

Neodontobutis lani, a new sleeper fish of the family Odontobutidae (Teleostei: Gobiiformes) from Guangxi, southern China

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Abstract

A new species, *Neodontobutis lani* (Odontobutidae) is described from the Zuojiang River, a tributary of the Xijiang River of the Pearl River basin, at Longzhou Town, Guangxi Zhuang Autonomous Region, Southern China. This species can be distinguished from other *Neodontobutis* species by following characters: anterior head flat, with interorbital width / eye diameter = 1.4–1.9 (vs. less than 1.4); several rows (vs. single row) of transforming ctenii on posterior edges of body scales; sensory papilla on lower jaw arranged in two oblong clusters (vs. two single lines). It can be distinguished from *Odontobutis* species by: separated right and left gill membrane (vs. joined); barbel-like projection present on sensory papillae. Molecular phylogenetic analysis of 2,076 nuclear coding loci indicates that *N. lani* is a sister species of *N. hainanensis*, the only *Neodontobutis* species that has been described from China.

Key words: *Neodontobutis*, freshwater sleepers, the Xijiang River

Introduction

The Odontobutidae is an early lineage of the Gobiiformes endemic to eastern and southeastern Asia, and includes six genera and approximately 15 species (Iwata, 2011; H. Li, He, Jiang, Liu, & Li, 2018). This family can be distinguished from other Gobiiformes by the following combination of characters: (1) separated pelvic fins; (2) scapula well developed, inserted between cleithrum and proximal radials; (3) longitudinally distributed lines of cephalic sensory papillae (Hoese & Gill, 1993; Iwata, 2011; H. Li *et al.*, 2018). According to molecular phylogenetic analyses, a monophyletic clade of the Odontobutidae comprises *Odontobutis* (8 species, distributed in China, Japan and the Korean Peninsula), *Perccottus* (1 species, distributed in northeastern China, the Far East part of Russia and the Korean Peninsula) and *Neodontobutis* (2 species and several controversial ones, distributed in southern China and the Indo-China Peninsula, see Table 1) (Iwata, 2011; Wu, 2008). These species share rather conserved morphology, including a large head, stout body, wide rounded pectoral fins and obscure, mottled coloration. They are typically small, benthic, ambush predators living in rivers, streams, lakes and ponds (Iwata, 2011).

Identification of genera and species of the Odontobutidae are usually based on external characteristics such as presence of cephalic sensory canals, shape and distribution pattern of cephalic sensory papilla, head shape, gill membrane and body squamation (Iwata, 2011; Wu, 2008). In this article, a new species of the Odontobutidae was identified and described using this same combination of characters. The new species was also included in molecular phylogenetic analyses of mitochondrial *cytochrome c oxidase subunit I (cox1)* and of 2,076 nuclear loci to evaluate its placement among close relatives.

TABLE 1. List of valid *Neodontobutis* species according to Iwata (2011) and controversial species which were currently placed in genus *Neodontobutis* according to Fishbase Website.

Species	Distribution	References
Valid		
<i>Neodontobutis hainanensis</i> (Chen, 1985) (Type species)	Southern China, Pearl River basin and Hainan Island.	(Chen <i>et al.</i> , 2002; Iwata, 2011; Wu, 2008)
<i>N. aurarmus</i> (Vidthayanon, 1995)	Northeastern Thailand and central Laos, Mekong basin.	(Iwata, 2011; Vidthayanon, 1995)
Controversial		
<i>N. tonkinensis</i> (Mai, 1978)	Northern Vietnam and Laos.	(Kottelat, 2001a, 2001b)
<i>N. ngheanensis</i> X. K. Nguyen et D. H. Nguyen, 2011	Vietnam, Lam river basin, Khe Khang stream.	(Khoa & Duc, 2012)
<i>N. macroptoralis</i> (Mai, 1978) syn. <i>Microperops macroptoralis</i> Mai, 1978	Vietnam	(Kottelat, 2001b)

Material and methods

Specimens were collected from a local market in Longzhou Town, Chongzuo City, Guangxi Zhuang Autonomous Region (Figure 1). According to the collector, they were caught from a local stream. Fresh specimens (Figure 2) were temporarily preserved in 95% ethanol. The right pectoral fin of each specimen was preserved in ethanol as a tissue sample for molecular analysis. Specimens were fixed in 10% formaldehyde, then preserved in 75% ethanol for morphological research. Type specimens are stored in the Ichthyology Collection, Shanghai Ocean University, Shanghai, China. More than 20 specimens had been obtained, but most of them were distorted due to dehydration. Therefore, only three relatively well-preserved specimens (holotype and two paratypes) were used to collect most morphological measurements and counts. Six specimens including type specimens were used in the molecular analysis. A total of 12 specimens including type specimens were used to calculate interorbital width / eye diameter in order to examine whether the head of this species flatter than its congeners. Collection numbers of specimens of the new species are as follows: 25911 (= SOU1801007-1), 25912, 25913 (= SOU1801007-3), 25914, 25915 (= SOU1801007-5), 25916, 259120, 259121, 259122, 259123, 259124, 259125.

Methods of morphological measurements, counts and description were performed according to Wu *et al.*, (2008). Measurements were taken using calipers (precision to 0.01mm). Distribution patterns of cephalic sensory organs were observed using a stereomicroscope. Characteristics of scales were observed under a microscope. Three non-type specimens were dissected for counting the number of gill rakers and vertebrae.

Genomic DNA was extracted using an Ezup Column Animal Genomic DNA Purification Kit (Sangon, Shanghai, China). Partial sequences (~1500 bp) of *cytochrome c oxidase subunit I (cox1)* gene were amplified from six individuals of the new species, including the holotype and paratypes, as well as several comparative specimens using Vazyme 2x Taq Plus Master Mix II (Sangon, Shanghai) with a forward primer, CCATTTTACCTGTGRCAATCACACG, and a reverse primer CAGAGCGGTTATGTRTCTGGCTTGAA, following the thermal cycle: initial denaturation at 98 °C for 2 min, 16 cycles of denaturation at 95 °C for 30 s, primer annealing at 58.8 °C for 40 s and extension at 72 °C for 1 min, then 16 additional cycles but with 56.8 °C as the annealing temperature; the final extension was 5 min at 72 °C. The amplified products were evaluated on 2 % agarose gels and sent to Genwiz (Suzhou, Jiangsu, China) for sequencing. Sequences of additional specimens were retrieved from the National Center for Biotechnology Information (NCBI) database (Table 1). A maximum likelihood *COI* tree including all genera of Odontobutidae except for *Terateleotris* was constructed using MEGA10, choosing the Tamura-Nei model with 1000 bootstrap replications (Kumar, Stecher, Li, Knyaz, & Tamura, 2018). The resulting tree was visualized in FigTree v.1.4.2 (Rambaut, 2013).

A cross-species gene capture method was used to collect data from nuclear loci (C. Li, Hofreiter, Straube, Corrigan, & Naylor, 2013). Library preparation for target gene capture process was performed following Meyer and Kircher (2010) with modifications to accommodate capturing target gene of divergent species (C. Li *et al.*, 2013).

Around 100–300 ng genome DNA was sheared to about 250 bp for NGS library preparation. To improve efficiency, specific short sequenced called “inline indexes” were designed on the 3’ end of both P5 and P7 adapters (Wang, Yuan, Huang, & Li, 2022), so individuals of the same species can be pooled for the gene capture process, and then reads of each individual can be identified by the inline index after sequencing. Gene capture protocol followed Li *et al.* (2013). A set of gobioid-specific capture probes comprising 4,434 target loci were used to enrich target loci (H. Li *et al.*, 2018). These capture probes, or “baits”, were synthesized by MYcroarray (Ann Arbor, Michigan; cat#, 150901-Li-Goby). Then samples were pooled in equimolar ratios for 2x 150 bp paired-end sequencing on an Illumina HiSeq X10 lane at Genwiz (Suzhou, Jiangsu, China).

Reads were assembled, aligned and filtered using Assexon (Yuan, Atta, Tornabene, & Li, 2019) with default parameters. Briefly, raw reads from each sample were separated according to the combination of index sequences. Reads of some other odontobutid species were retrieved from dataset of Li *et al.* (2018), and analyzed with data collected in this study. Low-quality bases and adaptor sequences were trimmed using trim_galore v0.4.1 (http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/). PCR duplicates were excluded using “-fastx_uniques” command in USEARCH v10.0.240 (Edgar, 2010). A set of 4,434 nuclear coding sequences of *Danio rerio* (Yuan *et al.*, 2019) was used as a reference species to parse reads to each gene file using UBLAST with a relaxed e-value of 1×10^{-4} . Reads of each locus were assembled into contigs using SGA (Simpson & Durbin, 2012). Contigs were then aligned to protein sequences of the reference using protein2dna model in the package of Exonerate (Slater & Birney, 2005). Orthologous genes were identified using a reciprocal blast method. The output amino acid (AA) sequences were aligned using MAFFT v7.369b (Katoh & Standley, 2013), and then the aligned AA sequences were translated back to DNA sequences afterward. Alignments with low quality were removed using filter.pl of the Assexon package (Yuan *et al.*, 2019).

A concatenated tree including representative species of all genera in the family Odontobutidae except for the genus *Terateleotris* was constructed using IQ-TREE under maximum likelihood criterion and the best partitioned scheme (Minh *et al.*, 2020). The model for each partition was selected using -MFP option and 1000 bootstrap replicates were performed. The resulting tree was rooted with *Rhyacichthys aspro* as an outgroup and visualized in FigTree v1.4.0 (Rambaut, 2013). Information of molecular samples and sequences used in the phylogenetic analysis are presented in Table 2.

Results

Neodontobutis lani, Zhou and Li, sp. nov.

Holotype. SOU1801007-1 (25911), female, 62.4 mm standard length, obtained from a local market and presumed to be collected from the Zuojiang River, a tributary of the Xijiang River of the Pearl River basin, at Longzhou Town, Chongzuo City, Guangxi, China, collected by J.H. Lan, October 2019 (Figure 3).

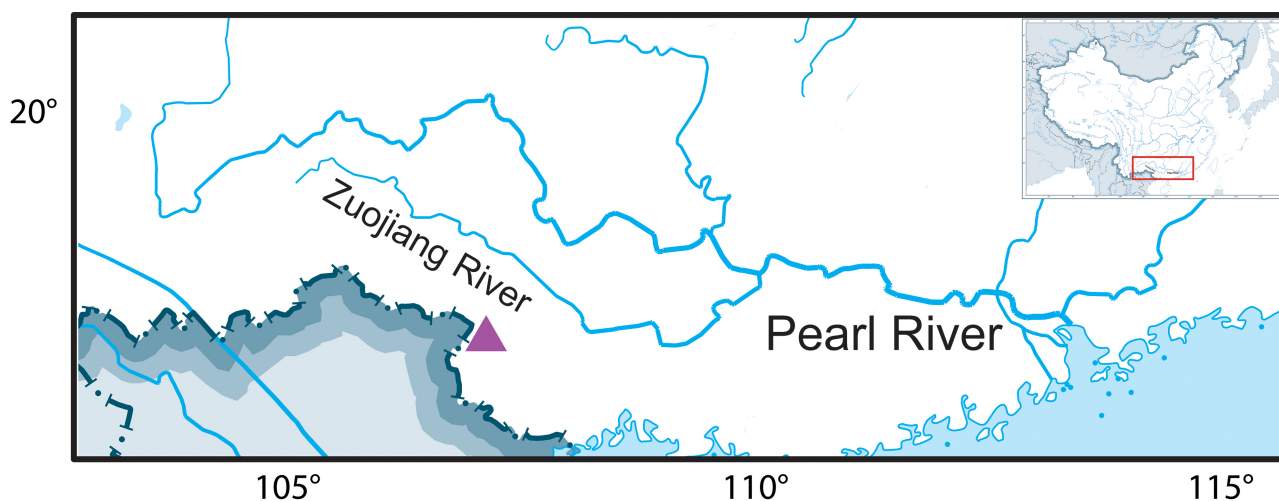


FIGURE 1. The sampling location of *Neodontobutis lani* sp. nov., indicated by the triangle. The inset shows the sampling region within the map of China.

TABLE 2. Details of comparative materials. Abbreviation of “Material type”: S = Specimen, G = Genome DNA, N = Raw reads of nuclear target gene capture dataset. M = Mitochondrial genome, for extracting *cox1* sequence.

Collection number or sequence ID	Material type	Species	Location
25902, 25904, 25905, 25461, 25462, 25464, 25465	S, G	<i>Neodontobutis hainanensis</i>	Chongzuo, Guangxi, China
25901, 25903, 25906	S	<i>N. hainanensis</i>	Chongzuo, Guangxi, China
20272, 20276, 20278, 202710, 202712	G	<i>N. hainanensis</i>	Hainan, China
25012	G	<i>Sineleotris saccharae</i>	Fangchenggang, Guangxi, China
CL1279_4	N	<i>N. hainanensis</i>	Yangjiang, Guangdong, China
CL632_1	N	<i>Perccottus glenii</i>	Harbin, Heilongjiang, China
CL808_11	N	<i>Odontobutis sinensis</i>	Guilin, Guangxi, China
CL1221_2	N	<i>O. potamophila</i>	Meicheng, Zhejiang, China
CL1275_4	N	<i>O. yaluensis</i>	Dandong, Liaoning, China
CL1224_3	N	<i>Sineleotris saccharae</i>	Huizhou, Guangdong, China
CL8_3	N	<i>Micropercops swinhonis</i>	Langxi, Anhui, China
CL_929	N	<i>Microdus chalmersi</i>	Wuzhishan, Hainan, China
CL303_1	N	<i>Rhyacichthys aspro</i>	
NC_021763.1	M	<i>Micropercops swinhonis</i>	
NC_022818.1	M	<i>Odontobutis sinensis</i>	
NC_036056.1	M	<i>O. haifengensis</i>	
NC_027583.1	M	<i>O. interrupta</i>	
NC_022706.1	M	<i>O. potamophila</i>	
NC_010199.1	M	<i>O. platycephala</i>	
NC_027160.1	M	<i>O. yaluensis</i>	
NC_045932.1	M	<i>Microdus chalmersi</i>	
NC_020350.1	M	<i>Perccottus glenii</i>	
NC_004414.1	M	<i>Rhyacichthys aspro</i>	

Paratypes. SOU1801007-3 (25913), female, 58.8 mm standard length; SOU1801007-5 (25915), male, 60.0 mm standard length. Collection locality and date same as the holotype.

Diagnosis. *Neodontobutis lani* can be distinguished from other *Neodontobutis* species by the following characteristics: anterior part of head relatively flat, with interorbital width / eye diameter = 1.4–1.9 (mean = 1.6), while the other *Neodontobutis* species possess rather compressed head and body, with mean interorbital width / eye diameter 1.0 in *N. hainanensis*, less than 1 in *N. auarumus*, 1.3 in *N. macroptoralis*, 1.2 in *N. tonkinensis*; sensory papilla on ventral tip of lower jaw form two oblong clusters (Figure 4c), whereas in other *Neodontobutis* papillae form two single lines; several rows of transforming ctenii on posterior edge of body scales (Figure 5a), whereas single row of transforming ctenii on scale of the other *Neodontobutis* (Figure 5b) (Chen, Kottelat, & Wu, 2002; Iwata, 2011; Kottelat, 2001b; Vidthayanon, 1995). This species can be distinguished from *Odontobutis* by: presence of barbel-like projection on sensory papilla (Figure 5c) (absent in *Odontobutis*), separated right and left gill membranes (joined in *Odontobutis*) and cycloid scales on predorsal region and abdomen (ctenoid scales in *Odontobutis*) (Iwata, 2011; Vidthayanon, 1995).

Description. Morphometric and meristic data for the holotype and paratypes are presented in Table 3 respectively. Dorsal fin rays VII; I, 9; anal fin rays I, 6–7; pectoral fin rays 15, pelvic fin rays I, 5; caudal fin rays 14–15; lateral scales 34–36; transverse scales 12–15; predorsal scales 18–19; gill rakers 3+6; vertebrae 28 (13+15).

Body stout, slightly compressed posteriorly. Head large, slightly flat, interorbital distance larger than eye diameter. Lower jaw slightly longer than upper. Gill membrane separated, attached to the isthmus. Cephalic sensory canals absent. Cephalic sensory papilla pattern is illustrated in Figure 4.

Dorsal spines nearly equal length except for the last shorter one, not reaching origin of the second dorsal fin.

Origin of the anal fin slightly posterior to the origin of the second dorsal fin. Pelvic fin short, close but separated. Pectoral fins and caudal fin wide, rounded. Most body scales ctenoid with several rows of transforming ctenii on the posterior edge. Predorsal scales and part of abdominal scales cycloid.

TABLE 3. Morphological characters for the type specimens of *Neodontobutis lani*.

Characters	SOU1801007-1	SOU1801007-3	SOU1801007-5
Counts			
Dorsal fins	VII;I,9	VII;I,9	VII;I,9
Pectoral fins	15	15	15
Pelvic fins	I,5	I,5	I,5
Anal fin	I,7	I,7	I,6
Caudal fin	15	15	14
Lateral scales	34	36	36
Transverse scales	12	15	13
Predorsal scales	18	19	18
Gill rakers	3+6 (counted on a non-type specimen)		
Vertebrae	13+15 (counted on a non-type specimen)		
Total Length	70.6	69.8	68.8
Standard Length	60.5	54.9	57.2
Percentages of standard length (%)			
Head length	36.7	37.7	38.1
Predorsal length	43.5	43.7	44.8
Snout – second Dorsal fin	63.3	63.8	64.0
Snout – anus	57.9	59.0	60.0
Snout – anal fin	63.6	66.3	64.3
Snout – Pelvic fins	35.5	37.3	36.2
Length of caudal peduncle	28.4	28.4	27.1
Depth of caudal peduncle	12.2	12.4	12.1
Body depth at first dorsal fin origin	20.8	22.4	21.9
Body width at anal fin origin	12.1	11.3	11.9
Length of first dorsal fin base	16.7	13.3	17.5
Length of second dorsal fin base	17.7	21.7	17.1
Length of anal fin base	14.9	16.6	12.1
Length of caudal fin base	13.4	14.0	12.8
Length of pectoral fin	18.3	17.9	19.4
Length of pelvic fin	14.5	16.8	15.9
Percentage of head length (%)			
Snout length	22.5	24.2	20.2
Postorbital length	57.2	59.9	59.6
Head depth on cheek (at the middle of the preopercular)	46.4	49.8	45.4
Maximum head depth	56.3	54.1	53.7
Maximum head width	55.0	56.5	54.6
Interorbital width	28.8	29.5	25.2
Eye diameter	18.5	17.9	17.0
Lower jaw length	29.7	36.7	34.4



FIGURE 2. *Neodontobutis lani* **sp. nov.**, a live uncatalogued individual.

Coloration: Head and body with dark brown mottling in live individuals. Several dark bars radiate from orbitals. Dark stripes on each side of body. A shiny, greenish blotch on upper side of operculum. A large dark blotch on base of caudal fin. Dorsal fins, anal fin and caudal fin mottled (Figure 2). Preserved specimens light brown, with stripes and irregular darker blotches on each side of body. Abdominal pale, slightly yellowish (Figure 3).



FIGURE 3. *Neodontobutis lani* **sp. nov.**, SOU1801007-1, holotype, 62.4 mm standard length, Longzhou Town, Guangxi. A. dorsal, B. lateral, C. ventral views.

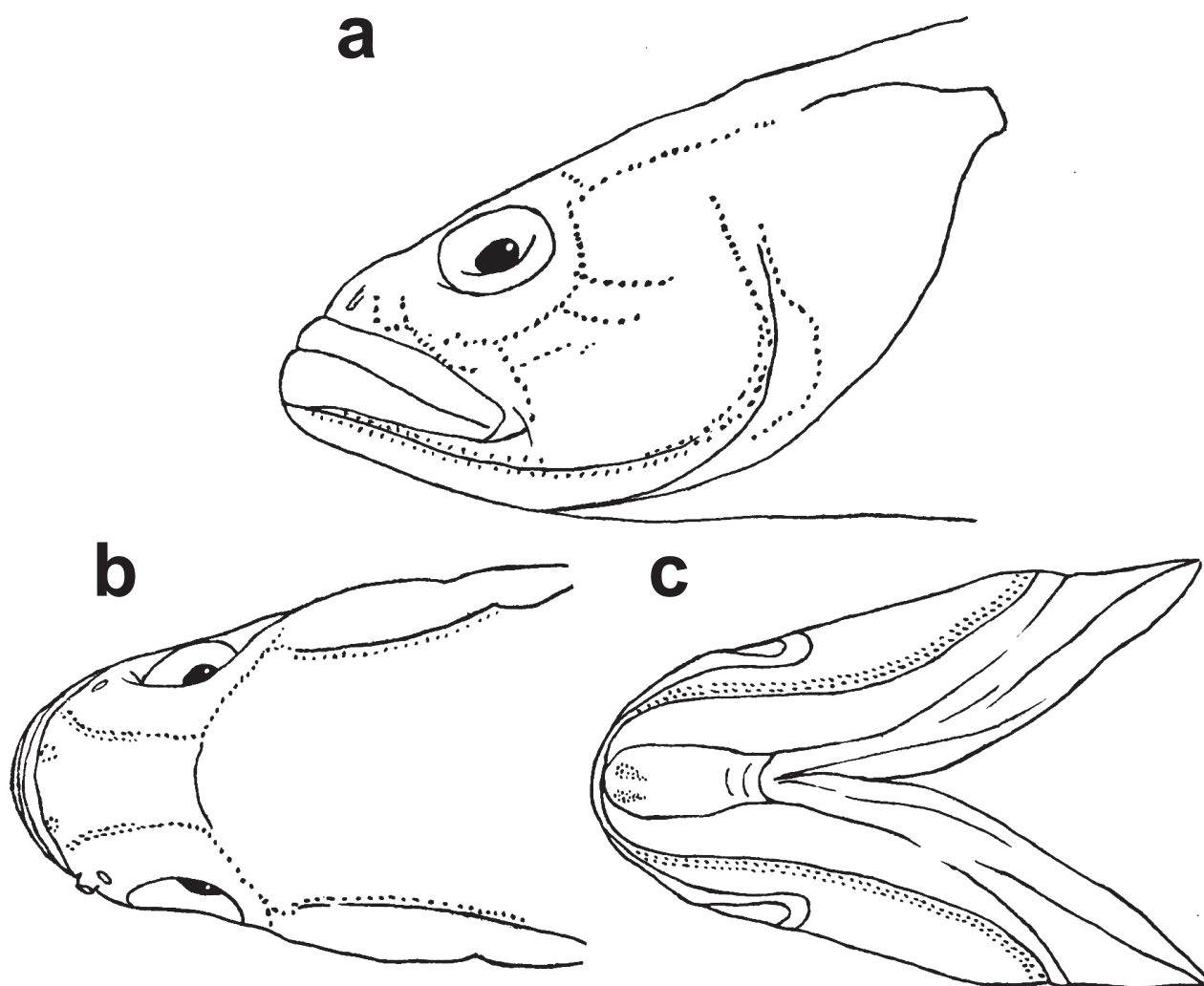


FIGURE 4. Patterns of main cephalic sensory papilla lines (row of spots) of *Neodontobutis lani* sp. nov. (SOU1801007-1, holotype) from lateral (a), dorsal (b) and ventral (c) view.

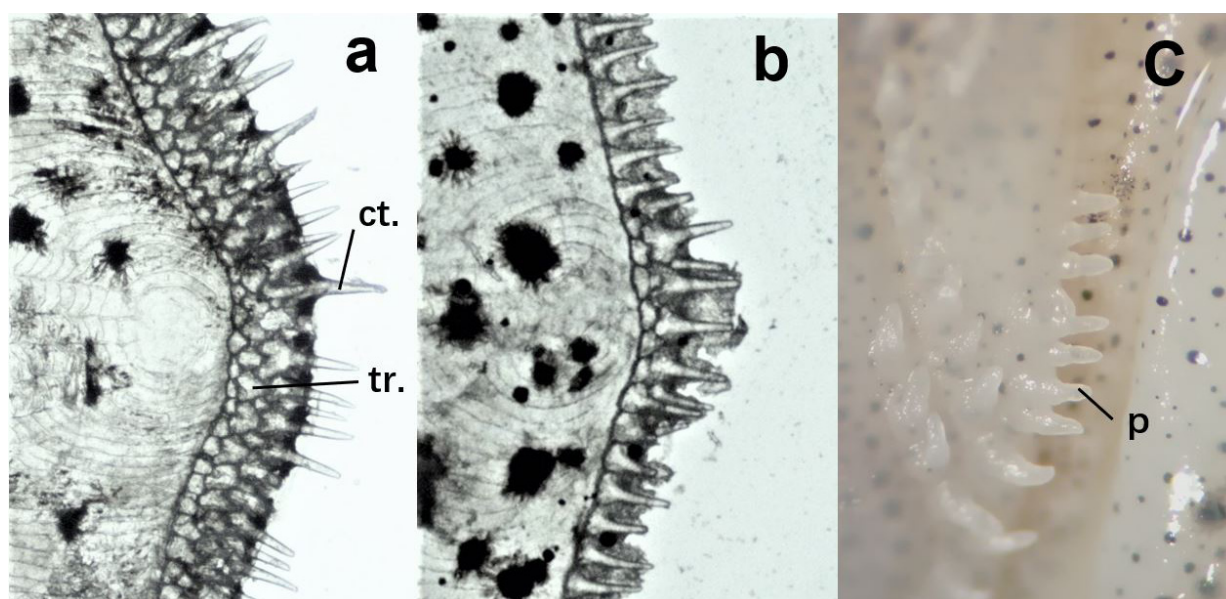


FIGURE 5. Posterior edge of scale on dorsal part of *Neodontobutis lani* SOU1801007-5, paratype (a) and *N. hainanensis* 25901 (b), ct, ctenii; tr, transforming ctenii. Sensory papilla on lower jaw of *N. lani* (c), p, sensory papillae.

Phylogenetic analysis. Four individuals of *Neodontobutis lani* (25912, 25913, 25914, 25916) and representative species of all genera in family Odontobutidae, except for the genus *Terateleoris*, were used in the concatenated nuclear gene tree reconstruction. *Rhyacichthys aspro* (Rhyacichthyidae) was used as the outgroup according to Li (H. Li *et al.*, 2018). For each sample, 1,027–1,688 loci from the 4,434 targeted ones were obtained after assembling. After excluding loci with more than 50% missing data, 2,021 loci were kept for phylogenetic analysis, the length of concatenated alignment was 336,090 bp (electronic appendix). The reconstructed maximum likelihood tree is shown in Figure 6. All genera of Odontobutidae involved in this research formed a monophyletic group. The genera *Neodontobutis*, *Perccottus* and *Odontobutis* were all monophyletic. *Neodontobutis lani* was the sister group of *N. hainanensis* and then formed a clade with *Perccottus glenii*. Aligned *COI* sequences can be found in electronic appendix. The *COI* gene tree which involved more individuals of *N. lani* and more *N. hainanensis* is shown in Figure 7. Its topological structure is same as the concatenated nuclear gene tree at genus level except for the position of genus *Microdous*. All *N. lani* individuals formed a distinct clade apart from *N. hainanensis*. Both the nuclear and mitochondrial trees suggest that *N. lani* is an independent species and should be placed in the genus of *Neodontobutis*.

Distribution. Specimens of *Neodontobutis lani* were bought from a farmers' market in Longzhou Town, Guangxi, which were collected by a local fisherman from tributaries of the Zuojiang River (Figure 1). The detailed distribution range of *N. lani* requires further exploration.

Biology. According to the measurement of specimens collected in this research, maximum size of *Neodontobutis lani* is approximately 62.4 mm standard length. An unidentified small fish (about 25mm standard length) was found in the mouth of a non-type specimen, indicating that *N. lani* is carnivorous. Ovaries with mature eggs were found in a non-type specimen (48.2mm standard length), holds approximately 414 eggs, with average diameter of 1 mm.

Etymology. The new species is named after Jiahu Lan, who found the specimens. The Chinese name is 蓝氏新沙塘鳢.

TABLE 4. Comparison on external characters of the new species *N. lani* to those of the genera *Neodontobutis*, *Perccottus* and *Odontobutis* (Iwata, 2011).

Trait	<i>Neodontobutis. lani</i>	<i>Neodontobutis</i>	<i>Perccottus</i>	<i>Odontobutis</i>
Barbel-like projection on sensory papilla	present	present	absent	absent
Sensory papilla distribution on lower jaw	two oblong patches	two single lines	two single lines	two patches
Interorbital width versus eye diameter	wider	narrower to slightly wider	nearly equal or slightly wider	wider
Right and left gill membrane	separated	separated	separated	united
Predorsal scales	cycloid	cycloid	cycloid	ctenoid
Predorsal scales extension to	posterior part of interorbital space	posterior part of interorbital space	posterior part of interorbital space	to anterior part of interorbital space
Transforming ctenii on body scale	several rows	single row	absent	several rows
Pre-vomerine teeth	absent	absent	present	absent

Discussion. The genus *Neodontobutis* was established by Chen *et al.* (2002), based on *N. hainanensis* (Chen *et al.*, 2002; Iwata, 2011; Wu, 2008). This genus can be distinguished from other genera of Odontobutidae mainly by the following characters: (1) sensory papilla forming a barbel-like projection, which is the most distinctive character; (2) right and left gill membranes separate; (3) rather compressed head and body, interorbital width narrower than eye diameter; (4) predorsal scales cycloid, reaching posterior part of interorbital space; (5) sensory papilla on lower jaw arranged in two single lines (Iwata, 2011). However, some relevant characters of *N. lani* do not fit the description above: *N. lani* possess relatively flat head, with mean interorbital width / eye diameter = 1.6 (range = 1.4–1.9), higher than that of *N. hainanensis* (mean = 1.0; range = 0.8–1.1), *N. auarumus* (1.0 in male, 0.9 in female) (Vidhayanon, 1995), *N. macroptoralis* (1.3) (Kottelat, 2001b) and *N. tonkinensis* (1.2) (Kottelat, 2001b). The mean value of interorbital width / eye diameter of *N. lani* was significantly higher than that of *N. hainanensis* ($P < 0.01$) (Figure 8), with the raw measurements can be found in electronic appendix. Moreover, several rows of transforming ctenii

present on posterior edge of body scale of *N. lani*, while only one row of transforming ctenii present on that part of the other *Neodontobutis* species (Iwata, 2011; Vidthayanon, 1995). In addition, sensory papilla on the lower jaw are arranged in two oblong clusters in *N. lani*, which differs from other *Neodontobutis* species, which have papioae in two single lines on the underside of the jaw (Iwata, 2011). Interestingly, these characteristics of *N. lani* closely align with character states described for *Odontobutis*. Some critical external characters of genus *Neodontobutis* were compared to that of *Perccottus* and *Odontobutis* in table 4 (Iwata, 2011).

Despite some similarities to *Odontobutis*, *Neodontobutis lani* was assigned to the genus *Neodontobutis* based on following reasons: (1) the most critical external characters, such as shape of sensory papilla (presence of barbel-like projection), connection of gill membrane (not connected) and predorsal scales (cycloid, ctenoid in *Odontobutis*) consistent with that of the genus *Neodontobutis*; (2) molecular phylogenetic analysis shows that *N. lani* and *N. hainanensis*, the type species of the genus *Neodontobutis*, formed a monophyletic group with a 100 bootstrap support (Figure 6 & 7). Due to inadequate taxon sampling, specimens and tissue samples of *N. auarmus* and other relevant species are not available for this study. The relationship of species of this genus needs further research.

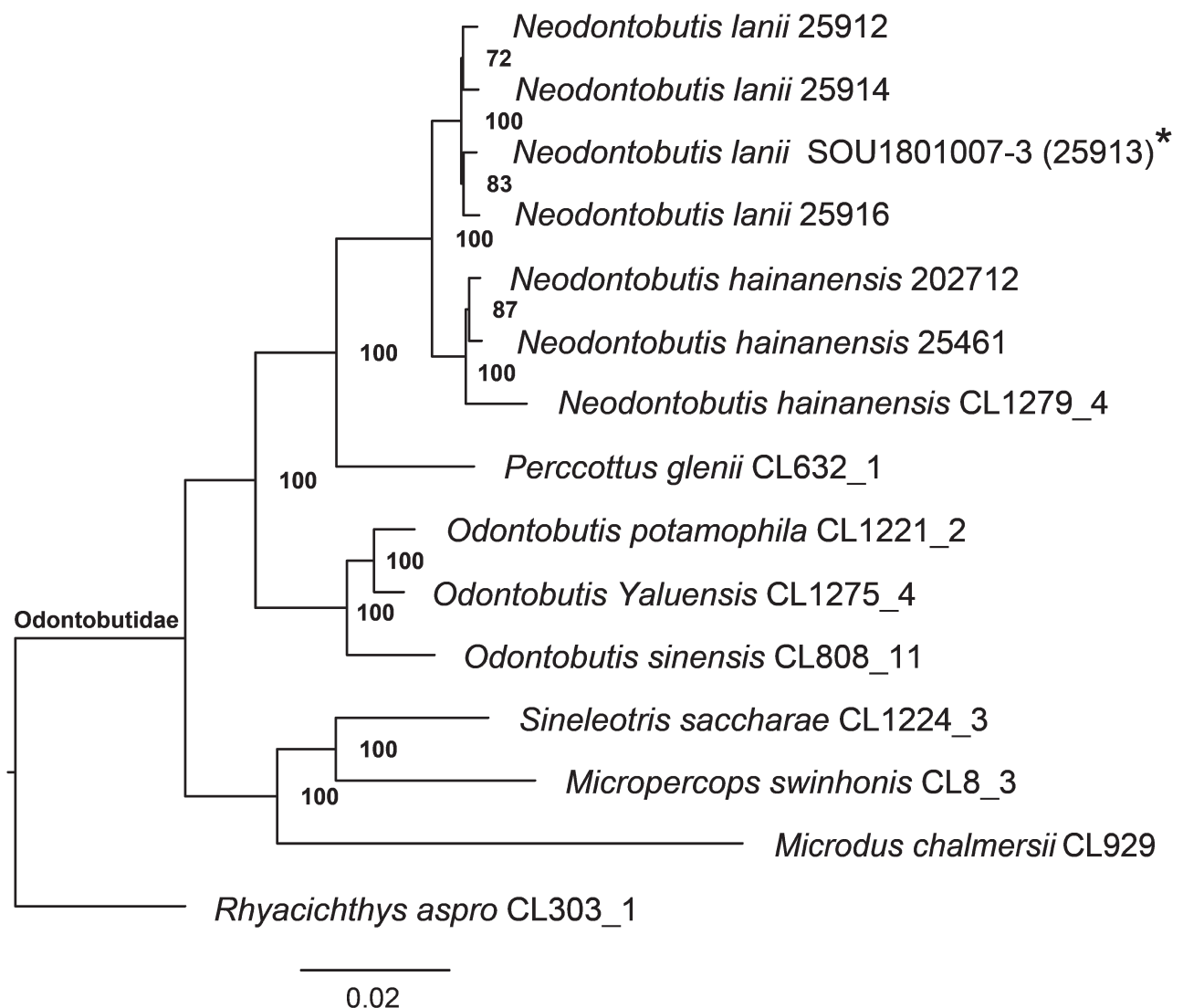


FIGURE 6. Concatenated maximum likelihood tree of *Neodontobutis lani*, and species representing all genus of the Odontobutidae, except for *Terateleotris*, based on 2,076 single-copy nuclear loci. *SOU1801007-3 is a paratype.

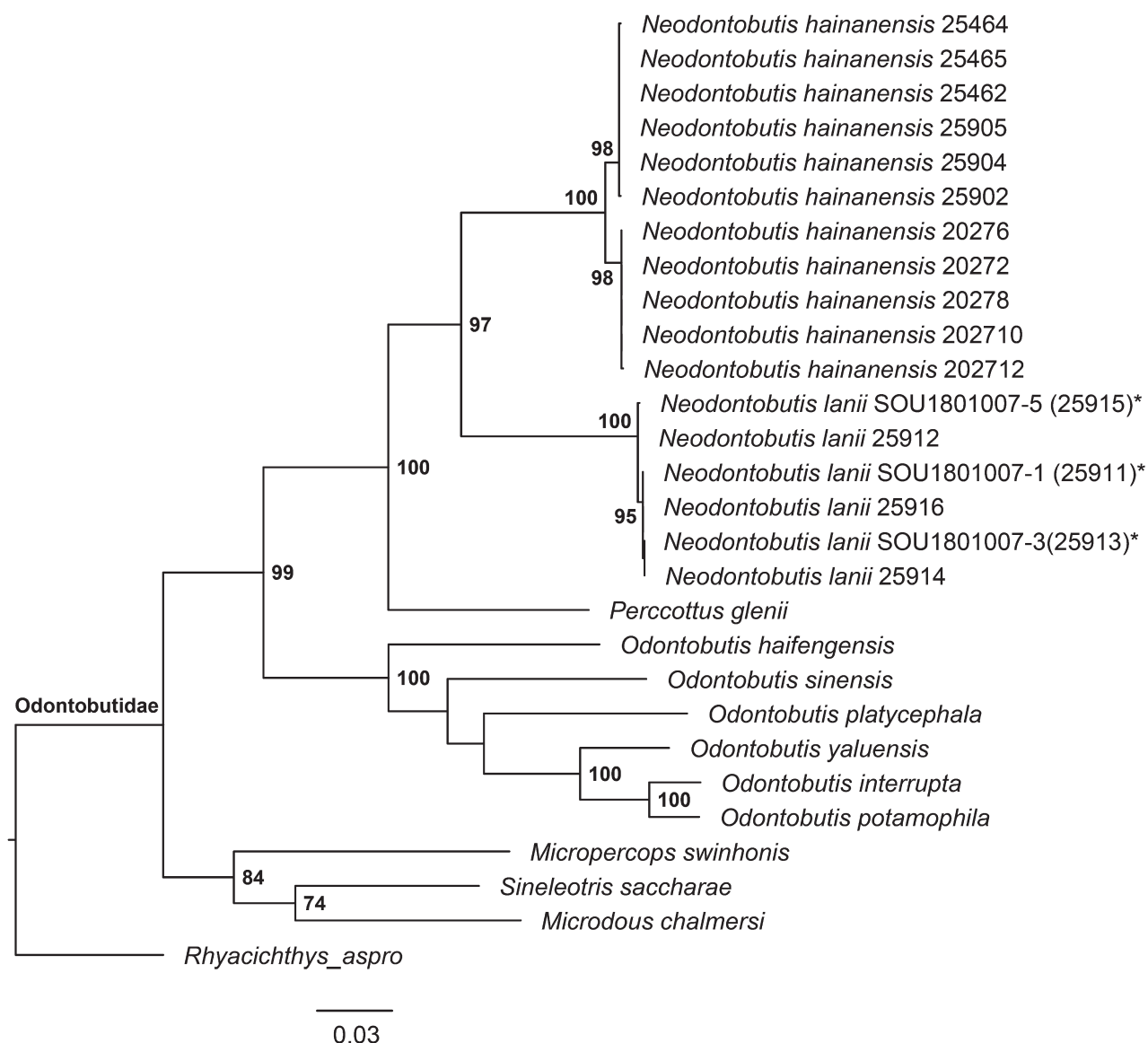


FIGURE 7. Maximum likelihood tree of *Neodontobutis lani*, and comparative species of the Odontobutidae, based on partial sequence (~ 1500 bp) of *cytochrome c oxidase subunit I (COI)* gene. *SOU1801007-1 is the Holotype, SOU1801007-3 and SOU1801007-5 are the paratypes.

Specimens of *Neodontobutis lani* were collected from Longzhou Town, the Zuojiang River basin, which is a tributary of the Pearl River. *Neodontobutis hainanensis* and *O. sinensis* are other Odontobutidae species distributed in the Zuojiang Basin, so the differentiation of their niches would be an interesting question. Moreover, other *Neodontobutis* species, *N. auarmus*, and *N. tonkinensis* and *N. ngheanensis*, which are tentatively placed in the genus *Neodontobutis* (Iwata, 2011), are distributed in the Indo-China Peninsula (Khoa & Duc, 2012; Kottelat, 2001a, 2001b; Vidthayanon, 1995). Investigation on the relationship of *N. lani* and other *Neodontobutis* species may shed light on the origin, diversification and dispersal of these taxa.

According to Li *et al.* (2018), southern China may be the region where the family Odontobutidae originated. Indeed, southern China (Guangdong, Guangxi and Hainan), and the adjacent Indo-China Peninsula, are the diversity centers of the Odontobutidae, which is home to 7 species (belonging to 5 genera) and 6 species (belonging to 3 genera) respectively, with 2 shared genera (Chen *et al.*, 2002; Iwata, 2011; Khoa & Duc, 2012; Kottelat, 2001a, 2001b; Wu, 2008). Due to their small size, benthic habit and low economic importance, as well as their remote distribution, diversity of Odontobutidae in Southern China and Indo-China Peninsula is understudied. Further exploration may uncover more new species from these regions.

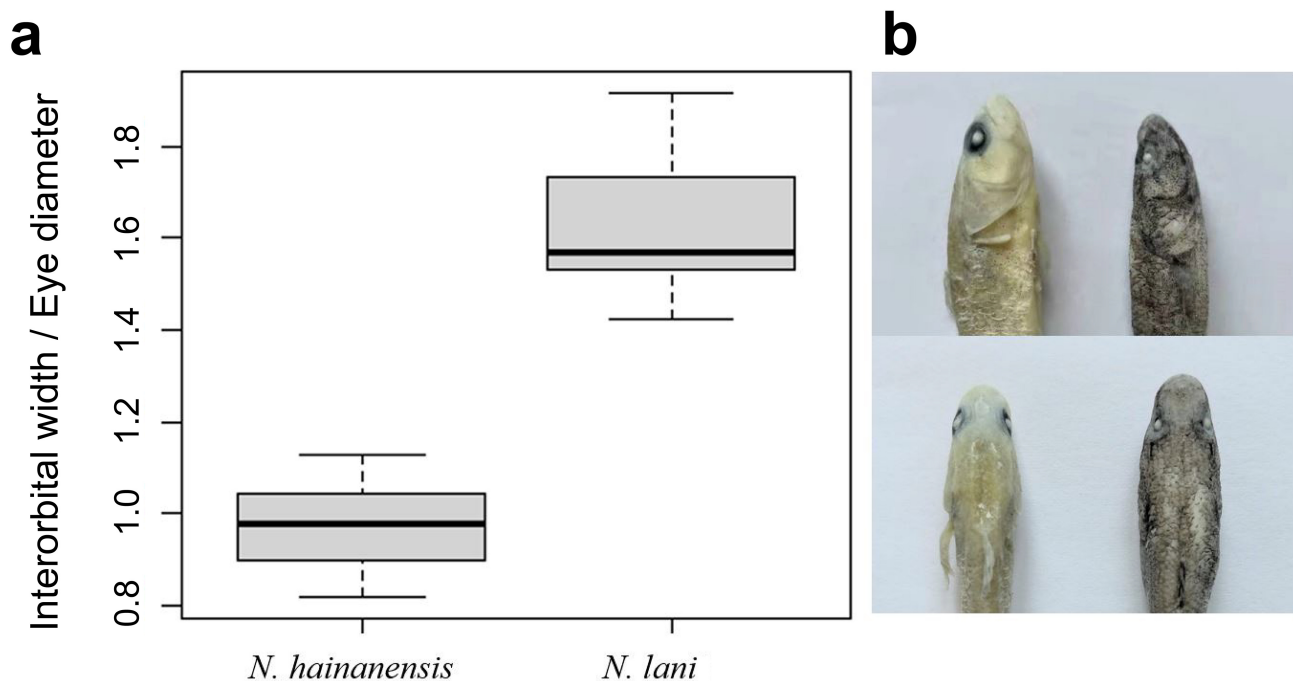


FIGURE 8. Comparison of the head region between *Neodontobutis lani* and *N. hainanensis*. (a) boxplot illustrates interorbital width / Eye diameter, including 12 individuals of *N. lani* (39.0–60.5 mm standard length) and 10 individuals of *N. hainanensis* (32.7–41.3 mm standard length), t value=11.171** (raw data can be found in electronic appendix). (b) Comparison of head shape of *N. hainanensis* (left, 41.3 mm standard length, 25901) and *N. lani* (right, 39.1 mm standard length, 259122).

Comparative material

Collection numbers of *Neodontobutis hainanensis* specimens for morphological comparison: 25901, 25902, 25903, 25904, 25905, 25906, 25461, 25462, 25464, 25465. All of these specimens were collected from Chongzuo City, Guangxi, China on the same date. Details of all materials used for molecular analyses, including collection number or sequence ID, species, material type and collection location are listed in Table 1.

Ethical approval

All animal procedures performed in this research were in accordance with the ethical standards of the Shanghai Ocean University.

Competing interests

The authors declare that they have no competing interests.

Author contributions

C.L. and Y.L. conceived and designed the study. M.Z and A.H. performed the experiments, analyzed the data, and prepared the manuscript. F. Wang prepared Figure 4. and conducted morphological measurements. All authors read and approved the final version of the manuscript.

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