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Brachygobius jennie, a new bumblebee goby (Teleostei, Oxudercidae) from Hengqin Island, Guangdong Province, China

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Abstract

A new species of bumblebee goby, *Brachygobius jennie* **sp. nov.** (Teleostei, Oxudercidae), is described based on 31 specimens collected from the Hengqin Island, Guangdong Province, China. This new species is distinguished from all other valid species of *Brachygobius* by its extreme small size (SL < 9 mm) and distinctive number, position, size and shape of its black bars. It possesses four complete (forming ring) or nearly complete post-cephalic, relatively narrow bars, two of which are located posterior to the base of the anal fin. The first bar behind the head extends pigmentation to the first dorsal fin but does not reach the midventral line. The second bar, chevron-shaped and located below the second dorsal fin, extends pigmentation onto that fin and ventrally reaches the midventral line and the anal fin. Cytochrome oxidase subunit I-based analyses, including six out of the eight valid species of *Brachygobius*, show *Brachygobius jennie* **sp. nov.** to be more than 10% genetically divergent from other species, supporting the morphological diagnosis and confirming its distinct taxonomic status. With a maximum size of less than 9 mm SL (based on observations of 106 specimens), the new species is not only the smallest species of *Brachygobius*, but also one of the smallest known gobioid fishes.

Key Words

Fish, Gobioidei, integrative taxonomy, new species, miniature vertebrates

Introduction

The tropical Asian genus *Brachygobius* Bleeker, 1874 (Teleostei, Oxudercidae), collectively known as bumblebee gobies, currently includes eight species (Miller 1989; Larson and Vidthayanon 2000; Larson 2001; Hoese et al. 2024; Fricke et al. 2026): *B. aggregatus* Herre, 1940, *B. doriae* (Günther, 1868), *B. kabiliensis* Inger, 1958, *B. mekongensis* Larson & Vidthayanon, 2000, *B. nunus* (Hamilton, 1822), *B. sabanus* Inger, 1958, *B. xanthomelas* Herre, 1937, and *B. xanthozona* (Bleeker, 1849). *Brachygobius* are small fishes with a typical standard length (SL) < 25 mm, preferentially occurring in brackish waters in mangrove forests, estuaries, and tidal streams, with two species fully adapted to freshwaters (i.e., *B. xanthomelas* and *B. mekongensis*) (Hoese et al. 2024; Froese and Pauly 2025). Larson (2001) and Hoese et al. (2024) commented on the classification of *Brachygobius*, highlighting that the taxonomy of this group is still only partially resolved. Yusri et al. (2025) used genetic data to examine the species diversity of *Brachygobius* in Malaysia and Southeast Asia and found evidence of undescribed species.

Although *Brachygobius* is widely distributed across tropical Asia, from India to the west to south Vietnam and the Philippines to the east, and all countries in-between, there is no published record of the genus from China. In April 2025, however, we collected small gobioid specimens from brackish mangrove habitats on Hengqin Island, the largest island in Zhuhai (86 km^2), located on the west bank of the Zhujiang River Estuary near Macau, China. An initial inspection of these specimens revealed that they belong to the genus *Brachygobius* as they ex-

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hibited a stout and robust body with a distinctive black banded color pattern, and all second dorsal and anal fin rays segmented (Hoese et al. 2024). Further investigation, integrating morphological and molecular evidence, showed this species is distinct from all other valid species of *Brachygobius* and is formally described herein.

Materials and methods

Specimen collection

One hundred and six specimens of *Brachygobius*, of which 31 were selected for the type series of the new species, were collected from shallow waters near the bank on Hengqin Island, Guangdong Province, China (Fig. 1), using D-type hand nets with a 30-cm handle, a 30 × 25 cm net mouth, 10-cm net depth, and 40-mesh nylon netting. Specimens were handled in compliance with Sun Yat-sen University ethical regulation. After capture, most specimens were immediately euthanized on ice, with three preserved in 95% ethanol (for genetic analysis) and the remainder in 5% formaldehyde (for morphological diagnosis). A few specimens were kept alive for several hours in a tank for a photographic session to document their live color pattern. They were then euthanized on ice and fixed in formaldehyde. Following two-week formaldehyde preservation, all specimens were thoroughly washed with tap water, transferred to 75% ethanol, and deposited in the collection of the Museum of Biology, Sun Yat-sen University (museum code: SYS), Guangdong, China.

Morphometrics and meristic counts

Morphometric measurements and meristic counts followed Wu et al. (2008) and Hoese et al. (2024), with modifications as follows. All specimens (< 9 mm SL) were examined under a stereomicroscope; images were captured with a digital camera connected to the microscope, and all measurements were taken from these images using ImageJ software. Standard length (SL), head length (HL), snout length, predorsal length, and preanal length were measured from the tip of the snout. Head depth and width were taken at the posterior margin of the preopercle, whereas body depth and width were measured at the origin of the anal fin. Head depth was measured from the posterior end of the supraoccipital, and head length from the anterior tip of the snout to the posterior margin of the opercle. Dorsal and anal fin base lengths were measured from the base of the first to the last ray. Head length and all other measurements were expressed in percentage (%) either of SL or HL. Meristic data, including longitudinal scale rows and fin rays, were counted under a stereomicroscope using transmitted light. Unsegmented fin rays (= spines) were counted using Roman numerals, whereas segmented rays (regardless of whether they are branched or not) were counted using Arabic numerals, following Larson (2001) and Hoese et al. (2024). Coloration was recorded from living and preserved specimens under natural light. Vertebral counts from three specimens were obtained using micro-CT imaging. Head canal pores and papillae rows were not recorded because they were difficult to observe in our small specimens, and the head lateral line system organization is not a diagnostic character for most species of *Brachygobius* (Larson 2001; Hoese et al. 2024).

DNA sequencing and comparative analysis

The barcode fragment of the mitochondrial cytochrome oxidase subunit I (COI) was determined for three specimens (Yi et al. 2017; Zhang et al. 2025). Total genomic DNA was extracted using the Marine Animal Tissue Genomic DNA Extraction Kit (TIANGEN, DP324-02). The COI nucleotide fragment was amplified using the primers Fish F1 (5'-TCAACCAACCACAAAGACATTGGCAC-3') and Fish R1 (5'-TAGACTTCTGGGTGGCCAAAGAATCA-3') (Ward et al. 2005). The PCR mixture consisted of 1 μL each of primers Fish F1 and Fish R1, 10 μL of PCR

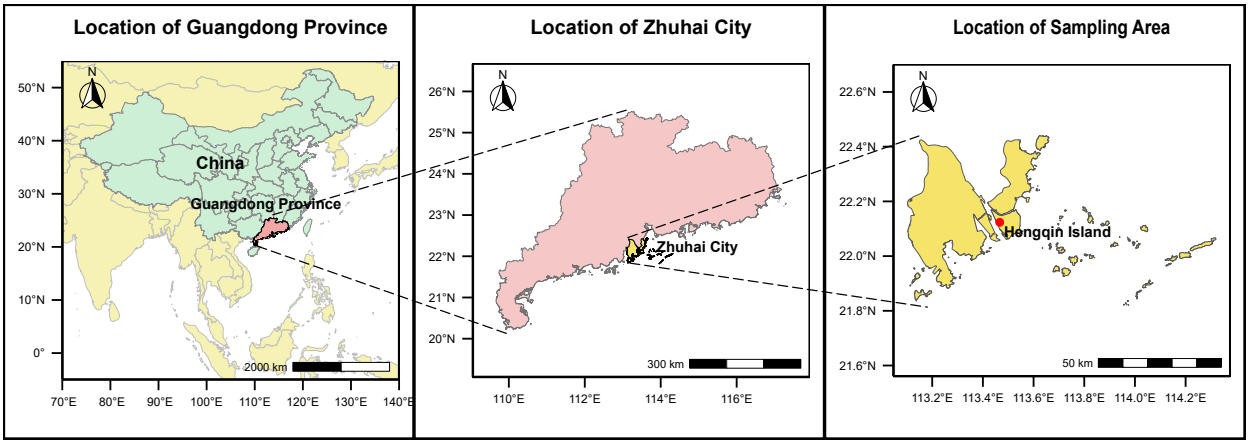

Figure 1. Left: Map of China showing the location of Guangdong Province in southern China. Middle: Enlarged map of Guangdong Province showing the location of Zhuhai City. Right: Map of Zhuhai City indicating the type locality of *Brachygobius jennie* sp. nov. on Hengqin Island (Geolocation coordinates 22.1236°N, 113.4663°E).

mix, 1 µL of DNA template, and 7 µL of ddH_2O. The PCR cycling protocol was as follows: initial denaturation at 95 °C for 5 min; 35 cycles of denaturation at 94 °C for 25 s, annealing at 55 °C for 25 s, and extension at 72 °C for 10 s; followed by a final extension at 72 °C for 5 min and storage at 4 °C. PCR products were bidirectionally sequenced by Shanghai Biotechnology Co., Ltd., using the same PCR primers. For each specimen, the forward and reverse chromatograms were first inspected and then combined. The resulting consensus sequences were deposited in GenBank under accession numbers PX690640, PX690641 and PX690642.

The new COI gene sequences from the newly collected specimens were combined with 29 COI sequences of *Brachygobius* archived in GenBank representing six of the eight valid species of the genus (the two missing species are *B. xanthozona* and *B. mekongensis*) (Table 1). Multiple sequence alignment was performed using MAFFT v7.526 with the automatic algorithm to optimize alignment accuracy (Katoh and Standley 2013). The best-fitting nucleotide substitution model (HKY+G+I) was selected using MEGA v12 (Kumar et al. 2024). Maximum likelihood (ML) analysis was conducted using RAxML-NG v1.2.2 (Kozlov et al. 2019), with 1,000 bootstrap replicates to evaluate nodal support. A specimen of *Gobiopterus brachypterus* (Bleeker, 1855) was used to root the phylogenetic tree. To assess both intraspecific and interspecific divergence, uncorrected genetic distances (= *p*-distances) were calculated using MEGA v12.

Results

Brachygobius jennie sp. nov.

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Figs 2–4; Table 2

Chinese name. 珍妮短虾虎鱼.

English name. Jennie's Bumblebee Goby.

Table 1. List of specimens of *Brachygobius* examined in the genetic analysis of this study. For each specimen, the species identification, COI sequence GenBank accession number, geographical origin and published (or unpublished) reference are provided. Accession numbers of sequences newly determined for this study are highlighted in bold.

Species name	Accession number	Geographical origin	Reference
Brachygobius jennie sp. nov.	**PX690640**	Hengqin Isl., China	This study
	PX690641	Hengqin Isl., China	This study
	PX690642	Hengqin Isl., China	This study
	KT951754	Leizhou Peninsula, China	Zhang et al. (2016)
Brachygobius xanthomelas	PV535654	Kelantan, Malaysia	Yusri et al. (2025)
	PV535655	Kelantan, Malaysia	Yusri et al. (2025)
	PV535657	Johor, Malaysia	Yusri et al. (2025)
	PV535658	Johor, Malaysia	Yusri et al. (2025)
Brachygobius sabanus	PV535659	Terengganu, Malaysia	Yusri et al. (2025)
	PV535660	Terengganu, Malaysia	Yusri et al. (2025)
	PV535665	Penang, Malaysia	Yusri et al. (2025)
	PV535663	Johor, Malaysia	Yusri et al. (2025)
	PV535666	Sabah, Malaysia	Yusri et al. (2025)
	PV535667	Sabah, Malaysia	Yusri et al. (2025)
Brachygobius doriae	PV535670	Sabah, Malaysia	Yusri et al. (2025)
	/	Brunei	HKL47, unpublished*
	KU674800	Perak, Malaysia	Huang et al. (2016)
Brachygobius kabiliensis	/	Brunei	HKL48, unpublished*
	PV535671	Sabah, Malaysia	Yusri et al. (2025)
	PV535672	Sabah, Malaysia	Yusri et al. (2025)
	PV535673	Sabah, Malaysia	Yusri et al. (2025)
Brachygobius cf. *aggregatus*	MW498533	Kedah, Malaysia	Zainal Abidin et al. (2021)
	MW498531	Kedah, Malaysia	Zainal Abidin et al. (2021)
	MW498532	Kedah, Malaysia	Zainal Abidin et al. (2021)
Brachygobius nunus	PX048535	Perak, Malaysia	Yusri et al. (2025)
	PX048536	Perak, Malaysia	Yusri et al. (2025)
	MK572081	Bangladesh	Rahman et al. (2019)
	MK572080	Bangladesh	Rahman et al. (2019)
Brachygobius sp "Bangkok"	PV535674	Bangkok, Thailand	Yusri et al. (2025)
	PV535675	Bangkok, Thailand	Yusri et al. (2025)
	MK628380	Mekong, Thailand	Panprommin et al. (2020)
	MK628381	Mekong, Thailand	Panprommin et al. (2020)
Gobiopterus brachypterus (Outgroup)	KX223902	/	Azmir et al. 2017

Note: *, publicly available from the BOLDSystems database (https://portal.boldsystems.org)

Figure 2. Photographs of: 1) a fresh non-type specimen of *Brachygobius jennie* sp. nov., showing: **a.** Dorsal; **b.** Lateral, and **c.** Ventral views and 2) the preserved holotype of *Brachygobius jennie* sp. nov., SYS p003206, 7.66 mm SL, from Hengqin Island, Guangdong Province, China, showing: **d.** Dorsal; **e.** Lateral, and **f.** Ventral views.

Table 2. Morphometric and meristic characteristics of the holotype and paratypes of *Brachygobius jennie* sp. nov. Right column: for each measurement and meristic count, the range is given, followed by the mean for measurement or the mode for meristic counts in parentheses. All rays in the second dorsal and anal fins are segmented, with the first ray unbranched (Hoese et al. 2024).

Morphological characteristics	Holotype	Paratypes and holotype n = 31
Morphometric measurements		
Standard length (SL, mm)	7.66	4.16–8.98 (6.57)
In % of SL		
Head length (HL)	18.9	15.8–19.7 (17.3)
Body depth	17.2	14.2–18.3 (16.5)
Distance between pectoral fin and pelvic fin insertions	16.1	13.3–17.4 (16.1)
Caudal peduncle length (CPL)	29.4	17.6–29.9 (24.9)
Caudal peduncle depth	13.1	9.4–13.2 (11.4)
Pectoral fin length	16.4	11.5–17.0 (13.9)
First dorsal fin length	13.2	9.2–13.8 (11.7)
Second dorsal fin length	16.3	9.4–16.9 (13.3)
Pelvic fin length	11.0	8.4–11.3 (9.8)
Anal fin length	15.3	7.2–15.3 (11.9)
Caudal fin length	17.2	9.1–18.3 (13.6)
Predorsal length	28.1	26.9–36.5 (31.0)
Prepelvic length	27.8	23.7–31.8 (27.0)
Preanal length	57.2	43.1–59.4 (53.2)
In % of HL		
Snout length	26.2	23.8–30.3 (27.3)
Eye diameter	37.2	35.4–45.1 (39.4)
Interorbital width	37.9	36.7–46.8 (39.9)
In % of CPL		
Caudal peduncle depth	44.4	43.1–53.4 (45.9)
Meristic counts		
First dorsal fin rays (all unsegmented)	VI	VI
Second dorsal fin rays (all segmented)	8	8
Anal-fin rays (all segmented)	8	8
Pectoral fin rays (all segmented)	14	12–16 (14)
Pelvic fin rays (all segmented)	5+5	5+5
Caudal fin rays (all segmented)	22	20–24 (22)
Vertebrae	24	24

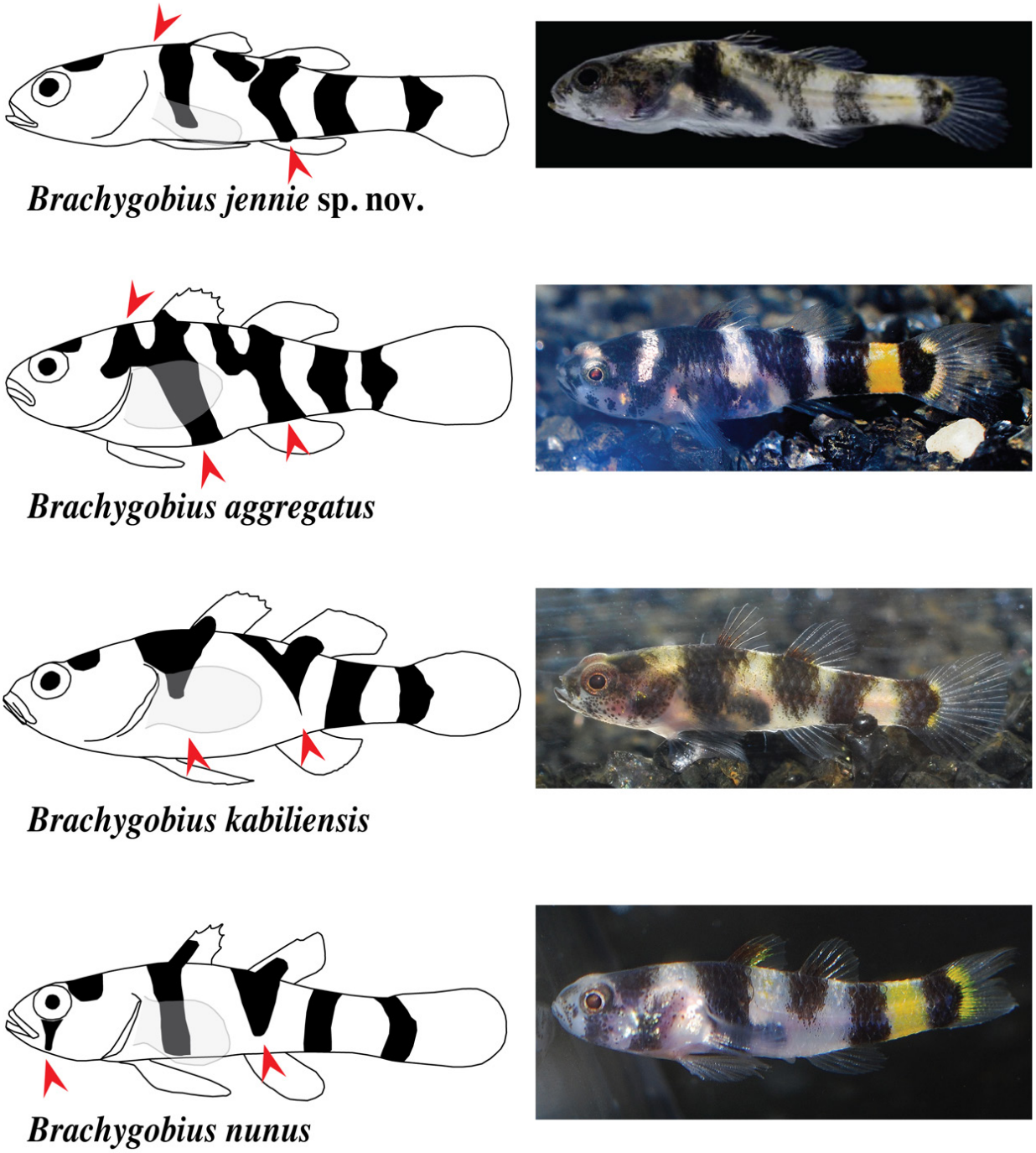

Figure 3. Diagnostic marking pattern comparison among all species of *Brachygobius* having four post-cephalic black bands, the last two located posterior to the base of the anal fin. Left column: schematic drawings highlighting diagnostic marking patterns based on information from type material and original descriptions; from top to bottom: *Brachygobius jennie* sp. nov. based on its description (this study); *B. aggregatus*, based on a drawing of a type specimen (plate 4 in Herre 1940); *B. kabiliensis*, based on the drawing of the holotype (fig. 19 in Inger 1958); and *B. nunus*, based on the drawing of a specimen of *Gobius alcockii* Annandale, 1906 (fig. 1 in Annandale 1906), a junior synonym of *B. nunus*, and the description of *B. nunus* by Hamilton (1822). Red arrowheads indicate diagnostic differences. Right column: photographs of live non-type specimens illustrating the corresponding left schematic patterns (all photos from the authors).

Type. ***Holotype.*** **China** • SYS p003206, 7.66 mm SL, sex undetermined; Hengqin Island, Guangdong Province, China, 113.4663°E, 22.1236°N; April 2025; Jiang-Yan Tian leg.

Paratypes. **China** • SYS p003207–p003236, 30 ex., 4.16–8.98 mm SL, all sex undetermined; same locality as holotype; April 2025; Jiang-Yan Tian leg.

Diagnosis. *Brachygobius jennie* sp. nov. can be readily distinguished from all congeners by the following characters: small size (SL < 9 mm) (vs maximum SL > 18 mm in all other species of *Brachygobius*); four complete (forming ring) or nearly complete post-cephalic, relatively narrow bars (vs three large bars in *B. xanthozona*, *B. doriae* and *B. sabanus* or five fainted bars in *B. mekongensis*), two of

which are located posterior to the base of the anal fin (vs only one in *B. xanthozona*, *B. xanthomelas*, *B. doriae* and *B. sabanus*). The first bar behind the head is narrow (vs wide in *B. aggregatus*) (Fig. 3), extends pigmentation to the first two rays of the first dorsal fin, but does not reach the midventral line. The second bar is chevron-shaped, located below the second dorsal fin, extending pigmentation onto that fin and, ventrally reaching the midventral line (vs not reaching the midventral line in *B. kabiliensis* and *B. nunus*) (Fig. 3). Predorsal scales present (vs absent in *B. mekongensis*) and black bars distinct (vs often fainted or indistinct in *B. mekongensis*).

Description. Morphometric data for the type specimens presented in Table 2. General morphology illustrated in Figs 2, 4. Data for holotype given in parentheses. A small species of *Brachygobius* (max SL < 9 mm). Two dorsal fins, first dorsal fin rays VI (all unsegmented; i.e., spines), first dorsal fin length 9.2–13.8% (13.2%) of SL; second dorsal fin rays 8 (all segmented; first unbranched, second to last branched), second dorsal fin length 9.4–16.9% (16.3%) of SL; anal fin rays 8 (all segmented; first unbranched, second to last branched), anal fin length 7.2–15.3% (11.9%) of SL; caudal fin rays 20–24 (22); pectoral fin rays 12–16 (14); pelvic fins fused forming a disc, pelvic fin rays 5+5; distance between insertions of pectoral and pelvic fins 13.3–17.4 (16.1%) of SL; body covered with ctenoid striated scales, longitudinal scale rows 24; predorsal scales present; vertebrae 24 (Suppl. material 1). Body short, longer than high, body depth 14.2–18.3% (17.2%) of SL; anterior part rounded, posterior part laterally compressed. Head large, head length 15.8–19.7% (18.9%) of SL; eye medium-sized, eye diameter 35.4–45.1% (37.2%) of HL, above midline with dorsal orbital edge above dorsal head profile; snout short, snout length 23.8–30.3% (26.2%) of SL; mouth large, terminal, slightly prognathous; jaws short, mouth corner below eye anterior half; jaws with 2 to 3 rows of small conical teeth; anterior nostril in tube near lip, posterior nostril not in tube, near front of orbit; gill opening narrow, to about depth of pectoral fin base.

Coloration. (Figs 2–4) In live and freshly collected specimens, body background whitish/cream, somewhat translucent (with the peritoneal cavity and vertebral column visible), with melanophores arranged into 1) four distinct post-cephalic black bars, complete or almost complete; 2) one black saddle mark between the dorsal fins never extending below midline but sometimes connected to the third bar, and sometimes 3) one small dorsal mark on the caudal peduncle between the last two bars. Bars relatively narrow with inter-bar spaces larger than bars, two of the bars located posterior to the base of the anal fin (no overlapping). The first bar behind the head narrow, nearly complete, extends pigmentation to the first dorsal fin but does not reach midventral line. The second bar, chevron-shaped, located below the second dorsal fin, extends pigmentation onto that fin and, ventrally, reaches the midventral line and anal fin. In some specimens, which may be males, caudal peduncle background between the last two bars yellowish, and two bright yellow spots on the upper and lower parts of the caudal fin base, just behind the fourth bar. Ventral region whitish without melanophores outside of the bars (Fig. 2). Scattered melanophores present on the scaled predorsal region, operculum, cheek, snout, and lower jaw. Occipital region black with a central white spot; dorsal orbital regions blackish. Pectoral, pelvic and caudal fins uniformly translucent. Base of the first two or three rays of the first dorsal fin pigmented black, first dorsal fin otherwise translucent; bases of the second to fourth rays of the second dorsal fin pigmented black, second dorsal fin otherwise translucent; bases of the second to fourth rays of the anal fin pigmented black, anal fin otherwise translucent.

Preserved specimens retain black markings, including the four black bars, but lose yellow coloration, with these regions turning cream/grey like the rest of the body background.

Sexual dimorphism. The sex of the available specimens could not be determined with certainty, no females carried eggs, and no obvious external sexual dimorphism was observed. The size at maturity is also unknown.

Distribution and habitat. *Brachygobius jennie* sp. nov. is currently known from the Hengqin Island, Guangdong Province, southern China. This extends the distribution of the genus *Brachygobius* northeastward, in a subtropical region. Species habitat consists of mangrove wetlands with clean brackish water, slow current, and silty bottom (Fig. 5). A COI sequence archived in GenBank (Accession number: KT951754) indicates that the species is also present in the Leizhou Peninsula, China (21.35°N, 110.55°E) (Zhang et al. 2016), approximately 300 km southwest of Hengqin Island. We note that this sequence was misidentified as *Butis butis* (Hamilton, 1822) in Zhang et al. (2016) and in GenBank; it should be corrected to *Brachygobius jennie* sp. nov.

Etymology. The specific epithet is in honor of Ms. Jennie Ruby Jane. The name recognizes her exceptional character and achievements, which proved source of inspiration for the first author who discovered the new species. The name is used as a noun in apposition. The Chinese vernacular name proposed for this new species is“珍妮短虾虎鱼”.

Sequence variation and molecular phylogeny

The COI gene sequences analyzed in this study ranged from 560 bp to 677 bp. The final alignment comprised 33 sequences, including three COI sequences of *Brachygobius jennie* sp. nov. newly generated for this study, each comprising 651 positions. Sequences longer than 651 bp were trimmed and sequences shorter than 651 bp were completed with “-.” The GC content varied between 41.5% and 47.7% across dataset. The average nucleotide composition was A = 26.4%, T = 30.2%, C = 25.7%, and G = 17.7%, with an A+T content accounting for 56.6%, indicating a moderate AT bias in the COI gene region.

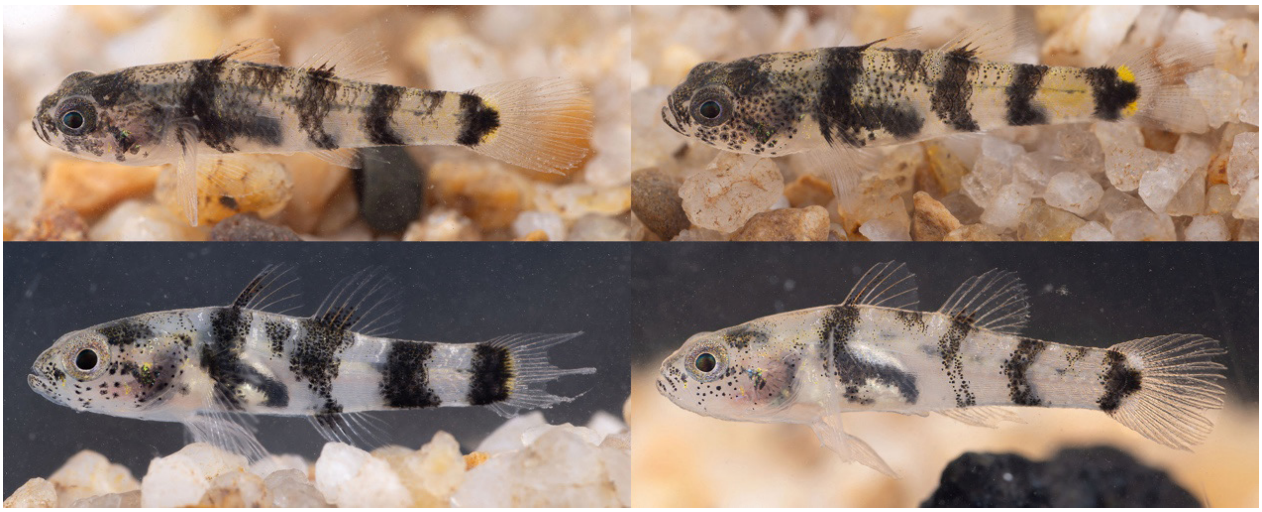

Figure 4. Live photographs of four paratypes of *Brachygobius jennie* sp. nov., illustrating intra-specific marking pattern. Photographed by Mr. Haocong Yang and Mr. Danyang Zhou.

Figure 5. Brackish mangrove habitat of *Brachygobius jennie* sp. nov. on Hengqin Island, Guangdong Province, southern China.

The COI-based ML phylogenetic tree of *Brachygobius* species is shown in Fig. 6. In this tree, the three COI sequences of *Brachygobius jennie* sp. nov. together with a GenBank archived sequence form a monophyletic group nested within *Brachygobius* (Bootstrap Support [BS] = 100%). *Brachygobius jennie* sp. nov. is closely related to *B. kabiliensis* (BS = 82%) although this latter species does not form a monophyletic group.

Brachygobius jennie sp. nov. genetically diverges from *B. kabiliensis* by 10.25% and from all other species of *Brachygobius* by 12.72–15.49% (Table 3). These values are well above the COI-based standard interspecific threshold of 2–3% (for example, see April et al. 2011). The intraspecific genetic distance among the three newly determined haplotypes of *Brachygobius jennie* sp. nov. is 0.00% (they are identical) indicating a large gap between intra- and interspecific differentiation.

Discussion

Based on combined morphological and molecular evidence, we recognize the first reported *Brachygobius* species from China as new and describe it herein as *Brachygobius jennie*.

Brachygobius jennie is classified within the genus *Brachygobius* based on the following combination of diagnostic morphological characteristics (Larson 2001; Hoese et al. 2024): a small (SL < 25 mm), robust body with a distinctive banded pigmentation pattern ("bumblebee-like"); first dorsal fin VI, second dorsal and anal rays 8, all segmented, the first one unbranched; caudal fin rays 20–24, all segmented. Our COI-based phylogenetic tree (Fig. 5) further supports the placement of *B. jennie* as a distinct lineage nested within *Brachygobius*.

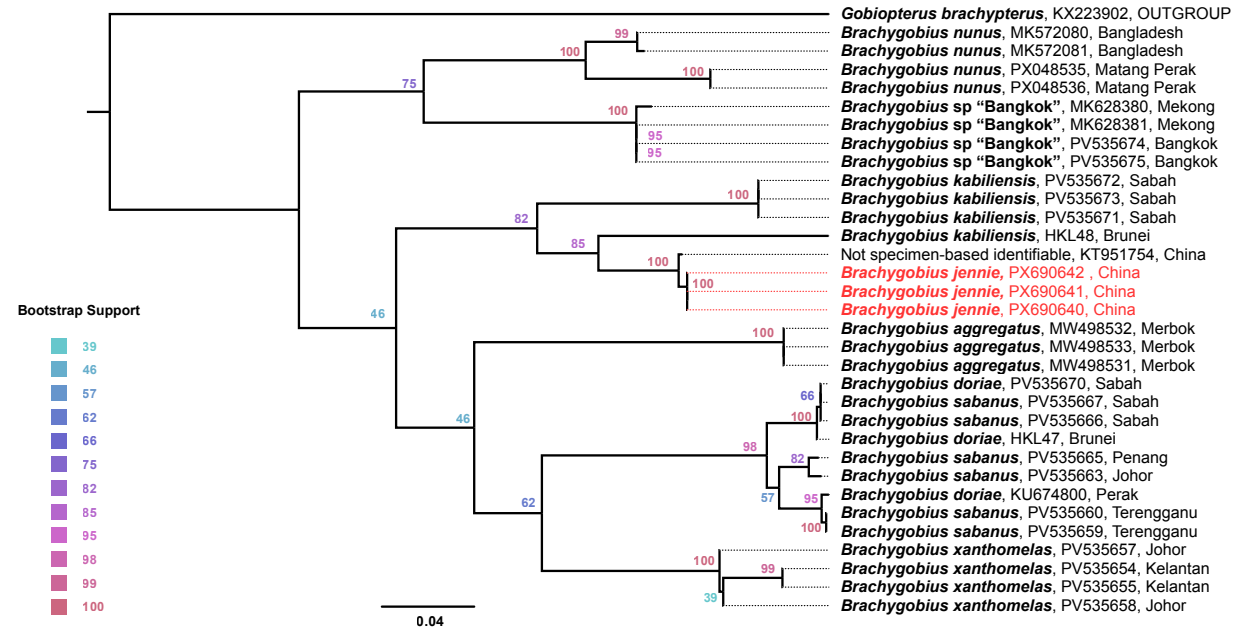

Figure 6. COI-based maximum likelihood (ML) phylogenetic tree of the genus *Brachygobius*, including *Brachygobius jennie* sp. nov. and six valid species. Branch lengths are proportional to the number of substitutions (scale bar represents 0.04 substitutions/position). Numbers at nodes are Bootstrap Supports (in %) for corresponding nodes (Bootstrap support strength is indicated by color coding). *Gobiopterus brachypterus* (GenBank sequence Accession number: KX223902) is used to root this tree. New sequences of the new species determined in this study are highlighted in red.

Table 3. Uncorrected genetic distances (in percentage) among (below diagonal) and within (diagonal, highlighted in bold) species of *Brachygobius*.

	1	2	3	4	5	6	7	8
1 *B. jennie* sp. nov.	**0.00**							
2 *B. xanthomelas*	13.86	**1.61**						
3 *B. sabanus*	12.72	11.97	**2.97**					
4 *B. doriae*	13.04	12.11	2.57	**2.63**				
5 *B. kabiliensis*	10.25	14.34	15.44	15.62	**6.14**			
6 *B. aggregatus*	15.09	12.78	14.49	14.76	15.37	**0.00**		
7 *B. nunus*	14.39	15.45	15.99	15.74	16.78	15.81	**3.96**	
8 *B.* sp. "Bangkok"	15.49	14.94	16.80	17.03	16.65	15.99	12.08	**0.33**

Within *Brachygobius*, the pigmentation pattern and body size of *B. jennie* are diagnostic (Figs 2–4). *Brachygobius jennie* can be readily distinguished from *B. xanthozona*, *B. doriae*, *B. sabanus*, and *B. xanthomelas* by the presence of two black bars posterior to the anal fin origin (vs only one in these other species) (Fig. 3) (Inger 1958; Hoese et al. 2024).

Morphologically, *B. jennie* most closely resembles *B. kabiliensis* a species restricted to Borneo Island (Inger 1958; Yusri et al. 2025). However, *B. jennie* differs from *B. kabiliensis*, and from *B. nunus* in that the second post-cephalic band reaches the midventral line (vs not reaching it in these two species) (Fig. 3). It also differs from *B. kabiliensis* and *B. nunus* by COI-based genetic distances > 10%.

Brachygobius jennie can be distinguished from *B. xanthozona* by a low number of longitudinal scale rows (24 in *Brachygobius jennie* vs 36 or more in *B. xanthozona*) and by its number of body bars (four in *B. jennie* vs three in *B. xanthozona*) (Larson 2001; Hoese et al. 2024).

Brachygobius jennie and *B. aggregatus* differ from each other in the width of the first black bar behind the head (Fig. 3). This bar is markedly broad in *B. aggregatus*, covering a large part of the predorsal region (Herre, 1940; Hoese et al. 2024; Yusri et al. 2025), but significantly narrower in *B. jennie*. The two species also show substantial molecular divergence, with a COI-based genetic distance of 14.3%.

Brachygobius mekongensis occurs throughout the lower and central Mekong basin in Southeast Asia (Larson and Vidthayanon 2000; Morioka and Sano 2009; Hoese et al. 2024). This species differs from *B. jennie* by having five narrow, often faintly defined or broken blackish to greyish bars (vs only four but always distinct black bars in *B. jennie*), and by the absence of predorsal scales (vs present in the new species) (Larson and Vidthayanon 2000).

In the phylogenetic tree (Fig. 6), *B. jennie* is resolved as the sister species of *B. kabiliensis*, consistent with their similar external morphology, but the two species differ

clearly in body size and in the shape of transverse body bars (Fig. 4). All remaining congeners form discrete, species-specific lineages with high bootstrap support and long branch lengths, except *B. doriae* and *B. sabanus*, which exhibit limited phylogenetic resolution. Interspecific COI divergences between *B. jennie* and other *Brachygobius* species (10.2–15.4%) are higher than typical values reported for gobioid fishes and corroborate the distinctiveness of the new species. Thus, concordant molecular and morphological evidence supports the recognition of *B. jennie* as a valid and evolutionarily distinct species within *Brachygobius*.

A distinctive and remarkable feature of *B. jennie* is its extremely small size. We examined more than 100 specimens, and none exceeded 9 mm SL. With a standard length of less than 9 mm, *B. jennie* is the smallest species of *Brachygobius* described so far. All other species in the genus are reported to reach at least 18.0 mm SL (Hoese et al. 2024).

The speciose suborder Gobioidei, which includes *Brachygobius*, is known to contain a few other miniature species with similarly tiny maximum sizes, such as *Schindleria brevipinguis* (Watson & Walker, 2004) and *Trimmatom nanus* (Winterbottom & Emery, 1981), both reaching a maximum of 10 mm SL, and *Pandaka pygmaea* (Herre, 1927), which grows up to only 11 mm SL (Froese and Pauly 2025). These species are usually reproductively mature at even smaller sizes. The maturity size of *B. jennie* is not yet known.

Outside Gobioidei, a few other fishes also exhibit extreme miniaturization, such as the parasitic males of the deep-sea family Linophrynidae (< 8 mm SL) (Froese and Pauly 2025).

Miniature vertebrate species are far more than biological curiosities. Their extreme size reduction often reflects profound changes in morphology, physiology, life history, and ecology (Hanken and Wake 1993). As such, they provide valuable insights into the developmental mechanisms and constraints that shape vertebrate form, the physiological trade-offs associated with miniaturization, and the ecological strategies that enable persistence at extremely small body sizes. Moreover, miniature taxa offer unique perspectives on evolutionary processes, including morphological simplification, paedomorphosis, genome size reduction, and niche specialization. Therefore, given its extremely small body size, *B. jennie* may represent a valuable model organism for investigating the evolutionary and developmental consequences of miniaturization in vertebrates.

Comparative material

Brachygobius sabanus: • USMFC (34) 00110, 22 specimens, 19.5–25.5 mm SL, Setiu, Terengganu, Malaysia (5.67°N, 102.71°E), 25 July 2023; *Brachygobius aggregatus*: • USMFC (34) 00020, 3 specimens, 14.0–15.5 mm SL, Semeling Bridge, Merbok, Kedah, Malaysia (5.68°N, 100.47°E), 19 April 2019; *Brachygobius nunus*: • USMFC (113) 00242, 9 specimens, 12.5–15.8 mm SL, Matang Mangrove Forest, Perak, Malaysia (4.843°N, 100.633°E), 29 March 2025; *Brachygobius kabiliensis*: • USMFC (113) 00274, 4 specimens, 11.9–13.8 mm SL, Kampung Kawang, Sabah, Malaysia (5.77°N, 116.00°E); *Brachygobius xanthomelas*: • USMFC (34) 00107, 4 specimens, 14.5–21.0 mm SL; Machang, Kelantan, Malaysia (5.77°N, 102.27°E), 16 September 2022.

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Supplementary material 1

Micro-CT image of the holotype showing overall skeletal morphology

Authors: Jiangyan Tian, Jianyong Wu, Chunliu Lan, Sébastien Lavoué, Jianrong Huang

Data type: png

Explanation note: The scan clearly illustrates the cranial structure, vertebral column, and fin-supporting elements, providing detailed osteological information for this species.

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Supplementary material 2

Morphological comparison of *Brachygobius jennie* sp. nov. with the genetically closest species

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Data type: docx

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