







## Research Article

# Clarification of Phylogenetic Relationships among Chinese Nemacheilids with Tube-Shaped Anterior Nostrils, with a Description of a New Genus and Two New Species

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Nostril structures are important characteristics for generic diagnosis in Nemacheilidae and Cobitidae. Based on phylogenetic analysis, we found that 10 genera within Nemacheilidae (*Eonemachilus*, *Guinemachilus*, *Lefua*, *Micronemacheilus*, *Oreonectes*, *Paranemachilus*, *Protonemacheilus*, *Traccatichthys*, *Troglonectes*, and *Yunnanilus*) shared tube-shaped anterior nostrils and formed a monophyletic group. Morphologically, the location between the anterior and posterior nostrils was divided into three types: (i) separated, i.e., a distance greater than 1.5 times the diameter of the posterior nostril; (ii) adjacent, i.e., a distance shorter than the diameter of the posterior nostril; and (iii) closely set, i.e., posterior margin of the anterior nostril connected to the anterior margin of the posterior nostril. Thus, the 10 genera can be distinguished based on nostril features. Additionally, we describe one new genus, *Guinemachilus* gen. nov., and two new species, *Guinemachilus pseudopulcherrimus* sp. nov. and *Paranemachilus chongzuo* sp. nov.

## 1. Introduction

At present, the family Nemacheilidae contains 792 species belonging to 49 genera [1], which are classified into five tribes, i.e., Lefuini, Nemacheilini, Triplophysini, Vaillantellini, and Yunnanilini, based on morphological characteristics [2]. However, the monophyly of these tribes is not well supported by complete mitochondrial genomes [3]. Especially when the genera within the Yunnanilini tribe are nested within the Lefuini tribe, while *Barbatula* Linck, 1790 and *Homatula* Nichols, 1925 within the Nemacheilini tribe are paraphyletic with respect to the Triplophysini tribe. To address these discrepancies, Du et al. [3] revised the classification of Yunnanilini using both morphological characteristics and molecular evidence, resulting in the placement

of the *Yunnanilus nigromaculatus* group into *Eonemachilus* and *Yunnanilus pulcherrimus* Yang, Chen, and Lan, 2004 into *Micronemacheilus* Rendahl, 1944. More recently, Wang [4] and Luo et al. [5] proposed that *Y. retrodorsalis* should be a member of *Troglonectes*, and *Heminoemacheilus* Zhu and Cao should be considered a synonym to *Paranemachilus* Zhu, 1983. Hence, *Heminoemacheilus zhengbaoshani* Zhu and Cao, 1987 and *Yunnanilus jinxiensis* Zhu, Du, Chen, and Yang, 2009 should be reassigned to *Paranemachilus*. Furthermore, Wang [4] recently reviewed the taxonomy of *Oreonectes* Günther, 1868 and *Troglonectes* Zhang and Zhao, 2016 based on morphological and molecular analyses, with the resulting phylogenetic tree suggesting that *Oreonectes* is not monophyletic, and *O. anophthalmus* Zheng, 1981 and *O. acridorsalis* Lan, 2013 should be placed in a new genus.

Luo et al. [5] established a new genus, *Karstsinnectes* Zhou, Luo, Wang, Zhou and Xiao, 2023, using *O. anophthalmus* as type species. Wang [4] further proposed that *Micronemacheilus*, *Oreonectes*, *Paranemachilus*, *Troglonectes*, and *Yunnanilus* be reclassified into the Yunnanilini tribe, with *Lefua* Herzenstein, 1888 remaining in the Lefuini tribe, and a new tribe (Traccatichthyini) be erected to contain the single genus *Traccatichthys*. Although Kottelat [6] suggested that *Protonemacheilus* Yang and Chu [7] is synonymous with *Physoschistura* or *Pteronemacheilus*, no evidence was provided to support this claim, and thus, *Protonemacheilus* is retained as valid in the current study.

In terms of morphology, Nemacheilidae loaches are distinguished by possessing two typical anterior nostrils: one valve-shaped and one tube-shaped. According to Yang and Chu [7], the anterior and posterior nostrils are either closely positioned or slightly separated, and the shape of the anterior nostrils (either tube- or valve-shaped) is a key diagnostic feature for genera. The presence of tube-shaped anterior nostrils is a characteristic shared by species within the Lefuini, Traccatichthyini, and Yunnanilini tribes [3, 4]. Furthermore, the closely set location and shape of the nostrils, the presence of lips with large papillae or furrows, the presence of scaled or scaleless cheeks, and the presence or absence of lateral and cephalic lateral lines are important generic diagnostic characteristics within these three tribes [3].

In April 2020 and 2021, several loach specimens were collected in the Guangxi Zhuang Autonomous Region (hereinafter referred to as Guangxi) of China. Morphological and molecular evidence supported these loach specimens as a new genus and two new species of the Yunnanilini tribe. Hence, the new genus, *Guinemachilus* gen. nov., and new species, *Guinemachilus pseudopulcherrimus* sp. nov. and *Paranemachilus chongzuo* sp. nov., are described herein. The phylogenetic relationships of Chinese nemacheilids with tube-like anterior nostrils are also discussed.

## 2. Materials and Methods

All current laws related to the Chinese Laboratory of Animal Welfare and Ethics (GB/T 35892-2018) were compiled within this study. Collection and preservation of specimens were done by following Li and Gao's [8] methods. Counts and measurements were performed by following the procedures of Kottelat [9] and Du et al. [3]. Species identifications were performed by following the procedures of Zhang et al. [10] and the original descriptions. The type specimens were deposited in the Guangxi Normal University (GXNU) and Kunming Natural History Museum of Zoology (KIZ-CAS).

Complete mitochondrial genes were sequenced by Shanghai Origine Bio-pharm Technology Co., Ltd. (China) following the standard Illumina procedures. Genome sequencing data were submitted to GenBank (accession numbers OQ024374–024381). We retrieved reference sequences of 37 nemacheilid species from the NCBI GenBank database for phylogenetic tree reconstruction, and *Parabotia fasciata* and *Leptobotia elongata* were as an outgroup (Supplementary Table S1). The MUSCLE was

used to align the gene sequence in MEGA v11 with default parameters [11] and subsequently concatenated. The mitochondrial genome was divided into 17 charsets. The best partitioning scheme for the 17 charsets and evolutionary models was estimated in PartitionFinder v2.1.1 [12] with the "greedy" algorithm [13]. Uncorrected pairwise distances between genera with tube-shaped anterior nostrils and species of *Guinemachilus* and *Paranemachilus* were calculated in MEGA v11. Using MrBayes in XSEDE (v3.2.7a) and CIPRES Science Gateway [14], Bayesian inference (BI) analysis was performed based on the best partition schemes and substitution models (Table 1). Bayesian phylogenetic inference analysis method was done following the method of Du et al. [15]. Node support for ML analysis was determined using 5000 ultrafast bootstrap replicates [16].

## 3. Results

**3.1. Phylogenetic Relationships.** The nemacheilids with tube-shaped anterior nostrils were divided into three highly supported clades. Clades 1 and 2, comprised of the Traccatichthyini and Lefuini tribes, respectively, were sisters to clade 3, comprised of the Yunnanilini tribe. The Yunnanilini tribe consisted of two subclades. Subclade 1 contained *K. parva* (Zhu and Zhu, 2014) and species of *Guinemachilus*, *Micronemacheilus*, and *Oreonectes*. Subclade 2 contained species of *Eonemachilus*, *Paranemachilus* (junior synonym of *Heminoemacheilus*), *Troglonectes*, and *Yunnanilus* (Figure 1).

Based on the phylogenetic tree, the new genus contained three species, i.e., *G. sp.*, *G. longibarbatulus* (Gan, Chen, and Yang, 2007), and *Guinemachilus pseudopulcherrimus* sp. nov., sisters to *Micronemacheilus*. *Heminoemacheilus* was not monophyletic. *Oreonectes*, *Micronemacheilus*, and *Guinemachilus* formed a monophyletic group, sisters to *K. parva*. The type species of *Heminoemacheilus*, *H. zhengbaoshani* Zhu and Cao, 1987 was clustered with species of *Paranemachilus*. Additionally, *Y. retrodorsalis* was grouped with *T. furcocaudalis* (Zhu and Cao, 1987), *T. daqikongensis* (Deng, Wen, Xiao, and Zhou, 2016), and *T. longibarbatulus* (Chen, Yang, Sket, and Algancic, 1998).

**3.2. Mitochondrial Differentiation.** Pairwise comparisons of complete mitochondrial genomes revealed that the average uncorrected *p* distances between genera with tube-shaped anterior nostrils ranged from 7.0% to 15.1% (average 12.8%, Table 2). Average uncorrected *p*-distances between species of *Guinemachilus* and *Paranemachilus* ranged from 5.8%–7.3% (average 6.7%) and 1.2%–6.4% (average 4.4%), respectively (Table 3).

**3.3. Systematics.** Within Nemacheilidae, species of the Yunnanilini, Lefuini, and Traccatichthyini tribes contain tube-shaped anterior nostrils. Among the *Guinemachilus*, *Lefua*, *Oreonectes*, *Paranemachilus*, and *Troglonectes* genera, the tip of the anterior nostril is elongated and barbel-like (Figures 2(f)–2(l) and 2(n)–2(s)) but is truncated without barbel-like elongation in other genera (Figures 2(a)–2(e) and 2(m) and 2(t)). In *Guinemachilus*, *Oreonectes*, and

TABLE 1: Best partition schemes and models based on PartitionFinder estimation.

Subset	Partitions	Model
1	tRNA genes	GTR+I+G
2	12S rRNA	GTR+I+G
3	16S rRNA	GTR+I+G
4	ND1 and ND6	GTR+I+G
5	ND2	GTR+I+G
6	COI	GTR+I+G
7	COII, COIII, and ND4L	GTR+I+G
8	ATP8	HKY+I+G
9	ATP6 and CYTB	GTR+I+G
10	ND3, ND4, and ND5	GTR+I+G
11	D-loop	GTR+I+G

*Troglonectes*, the barbel-like elongation of the anterior nostril is obvious and is longer than the depth of the nostril tube. In *Paranemachilus*, however, the barbel-like elongation is shorter than half of the depth of the nostril tube. The location between the anterior and posterior nostrils can be separated (a distance greater than 1.5 times the diameter of the posterior nostril; *Eonemachilus*, *Guinemachilus*, *Lefua*, and *Yunnanilus*, Figures 2(a)–2(h)), adjacent (a distance shorter than the diameter of the posterior nostril; *Micronemacheilus*, *Oreonectes*, *Paranemachilus*, and some species of *Troglonectes*, Figures 2(i)–2(r)), and closely set (posterior margin of the anterior nostril connected to the anterior margin of the posterior nostril; *Protonemacheilus* and *Traccatichthys* and some species of *Troglonectes*, Figure 2(s), 2(t)). In addition to nostrils, other useful generic diagnostic characteristics include the presence of lips with papillae or furrows; round, emarginate, or forked caudal fins; and specific color patterns.

3.4. *Guinemachilus* gen. nov. Type species. *Guinemachilus pseudopulcherrimus* sp. nov., new species.

*Diagnosis.* *Guinemachilus* differs from all other genera with tube-shaped anterior nostrils within the family Nemacheilidae by anterior and posterior nostrils separated; tip of anterior nostril with barbel-like elongation; barbel longer than tube height; lips smooth, with furrows but no papillae; cheeks scaleless; longitudinal stripe from snout to base of caudal fin absent; and posterior wall of swim bladder open.

*Etymology.* *Gui* is an abbreviation of Guangxi, and *Nemachilus* is a generic name within the family Nemacheilidae in reference to the nemacheilid loaches from Guangxi, China. “桂条鳅” is suggested as its Chinese common name.

*Remarks.* Phylogenetic analysis indicated that *Guinemachilus* belongs to the Yunnanilini tribe in Nemacheilidae. *Guinemachilus* differs from other genera in the Yunnanilini tribe by anterior and posterior nostrils separated (vs. adjacent in *Micronemacheilus*, *Oreonectes*, *Paranemachilus*, and *Troglonectes* and closely set in *Protonemacheilus*) and anterior nostril with barbel-like elongation (vs. truncated in *Eonemachilus* and *Yunnanilus*).

The phylogenetic tree assigned two confirmed species and one unidentified species to the genus *Guinemachilus*. The sequences of NC061031 and ON116504 were identified as *Y. bailianensis* in GenBank. However, tissue samples of NC061031 and ON116504 were collected from Laibin City (with the type locality of *Y. bailianensis* located in Bailian Cave, Liuzhou City). Although the phylogenetic tree and morphological characteristics support the Laibin specimens as belonging to *Guinemachilus*, the lack of physical specimens precludes a formal description of the species in this study.

3.5. *Guinemachilus pseudopulcherrimus* Du, Li, Luo, and Yu sp. nov. (Figures 3 and 4(a) and 4(b), Table 3). Holotype. GXNU-F20210008, 48.2 mm SL, 58.0 mm TL, Dongmiao Village, Du'An County, Hechi City, Guangxi, China, 23°58'59.3" N, 107°59'33.6" E, collector J.H. Lan, May 2021.

*Paratypes.* GXNU-F20210009–13, KIZ2022000024–27, 9 specimens, 44.7–51.3 mm SL, 55.3–62.0 mm TL; collected with holotype.

*Material used in molecular genetic analysis.* DLN20200283, DLN20210104, DLN20210107 and LJH01 (GenBank accession numbers: OQ024375–OQ024377, OQ024380) were collected from the type locality by F.G. Luo and J.H. Lan, January and April 2021.

*Diagnosis.* *Guinemachilus pseudopulcherrimus* sp. nov. differs from all other species of *Guinemachilus* by lateral line present with three or four lateral line pores (vs. absent in *G. sp.* and *G. longibarbatulus*).

*Description.* Morphometric data of type specimens of *Guinemachilus pseudopulcherrimus* sp. nov. are shown in Table 3. D, 4, 8; A, 3, 5; P, 1 8–9; V, 1, 6–7; C, 15–16. In total, 10 or 11 inner gill rakers, without outer gill raker on first gill arch. Cephalic lateral-line canals absent. Lateral line incomplete, and three or four lateral line pores present before base of pectoral fin.

Body cylindrical, deepest body depth in front of dorsal-fin origin, 16.9%–19.9% of SL. Head width slightly greater than depth. Snout length 32.4%–38.1% of lateral head length, shorter than postorbital length. Mouth inferior, lips smooth, with small furrows. Three pairs of barbels, inner rostral, outer rostral, and maxillary barbel extend to nostril, anterior margin, and posterior margin of the eye, respectively. Nostrils separated by a short distance, a distance longer than nostril tube height, anterior nostril with barbel-like elongation. Eyes present, diameter 21.2%–26.2% of lateral head length, suborbital groove absent in males.

Dorsal-fin origin anterior to pelvic-fin insertion, predorsal length 52.2%–56.0% of SL. Anal fin truncate. Pectoral fin length 50.1%–62.3% of distance between pectoral fin and pelvic-fin origin. Tip of pelvic fin one eye diameter from the anus. Distance between the anus to anal-fin origin approximately 1/3 of eye diameter. Caudal fin emarginate.

Body scaled, except the head, throat, and abdomen. Intestines short and straight. Two air-bladder chambers, anterior chamber encased in bony capsule, posterior chamber filling body cavity, anterior and posterior chamber connected by a slender tube.

*Color pattern.* Head and trunk with yellowish background color. Lower margin of the eye to dorsal head surface

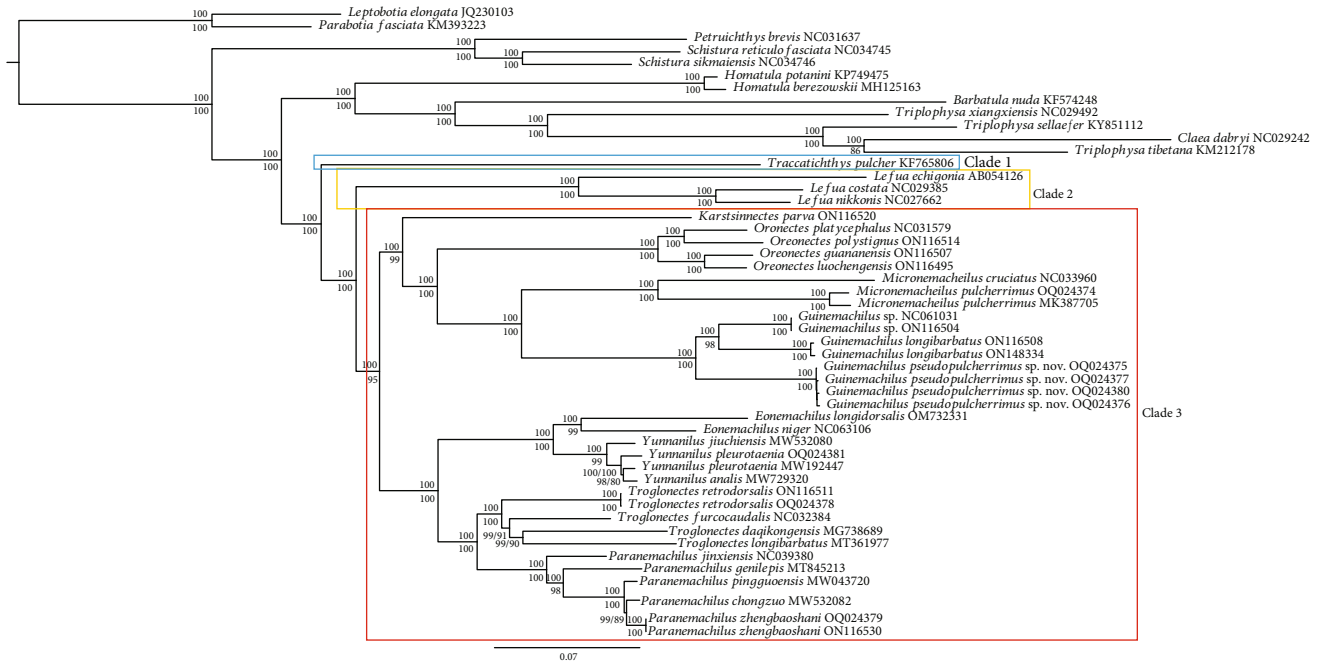


FIGURE 1: Bayesian phylogram of Nemacheilidae based on mitochondrial genomes of 37 nemacheilid species and two botiid species (outgroups). Numbers above and below branches are BPPs and ML bootstrap values, respectively.

TABLE 2: Uncorrected pairwise distances between Nemacheilidae genera with tube-shaped anterior nostrils based on complete mitochondrial genomes.

	1	2	3	4	5	6	7	8
1 <i>Traccatichthys</i>	—							
2 <i>Lefua</i>	0.1513							
3 <i>Oreonectes</i>	0.1418	0.1443						
4 <i>Micronemacheilus</i>	0.1480	0.1508	0.1332					
5 <i>Guinemachilus</i>	0.1423	0.1479	0.1297	0.1239				
6 <i>Eonemachilus</i>	0.1397	0.1388	0.1302	0.1386	0.1329			
7 <i>Yunnanilus</i>	0.1348	0.1343	0.1222	0.1320	0.1284	0.0742		
8 <i>Paranemachilus</i>	0.1315	0.1343	0.1219	0.1307	0.1252	0.1055	0.0929	
9 <i>Troglonectes</i>	0.1339	0.1375	0.1243	0.1340	0.1275	0.1088	0.0970	0.0703

TABLE 3: Uncorrected pairwise distances between species of *Guinemachilus* and *Paranemachilus* based on complete mitochondrial genomes.

	1	2	4	5	6	7
1 <i>Guinemachilus</i> sp.	—					
2 <i>Guinemachilus pseudopulcherrimus</i> sp. nov.	0.0676					
3 <i>G. longibarbatus</i>	0.0583	0.0734				
4 <i>Paranemachilus chongzuo</i> sp. nov.			—			
5 <i>P. zhengbaoshani</i>			0.0475			
6 <i>P. jinxiensis</i>			0.0505	0.0642		
7 <i>P. genilepis</i>			0.0256	0.0569	0.0503	
8 <i>P. pingguoensis</i>			0.0121	0.0477	0.0509	0.0319

dark brown, ventral head surface without color pattern. Predorsal trunk with 6–8 dark brown blotches, larger width than interspaces, sometimes fourth to sixth blotches con-

nected. Four to six wide dark brown blotches after dorsal fin fused with bars on flank forming saddles. Upper line of flank with 15–21 brown vertical dark brown bars or large

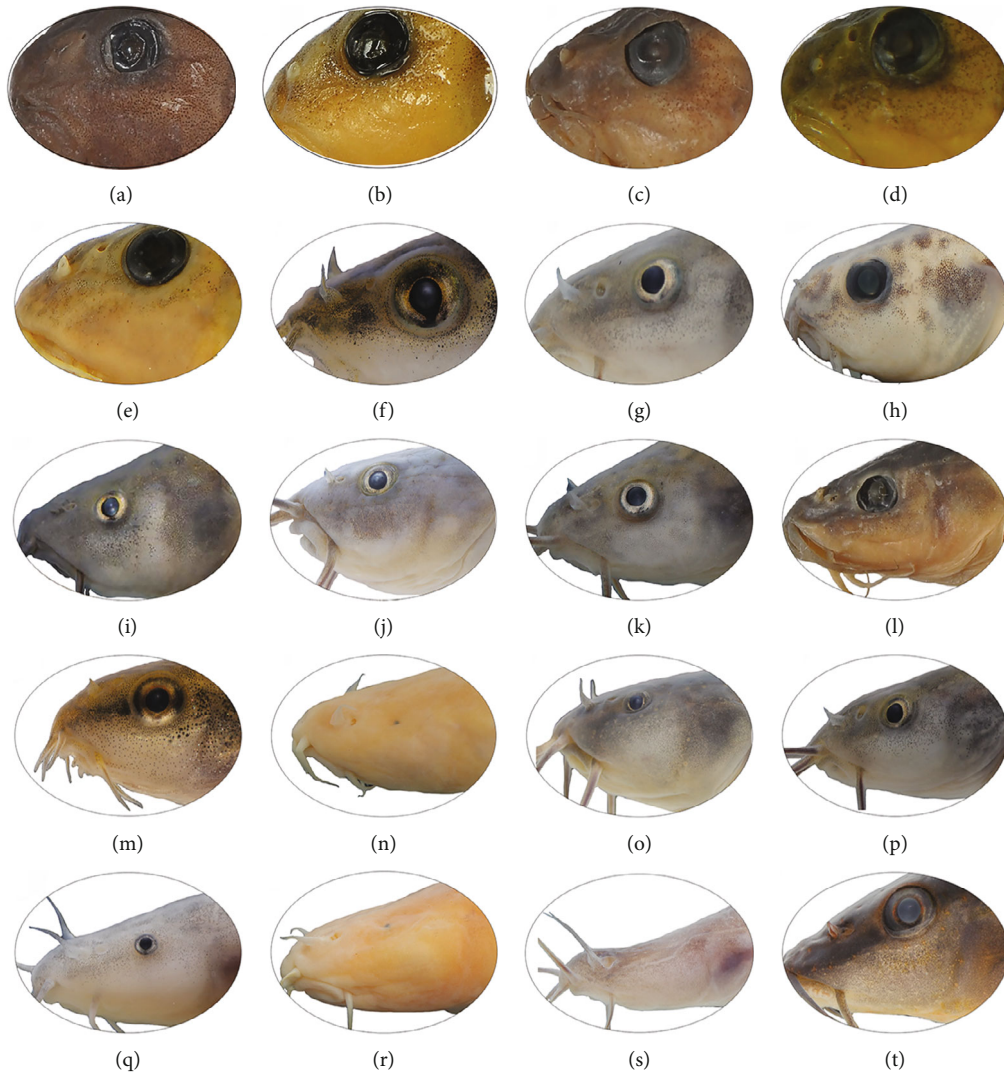


FIGURE 2: Location of anterior and posterior nostrils. (a–h) Separated: (a) *Eonemachilus niger*, KIZ1980001275; (b) *Eonemachilus baijiangensis*, uncatalogued 1<sup>st</sup>; (c) *Eonemachilus pachycephalus*, holotype, KIZ1982002824; (d) *Eonemachilus caohaiensis*, KIZ2006007730; (e) *Yunnanilus spanisbripes*, HRAS199504007; (f) *Guinemachilus pseudopulcherrimus* sp. nov.; (g) *Guinemachilus longibarbatus*; (h) *Guinemachilus* sp. (i–s). Adjacent: (i) *Paranemachilus genilepis*; (j) *Paranemachilus zhengbaoshani*; (k) *Paranemachilus pingguoensis*; (l) *Paranemachilus jinxiensis*; (m) *Micronemacheilus pulcherrimus*; (n) *Oreonectes luochengensis*; (o) *Oreonectes polystigmus*; (p) *Troglonectes retrodorsalis*; (q) *Troglonectes macrolepis*; (r) *Troglonectes barbatus*; (s) *Troglonectes lihuensis*. (t) Close set: *Traccatichthys pulcher*. (g–t) Uncatalogued, photograph from J.H. Lan.

spots. Blotches on flank narrower than interspaces, joined with saddles on back but below line between pectoral-fin base, the anus without pattern. One elliptical, dark brown spot at mid-caudal-fin base. Fin rays with tiny, dark brown spots. Fin membrane hyaline.

**Distribution and habitat.** *Guinemachilus pseudopulcherrimus* sp. nov. is currently only known from Dongmiao Village, Du'An County, Hechi City, Guangxi, China, in a tributary of the Hongshui River (23°58'59.3" N, 107°59'33.6" E). The species lives in a deep pool with water depths of 30–80 m and water temperatures of 20–25°C. In the rainy season, the pond changes to a slow-flowing river.

**Etymology.** *Pseudo* means fake and *pulcherrimus* is the species name of *Yunnanilus pulcherrimus*, in reference to

the similar color pattern between the two species. We suggest “似丽纹桂条鳅” as the Chinese common name.

**Remarks.** *Guinemachilus pseudopulcherrimus* sp. nov. differs from all other species of *Guinemachilus* by lateral line with three or four lateral line pores present (vs. absent in *G. longibarbatus* and *G. sp.*).

3.6. *Guinemachilus longibarbatus* (Gan, Chen, and Yang, 2007). *Yunnanilus longibarbatus*, Gan, Chen, and Yang, 2007: 322, Figure 1 (type locality: Gaoling Township, Du'An County, Guangxi, China).

**Diagnosis.** *Guinemachilus longibarbatus* differs from all other species of *Guinemachilus* by lateral line absent (vs. present

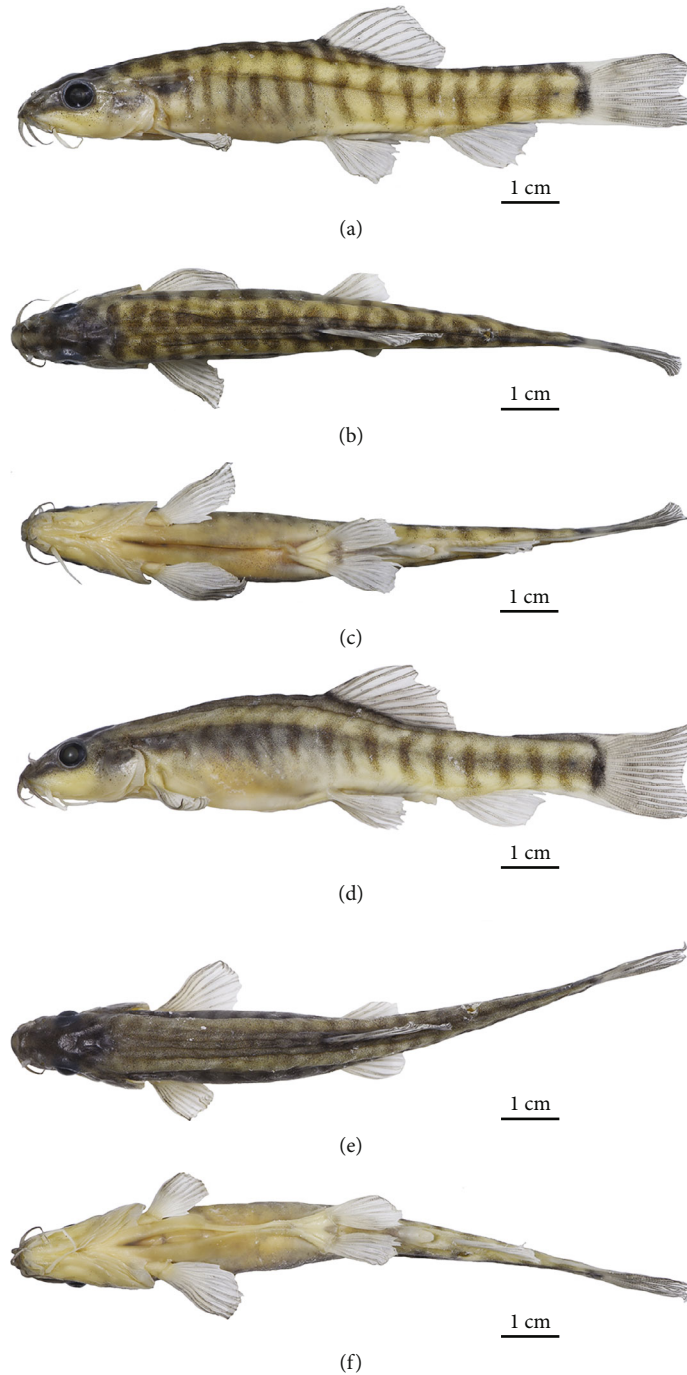


FIGURE 3: Standard three-sided views of *Guinemachilus pseudopulcherrimus* sp. nov. (a–c) Holotype GXNU-F20210008, 48.2 mm SL; (d–f) paratype KIZ2021000024, 50.0 mm SL. Scale = 1 cm.

in *Guinemachilus pseudopulcherrimus* sp. nov.) and outer gill raker on first gill arch absent (vs. present in *G. sp.*).

**Distribution.** Gaoling Township, Du'An County, Hechi City, Guangxi, China, in the Hongshui River, a tributary of the Pearl River.

**Remarks.** Du et al. [3] placed *Y. longibarbatu*s in *Heminoemacheilus* based on closely set anterior and posterior nostrils (= adjacent in this study). The phylogenetic tree

supported *Y. longibarbatu*s as a member of *Guinemachilus*. The nostril characteristics of the type specimens were rechecked by R. Min (Manager of the Fish Collection Room, Kunming Natural History Museum of Zoology, Kunming Institute of Zoology, Chinese Academy of Sciences). The type specimens of *Y. longibarbatu*s have adjacent (KIZ2003006024) and separated (KIZ2003006013, KIZ2003006015, and KIZ2003006022–6023) nostril types. Thus, we consider that

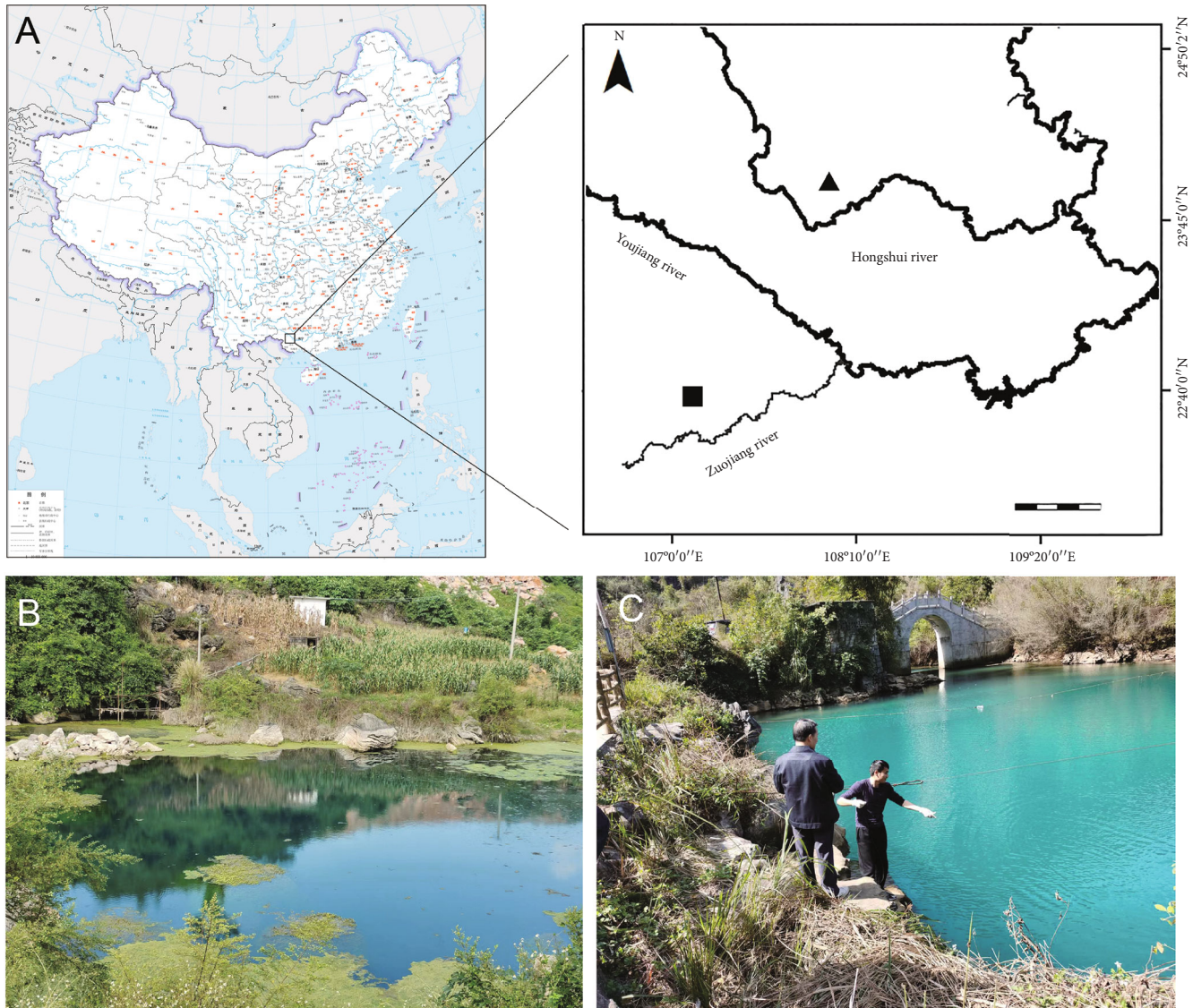


FIGURE 4: (a) Location of *Guinemachilus pseudopulcherrimus* sp. nov. (black triangle) and *Paranemachilus chongzuo* sp. nov. (black square) in Guangxi, China; (b) habitat of *Paranemachilus chongzuo* sp. nov.; (c) habitat of *Guinemachilus pseudopulcherrimus* sp. nov.

specimens with separated anterior and posterior nostrils may belong to *Guinemachilus*, while those with adjacent nostrils may belong to *Paranemachilus*.

3.7. *Paranemachilus* Zhu, 1983: 311 (type species: *Paranemachilus genilepis* Zhu, 1983: 311). *Paranemachilus* differs from all other genera within Nemacheilidae by anterior and posterior nostrils adjacent, base of anterior nostril tube-shaped, tip with barbel-like elongation, barbel length shorter than half of tube height.

**Remarks.** Based on the phylogenetic tree, the type species of *Heminoemacheilus*, *H. zhengbaoshani*, was sister to *Paranemachilus chongzuo* sp. nov. then stepwise clustered with other recorded species of *Paranemachilus*. Thus, the phylogenetic tree indicated that *Heminoemacheilus* was synonymous with *Paranemachilus*, with the valid genus name being *Paranemachilus*. Four species are recorded in *Paranemachilus*, i.e., *P. genilepis* and *P. jinxiensis* (cheeks with

scales) and *P. pingguoensis* and *P. zhengbaoshani* (cheeks scaleless). *Paranemachilus jinxiensis* differs from *P. genilepis* by caudal-peduncle length longer than depth (vs. shorter), and *P. zhengbaoshani* can be distinguished from *P. pingguoensis* by processus dentiformis present (vs. absent).

In April 2020, five specimens of *Paranemachilus* were collected in Daxin County, Chongzuo City, Guangxi, China. Morphological characteristics and molecular analyses indicated that these specimens are a new species of the genus *Paranemachilus*, which we describe below.

3.8. *Paranemachilus chongzuo* Du, Xu, Luo, and Zhou sp. nov. (Figures 5 and 4(a) and 4(c), Table 3). Holotype. KIZ2022000019, 54.2 mm SL, 65.5 mm TL, Daxin County, Chongzuo City, Guangxi, China, 22°37'21.72" N, 107°7'12.88" E, collected by F.G. Luo, April 2020.

**Paratypes.** KIZ2022000020–23, 4 specimens, 52.1–66.5 mm SL, 62.8–82.5 mm TL; collected with holotype.

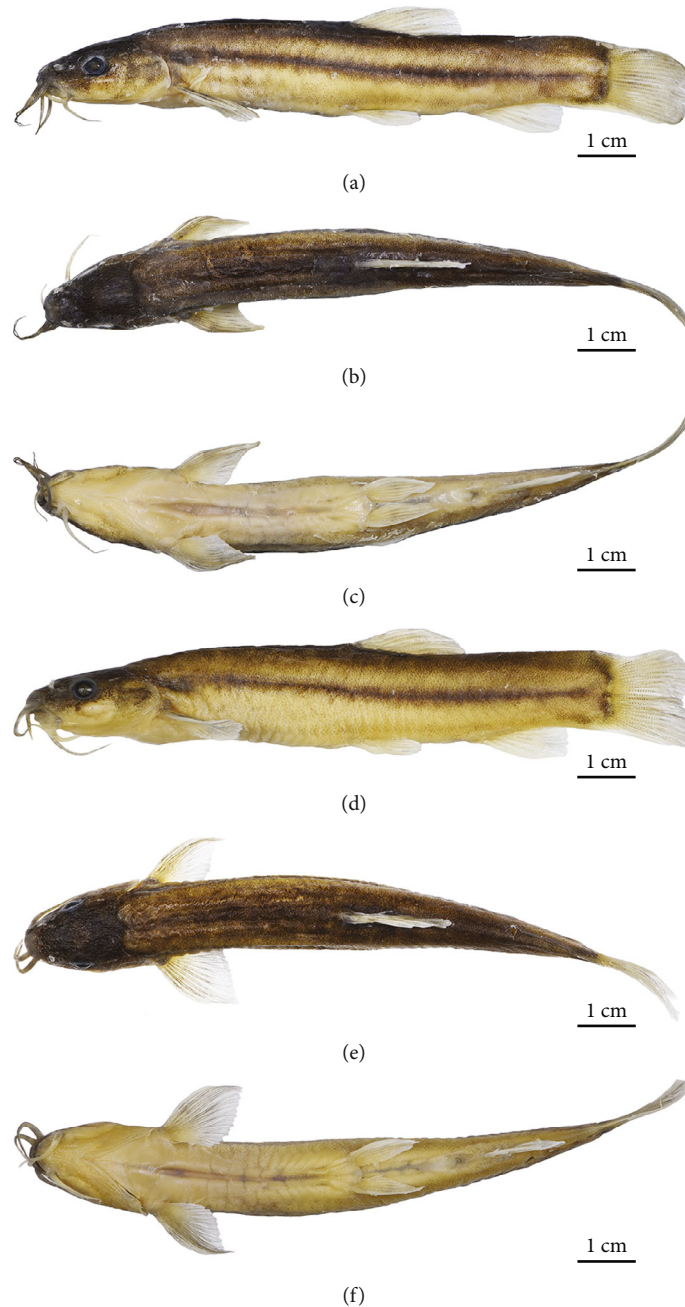


FIGURE 5: Standard three-sided views of *Paranemachilus chongzuo* sp. nov.: (a–c) holotype KIZ2022000019, 54.2 mm SL; (d–f) paratype KIZ2022000020, 52.1 mm SL. Scale = 1 cm.

KIZ2022000021 was sequenced (GenBank accession number: MW532082).

**Diagnosis.** *Paranemachilus chongzuo* sp. nov. differs from all other species of *Paranemachilus* by cheeks naked, 10 branched pectoral fins, cephalic lateral-line canals with three supraorbital pores, other pores absent, body depth 16.6%–17.6% of SL, head depth 45.1%–48.5% of lateral head length.

**Description.** Morphometric data from type specimens of *Paranemachilus chongzuo* sp. nov. are shown in Table 4. D, 3, 8; A, 3, 5; P, 1, 10; V, 1, 6; C, 16–17. First gill arch with one outer (in SL <70 mm, outer gill raker unclear) and 14–16

inner gill rakers, respectively. Cephalic lateral-line canals with four supratemporal, three supraorbital at base of anterior nostrils, 4 + 7–8 infraorbital, and zero preoperculo-mandibular pores. Lateral line incomplete, 4–8 lateral line pores present before tip of pectoral fin.

Body cylindrical, with deepest body depth in front of dorsal-fin origin, 16.6%–17.6% of SL. Head width slightly greater than depth. Snout length 32.5%–37.0% of lateral head length, shorter than postorbital length. Mouth inferior, lips smooth. Three pairs of barbels, inner and outer rostral, and maxillary barbel extending to posterior margin of the eye, posterior margin of preopercle, and posterior margin



TABLE 4: Morphometric and meristic data of *Guinemachilus pseudopulcherrimus* sp. nov. and *Paranemachilus chongzuo* sp. nov. Range, mean, and standard deviation (mean  $\pm$  SD) are presented for paratypes.

	<i>Guinemachilus pseudopulcherrimus</i> sp. nov.		<i>Paranemachilus chongzuo</i> sp. nov.	
	Holotype GXNU-F20210008	Paratypes (N = 9) GXNU-F20210009–13, KIZ2022000024–27	Holotype KIZ2022000019	Paratypes (N = 4) KIZ2022000020–23
Total length (mm)	58.0	55.4–62.0 (59.3 $\pm$ 1.7)	65.5	62.8–82.5 (72.8 $\pm$ 8.1)
Standard length (mm)	48.2	44.7–51.3 (49.0 $\pm$ 1.9)	54.2	52.1–66.5 (60.2 $\pm$ 6.0)
Percent of standard length				
Deepest body depth	17.8	16.9–19.9 (18.2 $\pm$ 0.9)	17.0	16.6–17.6 (17.0 $\pm$ 0.5)
Lateral head length	25.4	24.5–27.1 (25.6 $\pm$ 0.9)	24.7	22.6–24.8 (24.2 $\pm$ 1.0)
Predorsal length	52.3	52.2–56.0 (53.6 $\pm$ 1.2)	56.9	55.2–57.7 (55.9 $\pm$ 1.2)
Prepelvic length	54.9	54.8–59.4 (56.4 $\pm$ 1.4)	56.9	57.3–59.3 (58.3 $\pm$ 0.8)
Preanal length	76.0	75.2–77.9 (76.8 $\pm$ 0.8)	80.3	80.6–81.4 (81.0 $\pm$ 0.3)
Preanus length	73.7	73.6–75.2 (74.4 $\pm$ 0.6)	76.9	76.7–78.0 (77.1 $\pm$ 0.5)
Caudal peduncle length	13.9	13.9–15.6 (14.5 $\pm$ 0.7)	14.2	12.8–15.1 (13.6 $\pm$ 1.1)
Caudal peduncle depth	10.0	8.7–10.5 (9.8 $\pm$ 0.6)	13.1	11.0–13.1 (11.9 $\pm$ 1.0)
Head width	14.1	12.4–15.9 (13.9 $\pm$ 0.9)	13.8	13.2–14.6 (14.1 $\pm$ 0.6)
Percent of lateral head length				
Eye diameter	24.6	21.2–26.2 (22.8 $\pm$ 1.6)	18.1	15.7–19.4 (17.8 $\pm$ 1.6)
Interorbital width	22.7	20.9–28.9 (24.8 $\pm$ 2.8)	31.2	29.5–35.1 (33.0 $\pm$ 2.5)
Snout length	32.9	32.4–38.1 (35.4 $\pm$ 1.8)	34.8	32.5–37.0 (34.9 $\pm$ 2.0)
Head width	55.4	49.0–58.6 (54.5 $\pm$ 2.5)	55.9	57.2–59.8 (58.3 $\pm$ 1.1)
Head depth	54.4	46.6–55.1 (51.6 $\pm$ 2.7)	45.1	45.9–48.5 (47.0 $\pm$ 1.1)
Percent of caudal peduncle length				
Caudal peduncle depth	71.8	61.5–74.5 (67.4 $\pm$ 4.5)	91.9	82.7–96.8 (87.8 $\pm$ 6.2)
Dorsal-fin rays	4, 8	4, 8	3, 8	3, 8
Pectoral-fin rays	1, 9	1, 8–9	1, 10	1, 10
Pelvic-fin rays	1, 7	1, 6–7	1, 6	1, 6
Anal-fin rays	3, 5	3, 5	3, 5	3, 5
Caudal-fin branched rays	16	15–16	16	17

of opercula, respectively. Nostrils adjacent, tip of anterior nostril with weak barbel-like elongation. Eyes normal, diameter 15.7%–19.4% of lateral head length, suborbital groove in males absent.

Dorsal-fin origin anterior to pelvic-fin insertion, predorsal length 55.2%–57.7% of SL. Anal fin truncate. Pectoral fin length 45.0%–53.9% of distance between pectoral-fin and pelvic-fin origin. Distance between tips of depressed pelvic fins and the anus 1.2–1.6 times eye diameter. Distance between the anus and anal-fin origin 50.1%–107.8% of eye diameter. Caudal fin emarginate.

Body scaled, except the head. Intestines short and straight. Two air-bladder chambers, anterior chamber encased in bony capsule, posterior chamber filling body cavity, anterior and posterior chamber connected by slender tube, tube length about 2/3 of posterior chamber length.

**Color pattern.** Head and trunk with yellowish background color. Dorsal and lateral head surface dark brown, ventral head surface without color pattern. Body back and flank at lateral line dark brown. Flank between body back

and lateral line and below line between pectoral fin base and the anus yellowish, with tiny dark brown spots. Fin rays with tiny, dark brown spots. Fin membrane hyaline.

**Distribution and habitat.** Daxin County, Chongzuo City, Guangxi, China, in a tributary of the Zuojiang River. Water rises from the ground to form a pool, with a water temperature of 20–25°C.

**Etymology.** The specific name *chongzuo* is derived from the pinyin of its type locality, Chongzuo City. We suggest the Chinese common name “崇左异条鳅”.

**Remarks.** *Paranemachilus chongzuo* sp. nov. differs from other species of *Paranemachilus* by 10 branched pectoral fins (vs. 11–13 in *P. genilepis*, *P. pingguoensis*, and *P. zhengbaoshani* and 13–14 in *P. jinxiensis*), cephalic lateral-line canals with three supraorbital pores (vs. 9–12 in other species), preoperculomandibular pore absent (vs. present), body depth 16.6%–17.6% of SL (vs. greater than 18% in *P. jinxiensis* and *P. pingguoensis*), head depth 45.1%–48.5% of lateral head length (vs. greater than 50% in *P. pingguoensis* and *P. zhengbaoshani*), and cheeks scaleless (vs. scaled in *P. jinxiensis* and *P. genilepis*).

### 3.9. Identification key to Chinese Nemacheilids with Tube-Shaped Anterior Nostrils

- (1) Nostrils separated.....2  
 .....2
- . Nostrils adjacent or closely set.....5  
 .....5
- (2) Tip of anterior nostrils truncated.....3  
 .....3
- . Tip of anterior nostrils with barbel-like elongation.....4  
 .....4
- (3) Cephalic lateral-line canals and lateral line absent.....  
*Eonemachilus*
- . Cephalic lateral-line canals and lateral line present.....  
*Yunnanilus*
- (4) Longitudinal stripe from snout to base of caudal fin absent.....*Guinemachilus*
- . Longitudinal stripe from snout to base of caudal fin present.....*Lefua*
- (5) Nostrils adjacent.....6  
 .....6
- . Nostrils closely set.....9  
 .....9
- (6) Papillae on lips present .....  
 .....*Micronemacheilus*
- . Lips smooth, with furrows.....7  
 .....7
- (7) Barbel-like elongation of anterior nostrils shorter than half of depth of nostril tube.....-  
 .....*Paranemachilus*
- . Barbel-like elongation of anterior nostrils longer than depth of nostril tube.....8
- (8) Caudal fin rounded, dorsal fin with 6–7 branched rays.....*Oreonectes*
- . Caudal fin forked or emarginated, dorsal fin with 8–10 branched rays.....*Troglonectes*
- (9) Lips with large papillae.....  
 .....*Traccatichthys*
- . Lips smooth, with furrows.....  
 .....*Protonemacheilus*

## 4. Discussion

Nostril structures are important characteristics for generic diagnosis in Nemacheilidae and Cobitidae [3, 17]. Within Nemacheilidae, species in *Yunnanilini*, *Lefuini*, and *Traccatichthyini* share tube-shaped anterior nostrils [3, 5]. In our phylogenetic tree, species that shared tube-shaped anterior nostrils clustered together and formed a monophyly. Morphologically, the anterior nostrils of *Eonemachilus*, *Guinemachilus*, *Lefua*, and *Yunnanilus* were separated, *Micronemacheilus*, *Paranemachilus*, *Oreonectes*, and *Troglonectes* were adjacent, and *Protonemacheilus* and *Traccatichthys* were closely set. Furthermore, the tip of the anterior nostril exhibited a barbel-like elongation in *Lefua*, *Guinemachilus*, *Oreonectes*, *Paranemachilus*, and *Troglonectes*.

Du et al. [3] stated that *E. nigromaculatus* (Regan, 1904) (GenBank accession MW532081 or NC058283) was the most basal clade in the phylogenetic tree, but the tissue of *E. nigromaculatus* was misidentified. In this study, *E. longidorsalis* (Li, Tao, & Lu, 2000) and *E. niger* (Kottelat & Chu, 1988) clustered together as sister to *Yunnanilus*. The morphological characteristics and molecular analyses supported *Eonemachilus* as an independent genus. Additionally, the type species of *Heminoemacheilus*, *H. zhengbaoshani*, was clustered with species of *Paranemachilus*, indicating that *Heminoemacheilus* is synonymous with *Paranemachilus* [5]. The generic diagnostic characteristics of *Paranemachilus* include anterior nostrils tube-like, tip of anterior nostrils with weak barbel-like elongation, barbel length shorter than half of tube depth, anterior and posterior nostrils adjacent, and lips without papillae.

Wang [4] placed *Y. retrodorsalis* in *Troglonectes* based on molecular analysis. Additionally, the phylogenetic trees in Wang [4] and Luo et al. [5] showed that *Troglonectes* species could be divided into three clades, with clade 1 containing *T. retrodorsalis*, *T. shuilongensis* (Deng, Xiao, and Hou, 2016), and *T. sp.*; clade 2 containing type species of *Troglonectes*, *T. furcocaudalis* (Zhu and Cao, 1987), *T. barbatus* (Gan, 2013), *T. donglanensis* (Wu, 2013), *T. duanensis* (Lan, 2013), *T. macrolepis* (Huang, Du, Chen, and Yang, 2009), *T. microphthalmus* (Du, Chen, and Yang, 2008), and *T. translucens* (Zhang, Zhao, and Zhang, 2006); and clade 3 containing *T. daqikongensis* (Deng, Wen, Xiao, and Zhou, 2016), *T. lihuensis* (Wu, Yang, and Lan, 2012), and *T. elongatus* (Tang and Zhang, 2012) [4]. These *Troglonectes* species possess two different types of posterior nostrils: i.e., anterior margin of posterior nostril present (*T. retrodorsalis*, *T. furcocaudalis*, *T. barbatus*, *T. macrolepis*, and *T. microphthalmus*) and anterior margin of posterior nostril occupied by anterior nostril (*T. lihuensis*, *T. elongatus*, and *T. dongganensis*). However, the taxonomic status and systematic relationships of *Troglonectes* need further morphological and molecular clarification.

## Data Availability

The morphometric and meristic data of *Guinemachilus pseudopulcherrimus* sp. nov. and *Paranemachilus chongzuo* used to support the findings of this study have been

deposited in the ScienceDB repository (<https://www.scidb.cn/s/yEVFzq>). The photo and phylogenetic tree used to support the findings of this study are included within the article.

### Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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### Supplementary Materials

Supplementary materials contained the sequences used in this study with GenBank accession nos., locality, and references. These reference sequences in NCBI GenBank were retrieved for phylogenetic tree reconstruction. (*Supplementary Materials*)

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