal-fin rays without any variations (10) as reported in Li & Li (2011). However, the present study examined 31 specimens from the same locality reported in Li & Li (2011) (coded GP in the present study), and found 3 simple and 8-9 (8 in mode) branched anal-fin rays, 2 simple dorsal-fin rays above skin, and the second pectoral-fin ray branched (Tables 3-4).

Key to the species of *Tanichthys*

1a Origin of anal fin posterior to base of last dorsal-fin ray, mouth terminal ..... *T. thacbaensis*
1b Origin of anal fin anterior to base of last dorsal-fin ray, mouth superior 2
2a Black lateral stripe located on middle of flank.....3
2b Black lateral stripe located on dorsal half of flank.....4
3a Anal-fin margin orange, branched anal-fin rays 8 ..... *T. micagemmae*
3b Anal-fin margin white, branched anal-fin rays 9 ..... *T. kuehnei*
4a Dosal-fin margin white, branched anal-fin rays 8 in mode ..... *T. albonubes*
4b Dorsal-fin margin reddish-orange, branched anal-fin rays 9 in mode.....5
5a Anal-fin margin white, branched dorsal-fin rays 6 in mode ..... *T. albiventris*
5b Anal-fin margin golden, branched dorsal-fin rays 7 in mode..... *T. flavianalis*

Comparative materials

Tanichthys albonubes: DOS 202010801*, 15 specimens, 19.9-23.9 mm SL; DOS 202010802,

19 specimens, 19.7-24.9 mm SL; River Pa, tributary of River Bei, Pearl River system, in Aotou Town, Conghua District, Guangzhou City, Guangdong Province, China (Code: CH); 9 October 2020. DOS 202010803*, 15 specimens, 22.3-27.1 mm SL; DOS 202010804, 22 specimens, 20.1-24.5 mm SL; River Xi, Pearl River system, in Gaoming District, Foshan City, Guangdong Province, China (Code: FS); 11 October 2020. DOS 202010805*, 15 specimens, 18.4-25.3 mm SL; DOS 202010806, 16 specimens, 17.9-27.7 mm SL; River Qian, tributary of River Xi, Pearl River system, in Nanmu Town, Guiping City, Guangxi Province, China (Code: GP); 15 October 2020.

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