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A new species of *Yunnanilus* (Cypriniformes, Nemacheilidae) from Yunnan, southwest China

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Abstract

A new species of *Yunnanilus* is described from the Nanpanjiang River, Yunnan, China. The new species, *Yunnanilus polylepis*, can be distinguished from other species of *Yunnanilus* by the following combination of characteristics: Processus dentiformis absent; eye diameter smaller than interorbital width; outer gill raker absent and 10 inner gill rakers on first gill arch; whole trunk covered by scales; nine branched dorsal-fin rays; 10 or 11 branched pectoral-fin rays; six branched pelvic-fin rays. Despite our phylogenetic analysis, which sheds light on the complex relationships among *Yunnanilus* species, the majority of *Yunnanilus* species are restricted to more localized environments and habitats. It is urgent to address the environmental threats that jeopardize their survival, especially given their generally restricted distribution.

Key Words

Loach, mitochondrial gene, morphology, Nanpanjiang River, taxonomy

Introduction

Species belonging to the genus *Yunnanilus* Nichols, 1925 are primarily found in lakes, marshes, and slow-flowing waters, exhibiting an affinity for karstic regions, particularly in the Yunnan and Sichuan provinces of China (Du et al. 2021). Kottelat and Chu (1988), who identified eight valid species and six previously undescribed species from the Yunnan Plateau. Subsequently, Yang and Chen (1995) divided the species of *Yunnanilus* into the *Y. nigromaculatus* and *Y. pleurotaenia* species groups based on the absence or presence of lateral line and cephalic lateral line canals, respectively. Prokofiev (2010) classified the family Nemacheilidae into five tribes, i.e., Lefuini, Nemacheilini, Triplophysini, Vaillantellini, and Yunnanilini. However, the monophyly of the Yunnanilini tribe was not supported in subsequent studies, with Du et al. (2021) and Luo et al. (2023) revising the classification of Yunnanilini using both morphological characteristics and molecular evidence, resulting in the placement of the *Y. nigromaculatus* group into *Eonemachilus* Berg, 1938, *Y. pulcherrimus* Yang, Chen & Lan, 2004 into *Micronemacheilus* Rendahl, 1944, *Y. retrodorsalis* (Lan, Yang & Chen, 1995) into *Troglonectes* Zhang & Zhao, 2016, and *Y. jinxiensis* Zhu, Du & Chen, 2009 into *Paranemachilus* Zhu, 1983. In addition, Du et al.

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(2023) delineated the phylogenetic relationships among Chinese nemacheilids possessing tube-shaped anterior nostrils, categorizing the spatial relationship between the anterior and posterior nostrils into three distinct types, i.e., separated, adjacent, and closely set. Within this framework, Du et al. (2023) described a new genus, *Guinemachilus* Du et al., 2023, into which *Y. bailianensis* Yang, 2013 and *Y. longibarbatus* Gan, Chen & Yang, 2007 were placed. Subsequently, He et al. (2024) described a new species, *Yunnanilus Yangi* He et al., 2024, from Nanpan Jiang based on morphological and molecular data. Currently, 19 species of *Yunnanilus* have been recognized, with diagnostic characters including inferior mouth, anterior and posterior nostrils separated, anterior nostril base tube-shaped and tip without elongated barbel-like structure, and lateral line and cephalic lateral-line canals present (Kottelat and Chu 1988; Du et al. 2021, 2023).

In November 2023, 13 *Yunnanilus* specimens were collected from a tributary of the Nanpanjiang River in Huaning County, Yuxi City, Yunnan Province, China. Based on morphological characteristics and molecular evidence, these specimens represent a previously undescribed species of *Yunnanilus*. Herein, we provide a description of the new species and its comparison to congeners.

Materials and methods

All care and use of experimental animals complied with the relevant laws of the Chinese Laboratory of Animal Welfare and Ethics (GB/T 35892-2018). Specimens were rapidly euthanized by an overdose of anesthetic clove oil soon after being collected. Five specimens were preserved in 99% ethanol for molecular analyses, and eight specimens were stored in 10% formalin for morphological study. Specimens were deposited in the Kunming Natural History Museum of Zoology, Kunming Institute of Zoology (KIZ), Chinese Academy of Sciences (CAS).

All counts and measurements followed Kottelat (1990). The data were initially processed using Excel software for preliminary statistical analysis. Genomic DNA was extracted from fins fixed in ethanol. Partial sequences of the mitochondrial cytochrome c oxidase subunit I (COI) and cytochrome *b* (cyt *b*) were sequenced by Tsingke Biotechnology Co., Ltd. (China). All sequences were assembled by Seqman in the DNAstar software package and aligned in MEGA v11.0 (Tamura et al. 2021). Sequences have been submitted to GenBank (Accession Nos. PP254216–254236 for COI, PP262976–62996 for cyt *b*). The phylogenetic position of *Yunnanilus polylepis* sp. nov. was determined by maximum likelihood (ML) and Bayesian inference (BI) methods, which were implemented in the CIPRES Science Gateway (Miller et al. 2010). The ML was constructed in RAxML-HPC v8 (Stamatakis 2014). Selected was the rapid bootstrapping configuration, and the bootstrapping iterations were 1000. The BI tree was conducted by MrBayes in XSEDE v3.2.7a (Ronquist et al. 2012). Two runs were performed simultaneously with four Markov chains starting from a random tree. The chains were run for five million generations and sampled every 100 generations. The first 25% of sampled trees were discarded as burn-in, and the remaining trees were used to create a consensus tree and estimate Bayesian posterior probabilities (BPPs). The constructed phylogenetic trees were viewed and edited by FigTree v1.4.4 (Rambaut 2009).

Results

***Yunnanilus polylepis* sp. nov.**

https://zoobank.org/B34187E3-BE59-4EC6-90AE-72F3266CFD89

Type materials. *Holotype*. KIZ2023000009 (Kunming Natural History Museum of Zoology, KIZ, CAS), female, 43.7 mm standard length (SL), Qixitan Park, Panxi Town, Huaning County, Yuxi City, Yunnan, P. R China; Nanpanjiang River; 24.2434°N, 103.1221°E, C.S. Yang collected in November 2023.

***Paratypes*.** Seven specimens. KIZ2023000010–14, female, 31.9–37.1 mm SL, KIZ2023000039–40, male, 30.3–31.8 mm SL; same as holotype.

Other materials. DLN20230180–184, preserved in 99% ethanol for molecular study, same as type specimens.

Etymology. The specific name *polylepis* is derived from the characteristic of being entirely covered by scales Gender: Masculine. We suggest the Chinese and English common names as “多鳞云南鳅” and “densely scaled Yunnan loach,” respectively.

Diagnosis. The new species is distinguished from all other members of the genus based on the following characters: whole trunk covered by scales; processus dentiformis absent; eye diameter smaller than interorbital width; nine branched dorsal-fin rays; 10 or 11 branched pectoral-fin rays; six branched pelvic-fin rays; outer gill raker absent and 10 inner gill rakers on first gill arch.

Description. Morphometric and meristic data are given in Table 1. Whole trunk covered with small and dense tubercles. Greatest body depth anterior to dorsal-fin origin, posterior portion gradually compressed from dorsal-fin to caudal-fin base. Head length longer than depth and deeper than width. Snout slightly blunt, shorter than postorbital length of head. Eye diameter smaller than interorbital width, posterior nostril closer to anterior margin of eye than to tip of snout; anterior and posterior nostrils separated, distance greater than diameter of posterior nostril, base of anterior nostril tube-shaped, not elongated to barbel-like (Fig. 1I).

Body densely scaled except for head and thorax; pectoral-fin origin to pelvic-fin origin covered by smaller and sparse scales. Upper jaw processus dentiformis absent. Three pairs of barbels, two rostral pairs and one maxillary pair; inner rostral barbel reaching posterior nostril; outer rostral barbel reaching anterior margin of eye; maxillary barbel reaching posterior margin of eye.

Table 1. Morphometric and meristic data of *Yunnanilus polylepis* sp. nov.

Characters	Holotype	Paratypes (Mean±*SD*)
Total length (mm)	53.8	38.1–45.6 (42.2±2.8)
Standard length (mm)	43.7	30.3–37.1 (33.9±2.6)
Percent of standard length (%)		
Deepest body depth	15.7	16.3–19.6 (17.6±1.1)
Head width	13.4	12.6–15.7 (13.9±1.1)
Lateral head length	25.2	25.2–28.2 (26.5±1.0)
Prepelvic length	54.3	52.5–55.9 (54.3±1.4)
Preanal length	75.4	72.9–78.4 (76.0±2.1)
Preanus length	71.9	70.1–77.3 (73.7±2.3)
Caudal-peduncle length	12.5	11.6–13.7 (13.0±0.8)
Caudal-peduncle depth	10.1	10.0–11.5 (10.6±0.6)
Percent of lateral head length (%)		
Head width	53.0	49.9–57.7 (52.4±2.9)
Head depth	57.4	51.5–62.4 (57.9±4.1)
Eye diameter	19.6	16.7–27.0 (21.8±3.3)
Interorbital width	27.0	23.9–32.9 (29.4±3.0)
Snout length	39.4	31.1–41.7 (37.7±4.2)
Percent of caudal-peduncle length (%)		
Caudal-peduncle depth	80.8	73.2–88.7 (81.8±5.6)
Dorsal-fin rays	iv, 9	iv, 9
Pectoral-fin rays	i, 11	i, 10–11
Pelvic-fin rays	i, 6	i, 6
Anal-fin rays	iii, 5	iii, 5
Branched caudal-fin rays	16	15

Dorsal fin with four unbranched and nine branched rays; origin nearer to snout tip than to base of caudal fin; pectoral fin with one unbranched and 10 or 11 branched rays (mostly 10), inserted immediately anterior to vertical through posteriormost point of operculum; pelvic fin with one unbranded and six branched rays, tips of pelvic fin not reaching anus; anal fin with three unbranched and five branched rays, origin closer to anus; caudal fin emarginate, with 15 or 16 branched rays (mostly 15). Ten inner gill rakers, without outer gill rakers on the first gill arch; lateral line incomplete, with 15–20 lateral line pores, reaching between tip of pectoral-fin and dorsal-fin origin; cephalic lateral system with 13–14+3 infraorbital canal pores, 7–9 supraorbital canal pores, 6–8 supratemporal canal pores, and 9–10 preoperculomandibular canal pores.

Stomach U-shaped, intestine long and straight (Fig. 2B). Swim bladder divided into two chambers, anterior chamber covered by dumbbell-shaped bony capsule, posterior chamber developed, connected with anterior chamber by slender tube, tube length about half of posterior chamber length (Fig. 2A).

Coloration. In life, both sexes, head and trunk with grayish background color. Lower margin of eye to dorsal head surface dark brown, dorsal head with heart-shaped dark brown pattern, ventral head surface without color pattern.

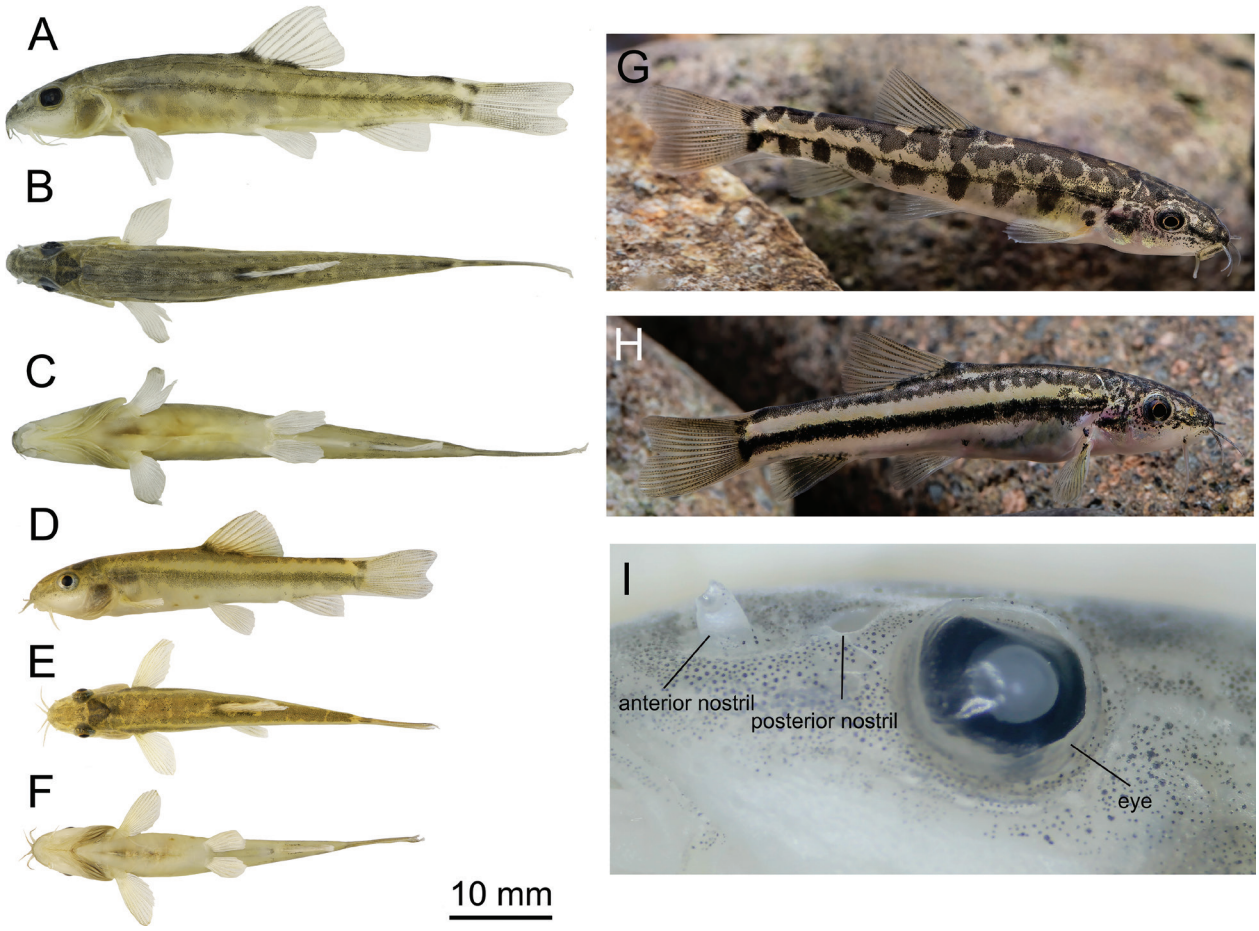

Figure 1. Morphometric characters of *Yunnanilus polylepis* sp. nov. **A–C.** Lateral, dorsal, and ventral views of female, holotype KIZ2023000009; **D–F.** Lateral, dorsal, and ventral views of male, paratype KIZ2023000041; **G–H.** Living photo of female and male; **I.** Location of anterior and posterior nostrils.

Predorsal trunk with five or six dark brown blotches, larger width than interspace. Four or five dark brown blotches after dorsal fin. Two elliptical, dark brown spots at base of dorsal fin, two dark brown spots at base of caudal fin. Fin rays with dark pigments, fin membrane hyaline. In females, upper line of flank with 12–14 dark brown large spots (Fig. 1G). In males, body with black longitudinal stripe on both sides (Fig. 1H). In formalin-fixed specimens, lateral stripe and blotches somewhat faded, body generally light yellow.

Distribution and habitat. *Yunnanilus polylepis* sp. nov. is currently only known from Qixitan Park, Panxi Town, Huaning County, Yuxi City, Yunnan, China; Nanpanjiang River (24.2434°N, 103.1221°E). This species inhabits a deep pool with water depths ranging from 3 to 8 m, characterized by a rich presence of macrophytes (Fig. 3). Other fish species present in the pool include *Discogobio brachyphysallidos* and *Sinocyclocheilus* sp. Despite its confined distribution, the population of *Yunnanilus polylepis* sp. nov. remains stable, largely due to the enforcement of a fishing ban within the park.

Genetic comparisons. Of the 1737 bp in combined alignment, *Y. polylepis* sp. nov. and *Y. pleurotaenia* were amplified in this study. These sequences were used for molecular phylogenetic analysis together with 34 complete mitochondrial genomes and six cyt *b* sequences from GenBank. *Parabotia fasciata* Dabry de Thiersant, 1872 and *Leptobotia elongata* (Bleeker, 1870), two botiid species, were used as outgroups. Given that BI and ML analyses produced overall identical topologies, only the BI tree with Bayesian posterior probabilities (BPP) and bootstrap support (BS) values are presented here (Fig. 4). The phylogenetic tree strongly supports samples of *Yunnanilus polylepis* sp. nov. to group into *Yunnanilus*. Furthermore, *Yunnanilus polylepis* sp. nov. was identified as a sister to the clade containing *Y. analis*, *Y. chuanheensis*, *Y. jiuchiensis*, and *Y. pleurotaenia* (BPP = 1; BS = 100). However, the molecular phylogenies do not support the monophyly of *Yunnanilus*. *Yunnanilus yangi* was weakly supported to be a sister group to *Eonemachilus* (BPP = 59; BS = 61), and then cladded together with those specimens of *Yunnanilus* (Fig. 4).

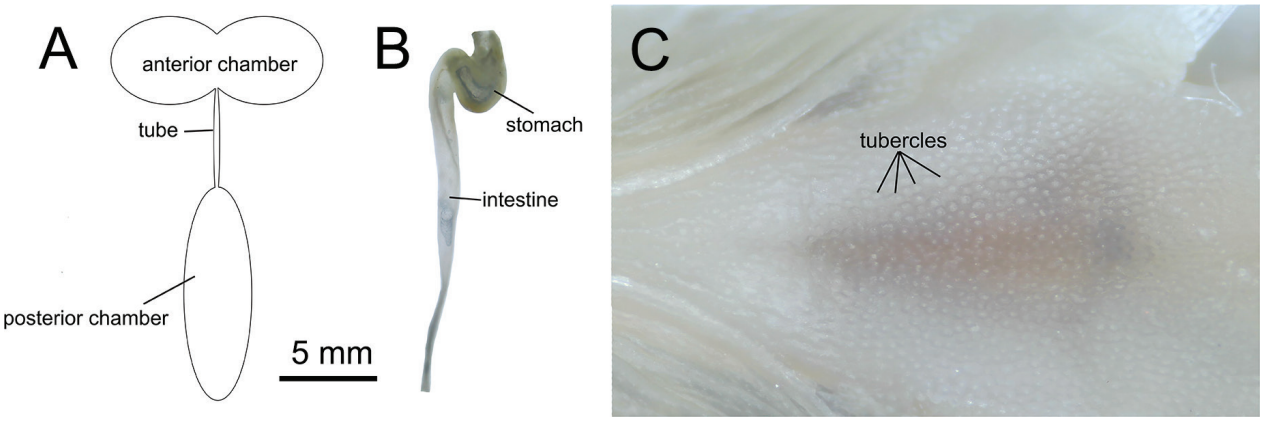

Figure 2. The air baldde (**A**), stomach and intestine (**B**), KIZ 2023000010, and tubercles on the trunk (**C**), KIZ2023000011 of *Yunnanilus polylepis* sp. nov.

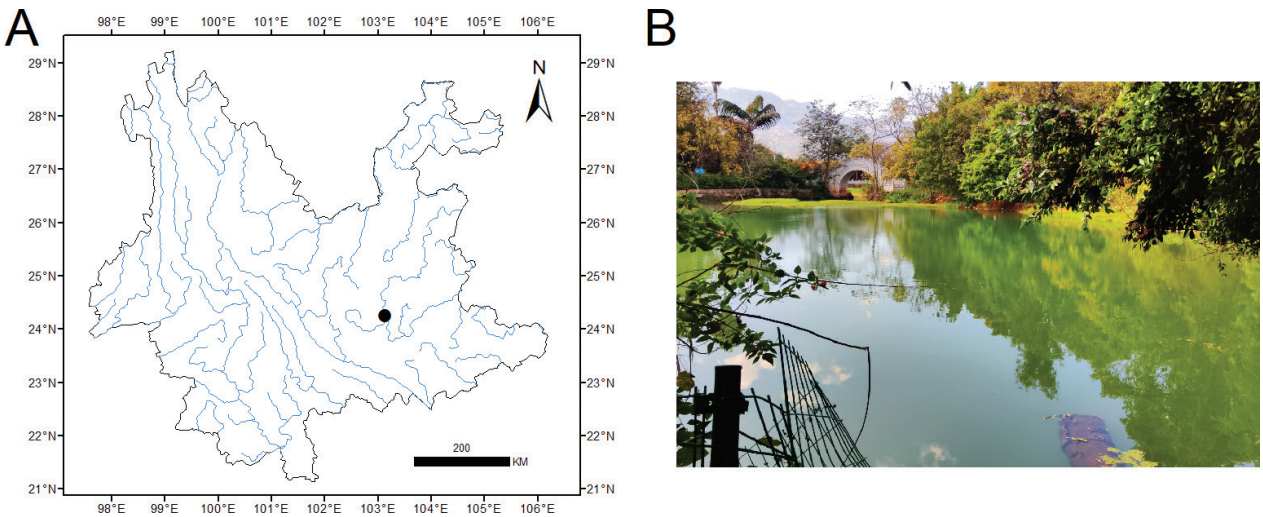

Figure 3. Type locality of *Yunnanilus polylepis* sp. nov. **A.** Distribution map; **B.** Habitat photo of the type locality at the time of collection.

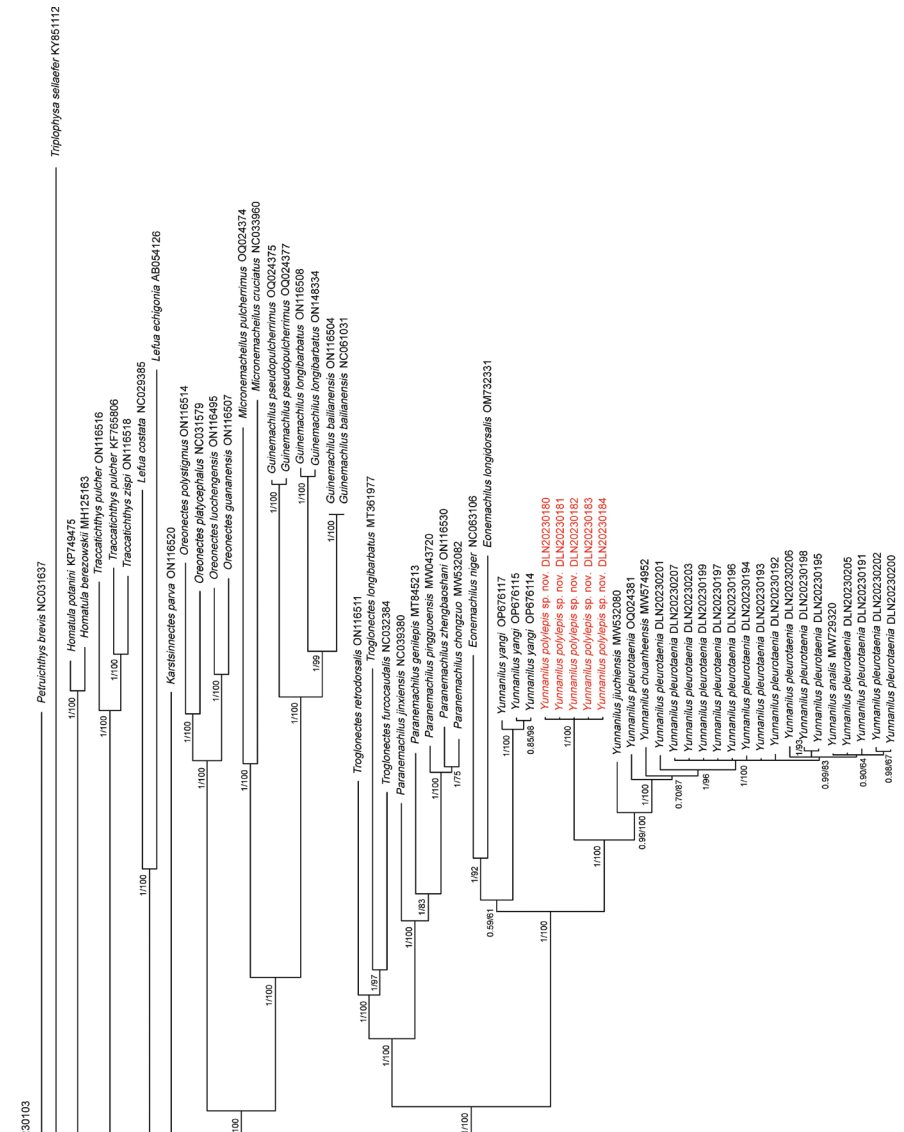

Figure 4. Bayesian phylogram of *Yunnanilus* based on a concatenated dataset of mitochondrial cytochrome c oxidase subunit I (COI) and cytochrome *b* (cyt *b*) sequences. The numbers on the branches represent BPPs from BI and bootstrap supports from ML.

Key to species of the genus Yunnanilus

1 Body scaleless 2
– Body covered by scales 7
2 Caudal fin forked 3
– Caudal fin emarginated 4
3 Outer gill raker present on first gill arch ..... *Y. forkicaudalis*
– Outer gill raker absent on first gill arch ..... *Y. discoloris*
4 Eye diameter smaller than interorbital width 5
– Eye diameter larger than interorbital width 6
5 Processus dentiformis present on upper jaw ..... *Y. paludosus*
– Processus dentiformis absent on upper jaw ..... *Y. yangi*
6 Eight inner gill rakers on first gill arch ..... *Y. analis*
– Ten inner gill rakers on first gill arch ..... *Y. beipanjiangensis*
7 Posterior trunk covered by scales 8
– Whole body covered by scales except head 11
8 Caudal fin forked, outer gill raker present on first gill arch ..... *Y. macrositanus*
– Caudal fin emarginated, outer gill raker absent on first gill arch 9
9 Processus dentiformis absent on upper jaw ..... *Y. sichuanensis*
– Processus dentiformis present on upper jaw 10
10 Interorbital width less than 25% of lateral head length, caudal peduncle length larger than 130% of its depth ..... *Y. nanpanjiangensis*
– Interorbital width larger than 26% of lateral head length, caudal peduncle length smaller than its depth ..... *Y. elakatis*
11 Eye diameter larger than interorbital width 12
– Eye diameter smaller than interorbital 13
12 Processus dentiformis absent on upper jaw; eight inner gill rakers on first gill arch ..... *Y. jiuchiensis*
– Processus dentiformis present on upper jaw, 10–12 inner gill rakers on first gill arch ..... *Y. longiballa*
13 Outer gill raker present on first gill arch 14
– Outer gill raker absent on first gill arch 15
14 Predorsal length 55%–58% of SL; 14–15 inner gill rakers on first gill arch ..... *Y. macrolepis*
– Predorsal length 51%–55% of SL; 11–12 inner gill rakers on first gill arch ..... *Y. spanisbripes*
15 Processus dentiformis absent on upper jaw 16
– Processus dentiformis present on upper jaw 17
16 Ventral area between pectoral and pelvic fins scaleless ..... *Y. chuanheensis*
– Ventral area between pectoral and pelvic fins covered by scales ..... *Y. polylepis* sp. nov.
17 12–13 branched pectoral fin rays; eye diameter less than 12% of lateral head length ..... *Y. macrogaster*
– Ten or eleven branched pectoral fin rays; eye diameter larger than 17% of lateral head length 18
18 10–13 inner gill rakers on first gill arch; six branched pelvic fin rays ..... *Y. pleurotaenia*
– Eight or nine inner gill rakers on first gill arch; seven branched pelvic fin rays ..... *Y. parvus*

Discussion

Yunnanilus polylepis sp. nov. possesses typical characteristics of the genus *Yunnanilus*, including an inferior mouth, anterior and posterior nostrils separated, a tube-shaped base of anterior nostrils that is not elongated into a barbel-like structure, and lateral line and cephalic lateral-line canals present (Du et al. 2021, 2023).

To date, 20 species of *Yunnanilus*, including the newly described species, have been recorded from the Yunnan and Sichuan provinces. Among these species, 12 occur in the Nanpanjiang River (*Y. analis* Yang, 1990, *Y. chui* Yang, 1991, *Y. elakatis* Cao & Zhu, 1989, *Y. forkicaudalis* Li, 1999, *Y. macrogaster* Kottelat & Chu, 1988, *Y. macrolepis* Li, Tao & Mao, 2000, *Y. macrositanus* Li, 1999, *Y. nanpanjiangensis* Li, Tao & Lu, 1994, *Y. paludosus* Kottelat & Chu, 1988, *Y. parvus* Kottelat & Chu, 1988, *Y. yangi* He et al., 2024, and *Yunnanilus polylepis* sp. nov.), six occur in the Yangtze River (*Y. discoloris* Zhou & He, 1989, *Y. jiuchiensis* Du, Hou, Chen & Yang, 2018, *Y. longibulla* Yang, 1990, *Y. pleurotaenia* (Regan, 1904), *Y. sichuanensis* Ding, 1995, and *Y. spanisbripes* An, Liu & Li, 2009), and one species each occurs in the Beipanjiang (*Y. beipanjiangensis* Li, Mao & Sun, 1994) and Honghe rivers (*Y. chuanheensis* Jiang, Zhao, Du & Wang, 2021). Within the genus, *Y. analis*, *Y. beipanjiangensis*, *Y. chui*, *Y. discoloris*, *Y. forkicaudalis*, *Y. paludosus*, and *Y. yangi* are characterized by the absence of scales, while *Y. elakatis*, *Y. macrositanus*, and *Y. nanpanjiangensis* are characterized by scales exclusively present on the caudal peduncle. Although *Yunnanilus polylepis* sp. nov., *Y. chuanheensis*, *Y. jiuchiensis*, *Y. longibulla*, *Y. macrogaster*, *Y. macrolepis*, *Y. parvus*, *Y. pleurotaenia*, and *Y. spanisbripes* share the

character of bodies covered by scales, *Y. chuanheensis* and *Y. jiuchiensis* are scaleless between the pectoral-fin and pelvic-fin bases. *Yunnanilus polylepis* sp. nov. can be further distinguished from *Y. chuanheensis* by six branched pelvic fin rays (vs. seven) and from *Y. jiuchiensis* by 10 inner gill rakers on the first gill arch (vs. eight) and an eye diameter smaller than the interorbital width (vs. larger). *Yunnanilus polylepis* sp. nov. can be distinguished from *Y. longibulla*, *Y. macrogaster*, *Y. macrolepis*, *Y. parvus*, *Y. pleurotaenia*, and *Y. spanisbripes* by processus dentiformis absent (vs. present). It can be further distinguished from *Y. longibulla* by eye diameter smaller than interorbital width (vs. larger), six branched pelvic fin rays (vs. seven or eight), caudal peduncle depth 12%–14% of SL (vs. 8%–10%), from *Y. macrolepis* and *Y. spanisbripes* by outer gill raker absent on first gill arch (vs. present), predorsal length 51%–55% of SL (vs. 56%–58% in *Y. macrolepis*), preanal length 73%–78% of SL (vs. 80%–82% in *Y. macrolepis*, 79%–82% in *Y. spanisbripes*), from *Y. macrogaster* by 10–11 branched pectoral fin rays (vs. 12 or 13), six branched pelvic fin rays (vs. seven), 10 inner gill rakers on first gill arch (vs. 13), body height 16%–20% of SL (vs. 22%–23%), eye diameter 17%–27% of lateral head length (vs. 12%), and from *Y. parvus* by nine branched dorsal fin rays (vs. eight), six branched pelvic fin rays (vs. seven), body height 15%–20% of SL (vs. 20%–23%).

Based on phylogenetic analysis, *Y. analis* was clustered with *Y. pleurotaenia*. However, due to the ambiguous geographical origin of *Y. analis* in GenBank (MW729320), its validity is not discussed in this study. Although molecular evidence from He et al. (2024) supported that *Y. yangi* belongs to *Yunnanilus*, the wrong sequence of *niger* was used in this publication (Du et al. 2023). Additionally, the phylogenetic tree weakly supported *Y. yangi* as a sister group to *Eonemachilus*; hence, the phylogenetic relationship between *Eonemachilus* and *Yunnanilus* will be discussed in the future. Despite the widespread distribution of *Y. pleurotaenia*, the majority of *Yunnanilus* species are restricted to more localized environments and habitats, including small ponds, streams, and lakes. While our phylogenetic analysis sheds light on the complex relationships among *Yunnanilus* species, it also underscores the urgency of addressing the environmental threats that jeopardize their survival, especially given their generally restricted distribution. Overfishing, agricultural runoff and siltation, organic pollution, habitat degradation, and increasing droughts have profoundly affected these endemic species. Notably, Jin et al. (2018) highlighted that the frequency, intensity, and impact of regional droughts have increased in Yunnan in recent years, with a particularly severe drought occurring in the spring and early summer of 2019 due to an abnormally delayed rainy season (Ding and Gao 2020). However, the specific effects of such droughts on the *Yunnanilus* population remain uncertain. Future studies focusing on taxonomic classification, biodiversity surveillance, and conservation assessments are deemed essential.

Nomenclatural acts registration

The electronic version of this article in portable document format represents a published work according to the International Commission on Zoological Nomenclature (ICZN), and hence the new name contained in the electronic version is effectively published under the Code in the electronic edition alone (see Articles 8.5–8.6 of the Code). This published work and the nomenclatural acts it contains have been registered in ZooBank LSIDs (Life Science Identifiers) and can be resolved, and the associated information can be viewed through any standard web browser by appending the LSID to the prefix http://zoobank.org/.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Z.-X. Q. and L.-N.D. measured the specimens, analyzed the data, conceived and designed the study, and prepared the manuscript. W.-H. S. analyzed the molecular data and constructed the phylogenetic tree. Z.-X. W. provided funding for the field survey. All authors read and approved the final version of the manuscript.

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