



Microdous hanlini (Gobiiformes: Odontobutidae), a new species of the fin-toothed sleepers from Guangxi, China

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Abstracts

Microdous (Gobiiformes: Odontobutidae) is a genus of freshwater sleepers distributed in southern China and Vietnam. There are two described species in this genus, *M. chalmersi* widely distributed in Hainan Island and Guangxi province of China and *M. amblyrhynchos* with a very restricted distribution range in Baise, Guangxi. It has been reported that *M. chalmersi* of Guangxi might be a cryptic species, which is different from *M. chalmersi* of Hainan. Here, we describe the cryptic species from Guangxi as a new species, *Microdous hanlini*, and compare it with the other species of *Microdous*. *Microdous hanlini* can be distinguished from *M. chalmersi* by its wider head (head width/head length = 0.51–0.58 vs. 0.48–0.53 in *M. chalmersi*) and wider interorbital width (interorbital width/head length = 0.15–0.19 vs. 0.09–0.13 in *M. chalmersi*). *Microdous hanlini* can be distinguished from *M. amblyrhynchos* by its longer snout, (snout length/head length = 0.29–0.35 vs. 0.26–0.28 in *M. amblyrhynchos*); slenderer head (head width/head length = 0.51–0.58 vs. 0.61–0.65); and protruding eyes (vs. not protruding). Phylogenetics analyses based on partial sequence of cytochrome c oxidase subunit I (COI) gene (~1500 bp) showed that *M. hanlini*, *M. chalmersi* and *M. amblyrhynchos* formed reciprocal monophyletic clades and *M. hanlini* is more closely related to *M. amblyrhynchos* than to *M. chalmersi*. Genetic distance between the three species is greater than the interspecific distance between some species of *Odontobutis*.

Key words: Odontobutidae, skin teeth, freshwater sleepers, taxonomy, cryptic species

Introduction

The family Odontobutidae is a small group of freshwater sleeper fishes distributed in East Asia from the Far East region of Russia to northern Laos and Vietnam, and in the Japanese archipelago. It was first established by Hoese and Gill (1993) based on three genera including *Odontobutis*, *Perccottus*, and *Micropercops*. Three other genera, *Sineleotris* (Herre, 1940), *Neodontobutis* (Chen et al. 2002) and *Terateleotris* (Shibukawa et al. 2001) were successively added to this family. Li et al. (2018) established the genus *Microdous* for *Phylipnus chalmersi* (Nichols and Pope, 1927), also named as *Sineleotris chalmersi* during their phylogenomic study of the Odontobutidae. *Microdous* is distinguished from other odontobutid genera by a combination of characters: lateral line absent (present in *Terateleotris*); barbel-like projection on sensory papilla absent (present in *Neodontobutis*); cephalic sensory canals complete (moderate in *Micropercops*, absent or reduced in *Odontobutis*); and absence of vertical bands on the sides and a dark band under eye (present in *Sineleotris*).

Subsequently, a new species, *Microdous amblyrhynchos* Hu & Li was discovered in Baise, Guangxi, which has a blunter snout and wider interorbital width than that of *M. chalmersi* (Hu, 2023). Furthermore, a population of *M.*

chalmersi of Guangxi was found divergent from a population of *M. chalmersi* of Hainan based on mitochondrial genome data, but no new species was described (Jiang et al., 2023). Recently, we collected nine specimens of “*M. chalmersi*” from Du’an Guangxi. We examined these specimens and compared them with *M. chalmersi* and *M. amblyrhynchos*. We also collected molecular data from the specimens and compared them with related species of the Odontobutidae, including representative species of *Microdous*, *Sineleotris*, *Micropercops* and *Odontobutis*. Here, we report a new species of *Microdous* based on analyses of morphometric characters and DNA barcode sequences.

Material and methods

Sampling

Nine specimens were collected from Du’an, Guangxi, China (23°55.94'N, 108°6.07'E) on March 2020 (Fig. 1). The left pectoral fins of three of them were clipped and used for molecular analysis (SOU1801015–1, SOU1801015–2, SOU1801015–3). Then all specimens were fixed in 10% formalin, and transferred to 75% ethanol to observe morphological data. All specimens were deposited in the Fish Collection of Shanghai Ocean University (SOU1801015–1~9; contact person: Dr Ya Zhang, email: zhangya@shou.edu.cn).

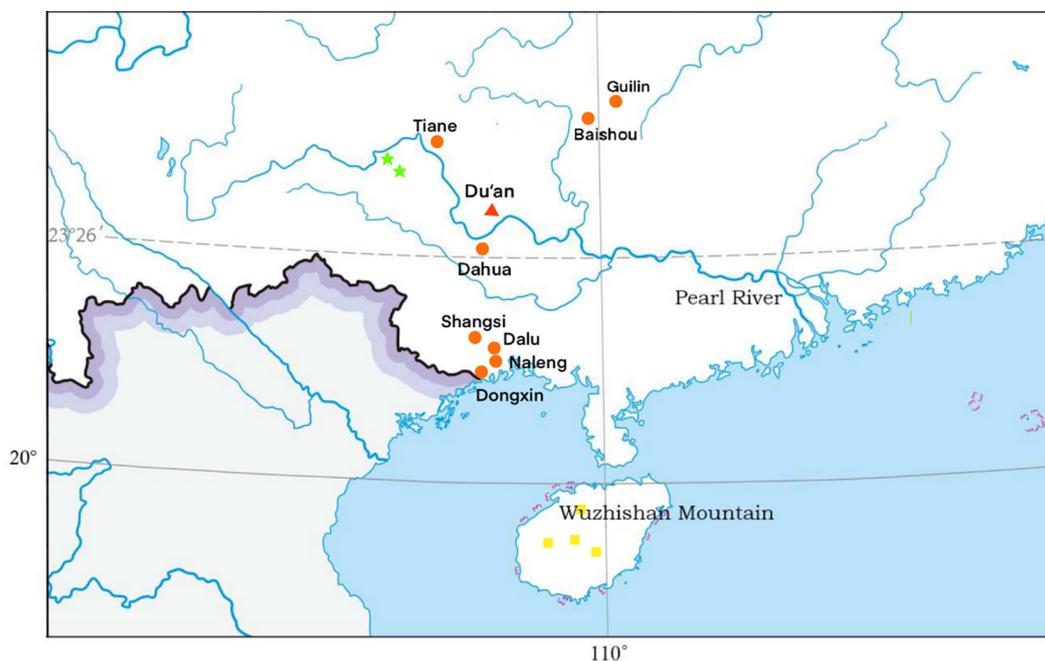


FIGURE 1. The sampling location of *Microdous hanlini* sp. nov., Du’an, indicated by the orange triangle. Additional distribution sites previously known for *M. hanlini* (orange dots; Gan et al., 2017; Zheng et al., 1981; Xiao et al., 2023; Wu et al., 1991; Wu et al., 1986.), *M. chalmersi* (yellow square; Wu and Ni, 1986) and *M. amblyrhynchos* (green star; Hu et al., 2023).

Morphological examination

Thirty-two morphological characters were examined, including eight meristic counts and 24 morphometric characters. Measurements were taken with an electronic caliper to 0.1 mm precision. Morphological measurements, counts, and observation followed Wu et al. (2008). Meristic counts involved fins rays of dorsal fins (D1/D2), anal fin (A), pectoral fins (P), pelvic fins (V), caudal fin (C), lateral scales (scale series located on the lateral line), transverse scales (scale series from origin of first dorsal fin to the abdomen) and predorsal scales (scale series from head to origin of first dorsal fin). Morphometric characters include total length (TL), standard length (SL), Preanal length (PL), caudal peduncle length, caudal peduncle depth, length of the first dorsal fin base, length of the second dorsal fin base, length of anal fin base, body depth at caudal fin base, pectoral fin length, pelvic fin length, body depth at the first dorsal fin origin, body depth at the anal fin origin, body width at first dorsal fin origin, body

width at the anal fin origin, head length, maximum head width, maximum head depth, head depth on cheek (at the middle of the preopercular), snout length, postorbital head length, eye diameter, interorbital width and lower jaw length. Morphological data were also taken for specimens of *M. chalmersi* and *M. amblyrhynchos* of Ichthyological Collections, Shanghai Ocean University, including type specimens (voucher numbers: SOU1801010–1~3, SOU1801010–7~8, 70504, v8791, v9228, 832384, v8792).

A specimen from the newly collected samples (SOU1801015–9) and a specimen of *M. chalmersi* (SOU1801017) were scanned using MicroCT Skyscan 1176 (Bruker, Belgium) with 45 kV tube voltage, 0.3-degree rotation step, and 8.7 µm pixel resolution. The three-dimensional renderings were created, visualized, and manipulated in the VG Studio Max v2.1 (Heidelberg, Germany), as the previously reported (He et al., 2016).

Molecular analyses

Genomic DNA was extracted from ethanol-preserved tissue using an Ezup Column Animal Genomic DNA Purification Kit (Sangon, Shanghai, China). The extracted DNA was checked by agarose gel electrophoresis for fragment size, and their concentration was quantified with a NanoDrop 3300 Fluorospectrometer (Thermo Fisher Scientific, Wilmington, DE, U.S.A.).

Partial sequence of cytochrome c oxidase subunit I (COI) (~500 bp), covering the whole barcoding region was amplified from three specimens (SOU1801015–1, SOU1801015–2 and SOU1801015–3). COI gene was amplified using a Vazyme 2x Taq Plus Master Mix II kit (Sangon, Shanghai, China) with a forward primer, 5'-CCATTTTACCTGTGRCAATCACACG-3' and a reverse primer 5'-CAGAGCGGTTATGTRTCTGGCTTGAA-3' according to Zhou et al. (2022). The PCR protocol was the follows: denaturation at 94 °C for 5 min, 30 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 1 min, with a final extension of 7 min at 72°C (Zhou et al. 2022). The size of amplified products was checked on 1 % agarose gels. Amplified product with one clear target band was sent to Azenta for sequencing (Suzhou, Jiangsu, China).

COI sequences from one *Sineleotris saccharae* (SOU1801016_2), one *Micropercops swinhonis* (NC_021763), two *Microdous chalmersi* (OQ319988, OQ319987), one "*S. chalmersi*" (NC_045932) and three *M. amblyrhynchos* (OP536373, OP536374, OP536375; specimen number SOU1801010-1~3) were retrieved from NCBI (<https://www.ncbi.nlm.nih.gov/>, accessed on March 2023) or amplified and sequenced following the protocol as above. *Odontobutis yaluensis* (NC_027160) and *O. haifengensis* (NC_036056) were used as outgroup taxa. All sequences were aligned using MUSCLE algorithm implemented in MEGA X (Kumar et al., 2018). The "MODELS" tool in the software MEGA X was used to search for the evolutionary model that best explains the variation between these sequences. The best substitution model found was HKY+I. A maximum likelihood (ML) tree was reconstructed with 100 bootstrap replications under the best model. The resulting trees were viewed and edited using FigTree v. 1.4.4 (Rambaut, 2018). Pairwise genetic distance within and between species was calculated as the p-distance between the three *Microdous* species, and six species of *Odontobutis* (NC_036056, NC_022818, NC_027583, NC_022706, NC_010199, NC_027160) were used to examine their relationship.

Results

Microdous hanlini Wang, He & Li, sp. nov.

Holotype. SOU1801015–8, female, 112.0 mm standard length (SL). Holotype obtained from a river of Du'an (23°55.94'N, 108°6.07'E), Guangxi, China; collected by J.-H. Lan, March 2020 (Fig.2).

Paratypes. SOU1801015–7, male, 84.3 mm SL; SOU1801015–1, male, 76.4 mm SL; SOU1801015–4, female, 82.3 mm SL; SOU1801015–3, male, 60.3 mm SL. Collection data same as for the holotype.

Etymology. The species name is named after Prof. Hanlin Wu in honor of his significant contributions to the study of the gobiiform fishes of China.

Diagnosis. The species *M. hanlini* can be distinguished from *M. chalmersi* (Nichols & Pope, 1927) by the following characteristics: wider head, head width/head length = 0.51–0.58 (vs. slender head, head width/ head length = 0.48–0.53); wider interorbital width, interorbital width (bone)/head length = 0.15–0.19 (vs. slender head and narrow interorbital width, interorbital width (bone)/head length = 0.09–0.13). The species can be distinguished from *M. amblyrhynchos* by the following characteristics: snout pointed, snout length/head length ratio 0.29–0.35 (vs. blunt

snout, snout length/head length ratio 0.26–0.28); slenderer head, head width/ head length = 0.51–0.58, (vs. head width/ head length =0.61–0.65); eye large and protruding outward (vs. eye not protruding) (Fig. 3, Table 1). The species *M. hanlini* can be distinguished from *Sineleotris saccharae* by absence of dark band under eye. The species *M. hanlini* can be distinguished from *Sineleotris namxamensis* by the following characteristics: lateral scale rows 40–43 (vs. 36–39); transverse scale rows 14–16 (vs. 12–14). These distinguishing features showed no difference between male and female individuals of *M. hanlini* (Table 2).

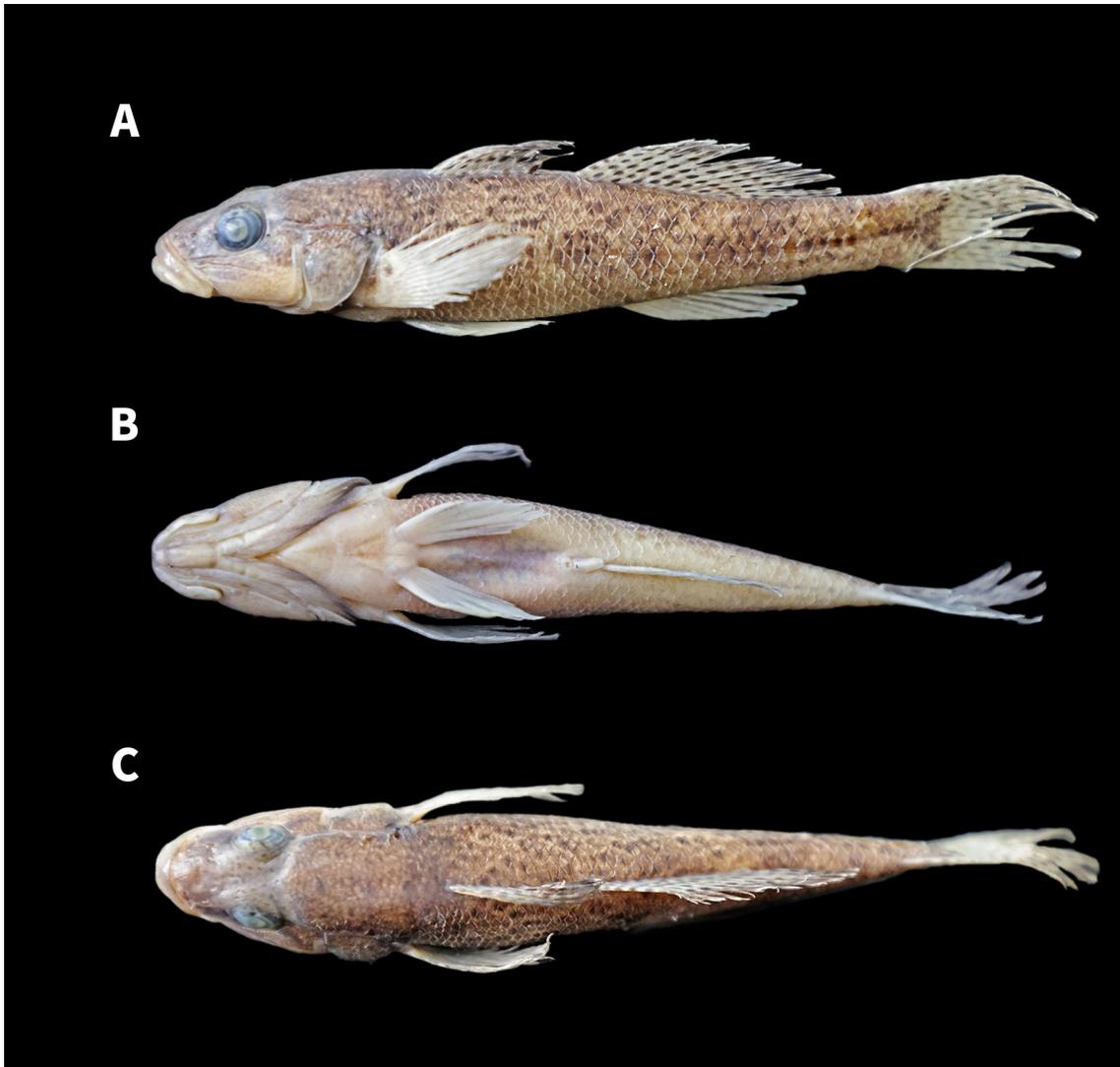


FIGURE 2. *Microdous hanlini* sp. nov., SOU1801015–8, holotype, 112.0 mm standard length, Du'an, Guangxi. **A** lateral **B** ventral **C** dorsal.

Description. First dorsal fin VIII or VIII; second dorsal fin I, 9–11; anal fin I, 7–9; pectoral fin rays 13–15; pelvic fin I, 4–5; caudal fin rays 14–16; longitudinal scale rows 40–43; transverse scale rows 14–16; predorsal scales 18–23 (Table 2).

Body stout, cylindrical front, slightly compressed posteriorly. Head medium large and blunt, height of the head equal to the width of the head. Eye large and protruding. Anterior end of the eye behind the posterior one-third of the upper jaw. Gill openings extending to under front part of eyes. Gill rakers thin and pointed. Cephalic sensory canals complete. Nostrils separated, two on each side, anterior nostril with a short tube, posterior nostril rounded, both located in front of the eye. Smooth or tiny teeth-like dermal projections in vomerine region (Fig. 4).

The origin of the first dorsal fin aligned with or slightly posterior to the origin of pelvic fin. The second dorsal fin originates before the origin of the anal fin. Caudal and pectoral fins oval. Pelvic fins separated, posterior ends not reaching anus. Lateral lines absent, body surface and opercula covered with weakly ctenoid scales. The body color of formalin-fixed specimens is grayish brown, with light-colored ventral surface, multiple dark spots on the dorsal fins, caudal fin and lateral surface of the body, and an irregular black spot on the upper base of the pectoral fins (Fig. 2).

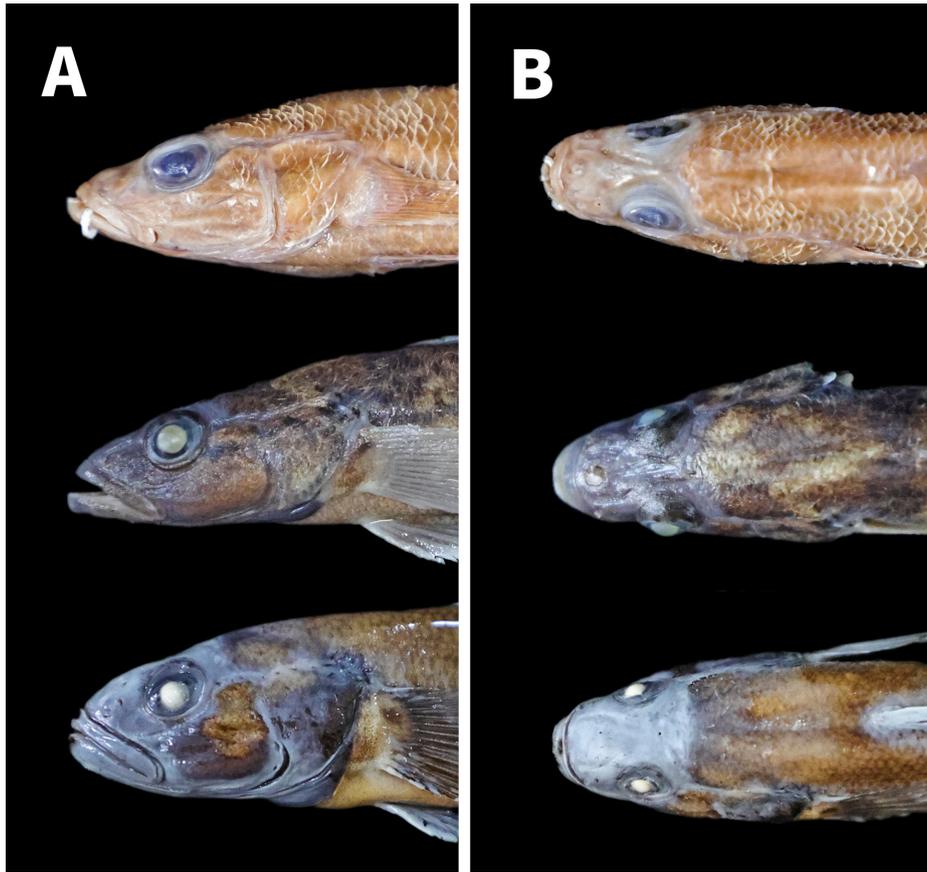


FIGURE 3. Lateral head (A) and dorsal (B) view of *Microdous chalmersi* (top, SL 83.0 mm, SOU 76V8792, Holotype), *M. hanlini* sp. nov. (middle, SL 84.3 mm, SOU1801015–7, Paratypes) and *M. amblyrhynchos* (bottom, SL 74.9 mm, SOU1801010–7, Holotype)

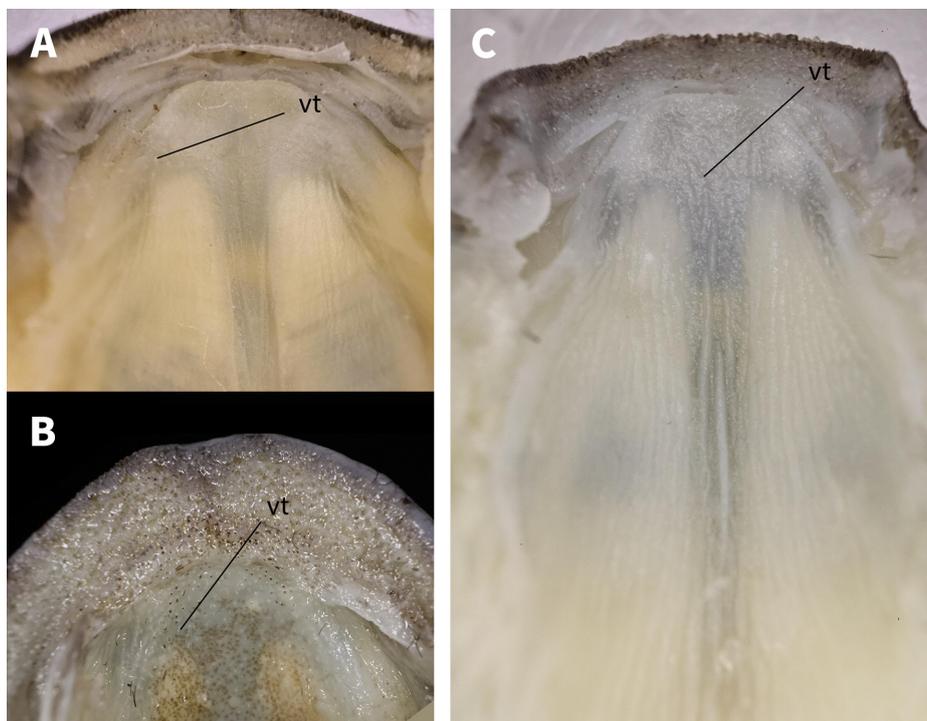


FIGURE 4. Vomerine region of **A** *Microdous hanlini* sp. nov. SOU1801015_10; **B** *M. hanlini* SOU1801015–2; **C** *M. chalmersi* SOU1801009–1. Abbreviation: vt, vomerine teeth-like dermal projections.

TABLE 1. Comparison of measurements of *Microdous hanlini* sp. nov. vs *M. chalmersi* and *M. amblyrhynchos*.

Species	<i>Microdous hanlini</i>			<i>M. chalmersi</i>			<i>M. amblyrhynchos</i>												
Specimen	SOU1801015-1	SOU1801015-2	SOU1801015-3	SOU1801015-4	SOU1801015-5	SOU1801015-6	SOU1801015-7	SOU1801015-8	70504	v8791	v9228	832384	v8792	SOU1801010-7	SOU1801010-8	SOU1801010-1	SOU1801010-2	SOU1801010-3	
Percentage of head length (%)																			
Maximum head width	52.17	51.95	57.34	55.09	58.34	56.06	51.36	58.01	48.86	49.23	53.33	48.34	49.60	60.98	62.73	64.87	57.95	62.16	
Interorbital width	14.75	18.74	17.47	16.81	16.77	18.58	14.87	15.39	8.67	11.92	13.33	12.31	9.73	26.78	26.2	27.23	26.92	26.13	
Snout length	30.97	33.15	35.27	28.8	34.09	31.40	30.75	34.24	31.45	34.17	39.59	31.07	33.30	26.39	27.33	27.32	27.78	27.03	
Percentage of head width (%)																			
Interorbital width	53.28	49.78	46.45	61.97	52.99	58.76	62.25	48.15	37.93	42.39	45.03	47.75	47.16	41.97	46.46	42.03	43.93	41.77	

TABLE 2. Morphological characters of the eight specimens of *Microdous hanlini* sp. nov.

Characters	SOU1801015-1 female	SOU1801015-2 male	SOU1801015-3 female	SOU1801015-4 male	SOU1801015-5 female	SOU1801015-6 male	SOU1801015-7 female	SOU1801015-8 male
Counts								
Dorsal fins	VIII, I-9	VIII, I-9	VIII, I-9	VIII, I-9	VIII, I-9	VIII, I-9	VIII, I-9	VIII, I-11
Anal fin	I-8	I-7	I-8	I-7	I-8	I-8	I-8	I-9
Pectoral fin rays	13	14	14	14	14	14	14	15
Pelvic fin	I-5	I-5	I-5	I-5	I-4	I-5	I-5	I-5
Caudal fin rays	15	15	14	15	15	16	16	15
Lateral scale (rows)	41	40	42	40	41	40	40	43
Transverse scale (rows)	15	16	15	15	15	15	14	15
Predorsal scales	20	22	20	22	23	21	22	18
Measurements (mm)								
Total length	76.4	115.1	112.3	82.3	82.5	80.3	84.3	111.8
Standard length	59.3	97.9	103.4	70.7	70.8	67.0	71.0	93.8
Preanal length	37.5	60.4	60.3	39.9	42.2	40.8	43.8	53.6
Caudal peduncle length	17.9	27.5	25.7	18.2	19.3	17.6	18.2	28.2
Caudal peduncle depth	5.1	9.5	10.2	6.5	6.7	6.8	6.4	9.3
Length of first dorsal fin base	7.37	12.3	12.13	8.25	9.1	9.53	9.57	13.34
Length of second dorsal fin base	9.5	18.1	17.7	11.6	10.8	11.6	12.1	18.5
Length of anal fin base	6.2	10.7	11.2	8.7	7.7	7.8	7.7	12.1
Body depth at caudal fin base	5.8	10.3	9.00	8.2	6.6	7.2	7.2	10.3
Pectoral fin length	11.7	23.3	22.2	15.7	14.0	14.3	16.8	21.9
Pelvic fin length	12.9	17.2	19.3	12.0	13.5	11.4	12.7	16.3
Body depth at first dorsal fin origin	11.4	18.0	17.8	12.6	13.6	11.9	13.9	16.4
Body depth at anal fin origin	8.2	15.7	16.9	10.8	9.4	10.4	11.2	15.4
Body width at first dorsal fin origin	8.6	14.5	14.7	10.1	10.6	9.3	10.5	14.8
Body width at anal fin origin	4.5	10.2	9.7	6.7	7.7	7.6	6.1	11.5
Head length	19.9	35.7	33.9	22.7	22.1	21.4	24.6	29.8
Postorbital head length	10.1	19.6	16.1	11.8	10.8	10.8	11.5	13.5
Maximum head width	10.4	18.5	19.5	12.5	12.9	12.0	12.6	17.3
Maximum head depth	10.2	17.5	18.9	13.5	11.6	11.6	12.6	17.5
Head depth at preopercle	7.9	14.8	15.3	10.9	8.8	8.9	10.2	14.5
Snout length	6.2	11.8	12.0	6.5	7.5	6.7	7.6	10.2
Eye diameter	5.5	6.7	6.6	5.0	5.5	4.6	5.1	6.6
Interorbital width	2.9	6.7	5.9	3.8	3.7	4.0	3.7	4.6
Lower jaw length	7.1	11.4	10.2	7.7	7.2	7.3	7.9	9.1

Coloration in life. Head brown with black dots on cheek. Body side dark brown with irregular black spots. Abdomen dark. Fins dim and transparent, with banded black spots. An irregular black fleck on upper part of base of the pectoral fins.

Coloration preserved. Body grayish brown, with light-colored ventral surface, multiple dark spots on the dorsal fins, caudal fin and lateral surface of the body, and an irregular black spot above the base of the pectoral fin. Ventral side of abdomen light brown. Head brown, cheek light brown, a horizontal black band crossing the eyes. Urogenital papilla whitish, with sparse black pigment.

Sexual dimorphism. Urogenital papilla elongates with a wide base, tapering and with a narrow tip. No distinct opening in male; clear ovipore in female (Fig. 5).

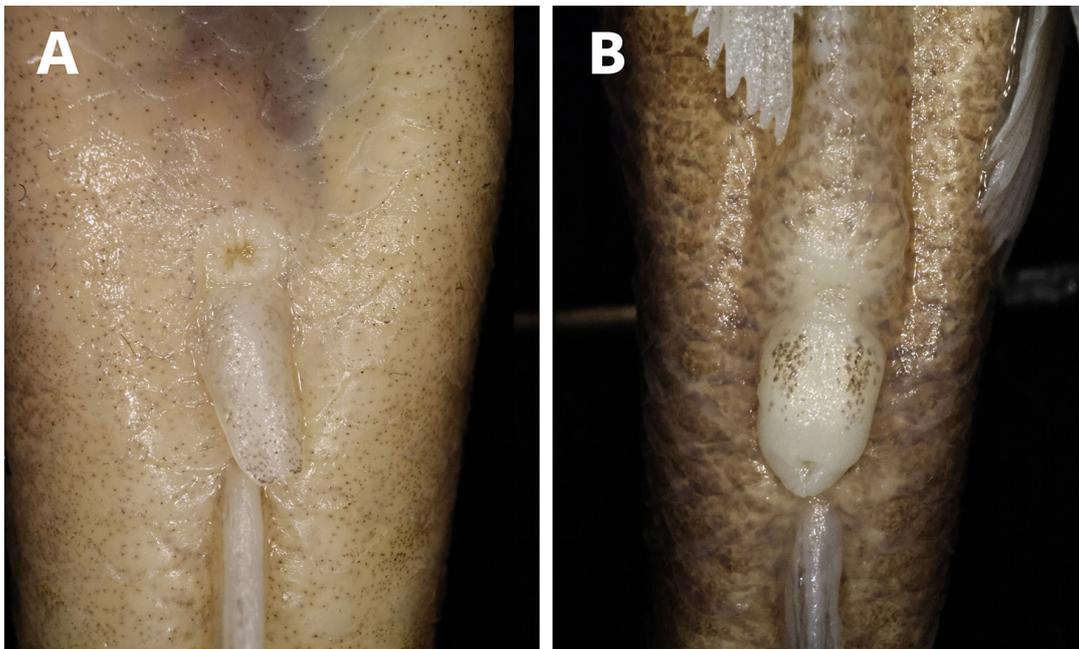


FIGURE 5. Urogenital papilla of *Microdous hanlini* sp. nov. SOU1801015–8 (A, male) and SOU1801015–7 (B, female).

Cephalic sensory canal system. Oculoscapular canal with three preorbita (anterior interorbital) pores A', B and C, single interorbital pore D, single postorbital (posterior interorbital) pore E, and seven pores F to L extending posteriorly. Preopercular canal with five pores M to Q (Fig. 6).

Cephalic sensory papillae. Neuromasts numerous, small and densely set in mostly longitudinally arranged rows (Fig. 6).

Head skeleton. The skeletons of *M. hanlini* and *M. chalmersi* were investigated using X-ray microtomography (Fig. 7). A pair of the frontal bones constitutes the majority of the skull roof. The frontal includes a narrow anterior supraorbital section and a widened postorbital section (or posterior section). The ratio of the width of anterior section to postorbital section is much smaller in *M. hanlini* than that of *M. chalmersi*.

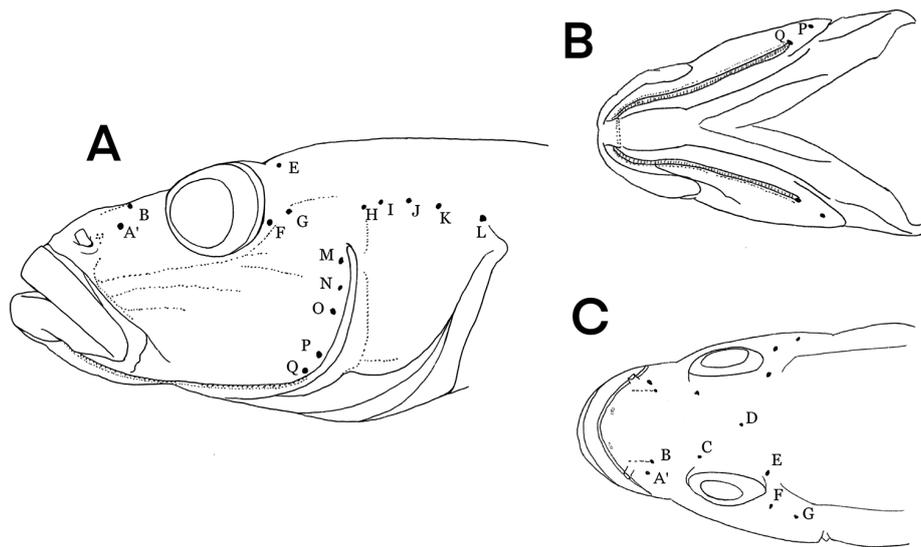


FIGURE 6. Patterns of main cephalic sensory pores (black spots) and papilla lines (rows of dots) of *Microdous hanlini* sp. nov. in lateral (A); ventral (B) and dorsal (C) view. Preorbital pores A', B, C; interorbital pore D; postorbital pore E; pores of posterior section of oculoscapular canal F, G, H, I, J, K, L; preopercular pores Q, P, O, N, M.

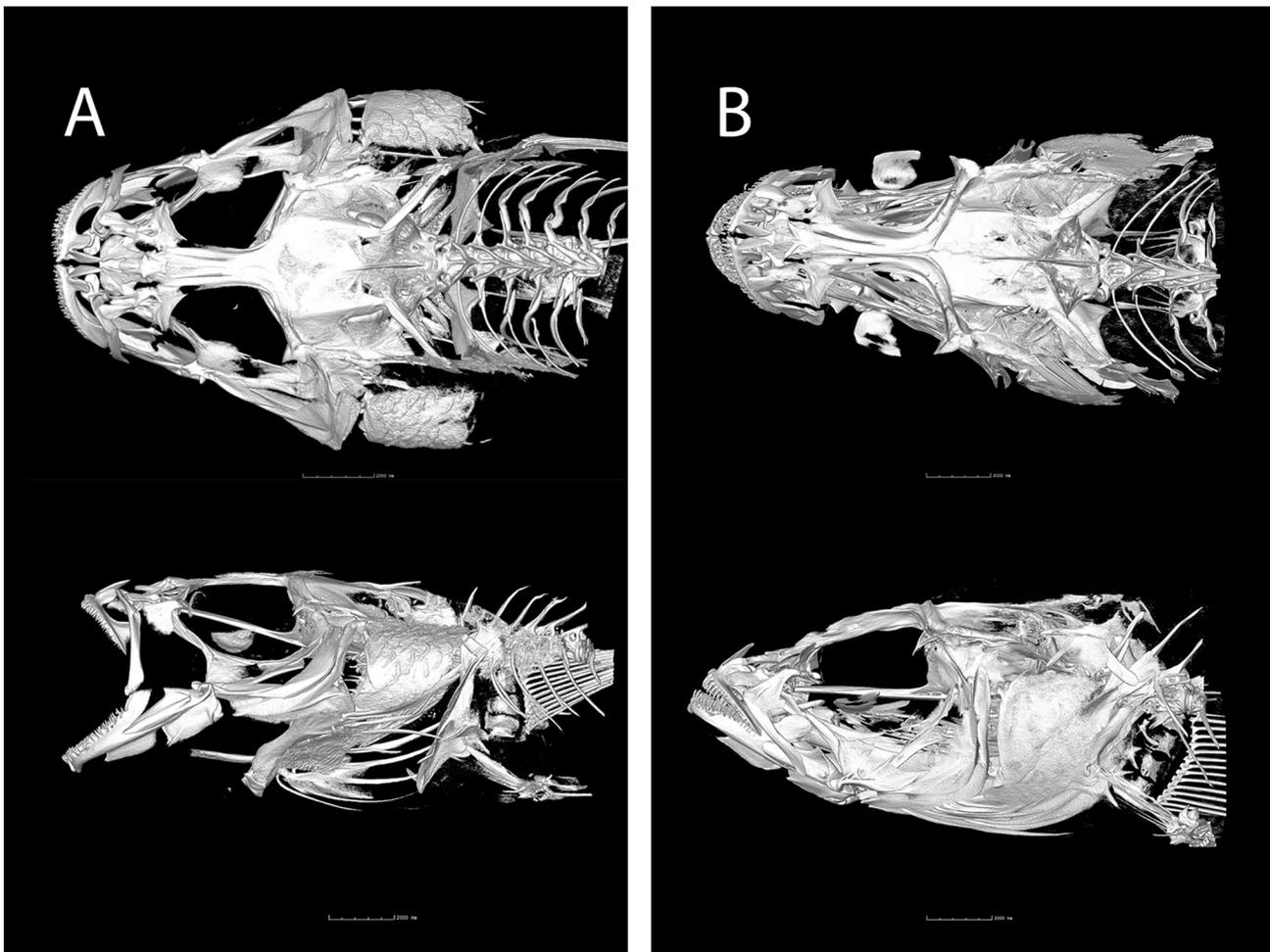


FIGURE 7. Head skeleton of *Microdous hanlini* sp. nov. (SOU1801015–9) (A) and *M. chalmersi* (SOU1801017) (B) based on microCT data.

COI analyses and genetic distance. The COI sequence of three paratype specimens were lodged in GenBank with accession number PP481989-PP481991, and the *Sineleotris saccharae* as OQ382855. The results showed that the three specimens of *M. hanlini* (SOU1801015-1~3) and the “*Sineleotris chalmersi*” sequence from Guangxi formed a monophyletic clade with a bootstrap value of 100 (Fig. 8), suggesting that the “*Sineleotris chalmersi*” was erroneously named for *M. chalmersi*, but is actually a sample of *M. hanlini* (Wang et al., 2019). *Microdous hanlini* then formed a closer sister relationship to *M. amblyrhynchos* than to *M. chalmersi* (Fig. 8).

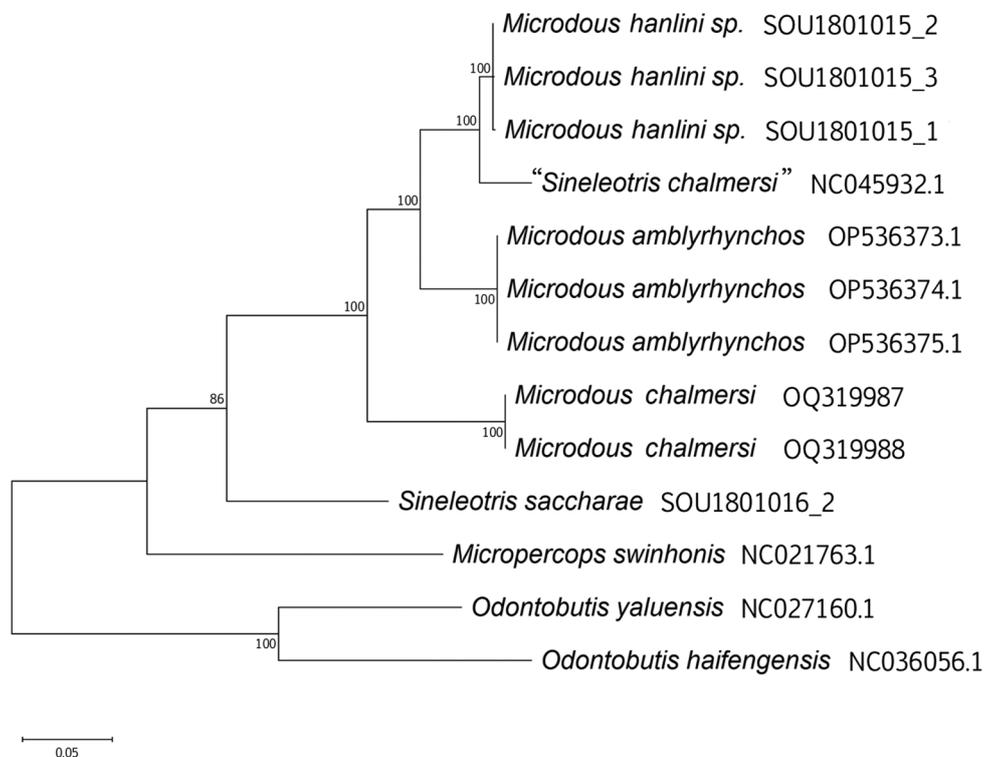


FIGURE 8. Maximum-likelihood (ML) tree of *Microdous hanlini* sp. nov. and other species of the Odontobutidae, *O. yaluensis* and *O. haifengensis* as outgroups. The numbers by the nodes represent the bootstrap support values.

The intraspecific genetic distance of *M. hanlini* was 0.00065~0.03361, which is much smaller than that between *M. hanlini* and *M. chalmersi* from Hainan (0.10759~0.11125) (Table 3). The average genetic distance between *M. hanlini* and *M. amblyrhynchos* is 0.07283 (0.06878~0.08361) (Table 3). The average genetic distance between *M. hanlini* and *M. chalmersi* is similar or greater than the genetic distance between some species of *Odontobutis* (Table 4).

Distribution. Specimens of *M. hanlini* examined in this study were collected from Du’an, Guangxi province (Fig. 1). Other documented collection records of “*M. chalmersi*”, “*Sineleotris chalmersi*” or “*Perccottus chalmersi*” in Guangxi include nearby regions of the Liu River, the Gui River, the Zuojiang River, the Youjiang River, the Hongshui River and the Beilun River (Gan et al., 2017). The detailed distribution range of *M. hanlini* requires further exploration.

Biology. According to the measurements of all specimens obtained in this research, the maximum size of *M. hanlini* collected was 115.1 mm in standard length. A smaller *M. hanlini* was found in the stomach after dissecting one specimen of *M. hanlini*, suggesting that *M. hanlini* is carnivorous.

Habitat. *Microdous hanlini* was collected from a rapid-flowing spring, which has rubble bottom substrate with a vegetation-covered bank. Water depth 20–40 cm (Fig. 9).



FIGURE 9. Habitat of *Microdous hanlini* sp. nov.. An unnamed stream of Du'an (23°55.94'N, 108°6.07'E), Guangxi, China.

TABLE 3. Genetic distance between *Microdous hanlini* sp. nov. (SOU1801015-1~3, NC_045932.1), *M. chalmersi* (OQ319988, OQ319987), and *M. amblyrhynchos* (SOU1801010-1~3, GenBank number OP536373.1~ OP536375.1).

Species	<i>M. hanlini</i>			<i>M. chalmersi</i>		<i>M. amblyrhynchos</i>			
	SOU1801015-1	SOU1801015-2	SOU1801015-3	NC_045932.1	OQ319987	OQ319988	OP536373.1	OP536374.1	OP536375.1
SOU1801015-1									
SOU1801015-2	0.00130								
SOU1801015-3	0.00196	0.00065							
NC_045932.1	0.03500	0.03355	0.03362						
OQ319987	0.10927	0.10759	0.10773	0.11125					
OQ319988	0.10927	0.10759	0.10773	0.11125	0.00000				
OP536373.1	0.07013	0.06878	0.06878	0.08361	0.10722	0.10722			
OP536374.1	0.07013	0.06878	0.06878	0.08361	0.10722	0.10722	0.00000		
OP536375.1	0.07013	0.06878	0.06878	0.08361	0.10722	0.10722	0.00000	0.00000	

TABLE 4. Genetic distance between six species of *Odontobutis*.

	<i>O. haifengensis</i>	<i>O. sinensis</i>	<i>O. interrupta</i>	<i>O. potamophilus</i>	<i>O. platycephala</i>	<i>O. yaluensis</i>
<i>O. haifengensis</i>						
<i>O. sinensis</i>	0.1442					
<i>O. interrupta</i>	0.1554	0.1370				
<i>O. potamophilus</i>	0.1527	0.1286	0.0393			
<i>O. platycephala</i>	0.1501	0.1331	0.1288	0.1351		
<i>O. yaluensis</i>	0.1521	0.1292	0.0747	0.0759	0.1281	

Comparative material. All *M. chalmersi* and *M. amblyrhynchos* specimens examined for morphological comparison were loaned from the Fish Collection of the Shanghai Ocean University, China with the registration tags 76V8791, 76V8792, 76V9228 from Qiongzong, Hainan, May 1976; HN832384 from Changjiang, Hainan, May 1983, and 070504 from Wuzhishan, Hainan, May 1983; SOU1801009–1, from Wuzhishan, Hainan, August 2018; SOU1801010 (1~3, 7~8) from Baise City, Guangxi.

Discussion

Microdous chalmersi was described as *Philypnus chalmersi* (Nichols and Pope, 1927) originally from Hainan Island of southern China. However, *Philypnus* is an obsolete genus name established for the eleotrid *Gobiomorus dormitor* (Jordan and Gilbert, 1882) in the Western Hemisphere. *Philypnus chalmersi* has been shown genetically far apart from *G. dormitor* and nested within the Odontobutidae, so the name of *P. chalmersi* has been corrected to *M. chalmersi* (Li et al., 2018). Traditionally, *Microdous chalmersi* was thought to be distributed in Hainan Island and the Pearl River basin of China and northern Vietnam (Wu and Ni, 1986). Recent analysis of mitochondrial genome sequences suggested a potential cryptic species (Jiang et al., 2023), which has been confirmed by thorough examination of the newly collected specimens in this study. Wang et al. (2019) reported a complete mitochondrial genome for *Sineleotris chalmersi*. The specimen was collected from Dahua Yao Autonomous County (23°33'35.36"N, 107°20'36.06"E) in the upper Pearl River (Guangxi, China). Our phylogenetic studies including this specimen indicated that it is a specimen of *M. hanlini*.

Microdous hanlini can be distinguished from other species in the genus by: head width and interorbital width; longer snout; eye protruding outward. Phylogenetics analyses showed that *M. hanlini* and *M. chalmersi* formed reciprocal monophyletic clades and *M. hanlini* had a closer sister relationship to *M. amblyrhynchos* than to *M. chalmersi* (Fig. 8). *Microdous hanlini* and *M. amblyrhynchos* occur closely in geological distance and probably have sympatric distribution. The mechanism of their differentiation requires further study.

Sineleotris namxamensis has no dark band under eye but a black fleck at the base of pectoral fin (Chen & Kottelat 2004), which is consistent with the species of *Microdous* but not *Sineleotris*. Therefore *S. namxamensis* may be a species of *Microdous*. More samples should be collected from Lao PRD and Vietnam to compare using both morphological characters and molecular data.

Ethical approval

All animal procedures performed in this research were done in accordance with the “Ethical Standards of the Shanghai Ocean University (2020)”.

Competing interests

The authors declare no competing interests.

Author contributions

F. Wang and C. Li conceived and designed the study. M. Wei collected the specimens and analyzed morphological data. Y. He performed the CT-scan. F. Wang performed the molecular experiments, conducted morphological measurements, analyzed the data, and prepared the manuscript. All authors edited and approved the final version of the manuscript.

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