

Yunnanilus chuanheensis, a new loach species (Cypriniformes: Nemacheilidae) from the upper Lixianjiang River in Yunnan, China

DEAR EDITOR,

A new loach species (Cypriniformes: Nemacheilidae: Yunnanilini), *Yunnanilus chuanheensis* **sp. nov.**, was caught from Chuanhe in the upper reaches of the Lixianjiang River, a tributary of the Red River in Nanjian County, Yunnan Province, China. This species is a member of the traditional *Y. pleurotaenia* species group based on the presence of a lateral line and cephalic lateral-line canals. It can be distinguished from other species in the *Y. pleurotaenia* species group by the following characters: lips without papillae, anterior and posterior nostrils separated, whole body scaled, eye diameter smaller than interorbital width, outer gill raker absent on first gill arch, eye diameter greater than 18% of head length, 10–11 inner gill rakers on first gill arch, and lateral line not extending to vertical through dorsal fin insertion. To the best of our knowledge, this is the first *Yunnanilus* species recorded from the Red River drainage.

Loaches belong to the suborder (sometimes called superfamily) Cobitoidei within the order Cypriniformes. They are small benthic fish known throughout Eurasia, with high morphological and habitat diversity (Kottelat, 2012). The family-level phylogenetic relationships within Cobitoidei, as well as taxonomic classification and hierarchical relationships at the subfamily, tribe, and genus level, have been frequently discussed, but remain unresolved (see Kottelat, 2012; Prokofiev, 2010). The subfamily Nemacheilinae, which is mainly distributed in China and Southeast Asia, is one of the most diverse lineages of the superfamily Cobitoidei (Freyhof & Serov, 2001). It is also one of the most taxonomically complex groups, with poorly studied systematic and phylogenetic relationships (Prokofiev, 2010).

Loaches in Yunnanilini, also known as the *Yunnanilus* group

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(sensu Prokofiev, 2004), represent a Nemacheilinae tribe (erected by Prokofiev, 2010) that includes eight nominal generic taxa (*Yunnanilus* Nichols, 1925; *Eonemachilus* Berg, 1938; *Micronemacheilus* Rendahl, 1944; *Paranemachilus* Zhu, 1983; *Heminoemacheilus* Zhu and Cao, 1987; *Petruichthys* Menon, 1987; *Protonemacheilus* Yang and Chu, 1990; and *Traccatichthys* Freyhof and Serov, 2001). Yunnanilini is characterized by several features: i.e., bony capsule of the swim bladder is open posteriorly, swim bladder is stretched in part, pre-ethmoid-l is present, pelvic fin contains three radials, occipital part of the skull is not elongated, scale cover is well developed, and abdominal axillary lobe is not well developed (Prokofiev, 2010); however, the inter-generic diagnostic characteristics are debatable, and the phylogenetic relationships, especially the validity of some nominal genera, remain unclear.

Yunnanilus, the type genus in Yunnanilini, has 38 nominal species, including 34 species considered valid according to FishBase (Froese & Pauly, 2019). Yang & Chen (1995) previously divided *Yunnanilus* into the *Y. nigromaculatus* and *Y. pleurotaenia* species groups based on the absence or presence of lateral lines and cephalic lateral-line canals, respectively. Species described since have usually been assigned to either group according to their combination of diagnostic characters. According to the species descriptions and validities so far, those belonging to the *Y. nigromaculatus* species group include *Y. altus*, *Y. bailianensis*, *Y. baijiangensis*, *Y. caohaiensis*, *Y. longibaratus*, *Y. longidorsalis*, *Y. niger*, *Y. nigromaculatus*, *Y. niulanensis*, *Y. obtusirostris*, *Y. pachycephalus*, *Y. qujinensis*, and *Y. yangzonghaiensis*. Correspondingly, all other species in

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Yunnanilus belong to the *Y. pleurotaenia* species group.

During an ichthyological sampling of the Lixianjiang River (upper Red River) drainage by the first two authors, several loach specimens were collected. The specimens showed typical characters resembling the genus *Yunnanilus*, including an inferior mouth, separated anterior and posterior nostrils, and tube-like anterior nostrils that were not elongated into a barbel-like structure. Through morphological and molecular examination of the specimens, we identified a new species in the *Y. pleurotaenia* species group, described herein as *Yunnanilus chuanheensis* sp. nov.

Specimens were first preserved in 10% formalin then transferred into 75% alcohol and deposited in the Kunming Natural History Museum of Zoology, Kunming Institute of Zoology (KIZ), Chinese Academy of Sciences. Measurements and counts were taken point-to-point with a digital caliper (0.1 mm precision) on the left side of the fish whenever possible. Methods of counts and measurements followed Chu & Chen (1989). The table of morphometric and meristic data followed Du et al. (2015).

To evaluate the genetic distance between the new species and its congeners and to test its phylogenetic position, the mitochondrial cytochrome b (cyt b) gene was sequenced from one individual (GenBank accession No.: MW574952). All cyt b sequences in *Yunnanilus* deposited in NCBI were downloaded as background information for molecular analysis. Inter-specific pairwise genetic distances were calculated using Kimura's two-parameter (K2P) model (Kimura, 1980) in MEGA X (Kumar et al., 2018), and a simple maximum-likelihood (ML) tree was constructed to illustrate the phylogenetic relationships using the general time reversible (GTR) model as the best substitution model in MEGA X.

Taxonomy

Yunnanilus chuanheensis sp. nov. Jiang, Zhao, Du & Wang

<http://zoobank.org/A7398CE3-7F29-458B-A928-193271935C6E>

Figure 1A–G; Table 1.

Holotype: KIZ 2016007379, 46.8 mm standard length (SL); China, Yunnan Province, Nanjian County, Baohua Town; Chuanhe, upper reaches of the Lixianjiang River, a tributary of the Red River (N24°52'07.2", E100°28'09.6"; ~1 844 m a.s.l.); 28 April 2016; Wan-Sheng Jiang, Ya-Peng Zhao, and Shu-Sen Shu col.

Paratypes: KIZ 2016007380–90, 11, 35.4–49.4 mm SL, same data as holotype. Tissues of the pectoral fin (right side) of some specimens were taken for DNA sequencing.

Diagnosis: The new species belongs to the *Y. pleurotaenia* species group and is distinguished from the *Y. nigromaculatus* species group based on lateral line and cephalic lateral-line canals present (vs. sensory canals absent). For the other species within the *Y. pleurotaenia* species group, *Yunnanilus chuanheensis* sp. nov. can be distinguished from: (1) *Y. cruciatus* and *Y. pulcherrimus* by papillae on lips absent (vs.

present); (2) *Y. jinxiensis* by anterior and posterior nostril separated (vs. closely placed); (3) *Y. analis*, *Y. beipanjiangensis*, *Y. chui*, *Y. discoloris*, *Y. elakatis*, *Y. forkicaudalis*, *Y. macrositanus*, *Y. nanpanjiangensis*, *Y. paludosus*, and *Y. sichuanensis* by whole body scaled (vs. scaleless or only caudal peduncle scaled); (4) *Y. jiuchiensis* and *Y. longibulla* by eye diameter smaller than interorbital width (vs. larger); (5) *Y. ganheensis*, *Y. macrolepis*, and *Y. spanisbripes* by outer gill raker on first gill arch absent (vs. present); (6) *Y. macrogaster* by eye diameter greater than 18% of head length (vs. smaller than 12%); (7) *Y. parvus* by 10–11 inner gill rakers on first gill arch (vs. 8–9); and (8) *Y. pleurotaenia* by lateral line not extending to vertical through dorsal fin insertion (vs. extending to).

Description: Morphometric and meristic data are presented in Table 1. Body slender. Anterior part of body to dorsal-fin origin cylindrical; caudal peduncle laterally compressed; deepest body depth usually at point anterior to dorsal-fin origin. Head depth greater than width. Snout length less than or equal to postorbital length. Anterior and posterior nostrils widely separated, anterior nostril with fleshy flap forming tube at tip. Eyes normally developed, middle of lateral head length; eye diameter smaller than interorbital width, interorbital space slightly convex. Mouth inferior. Upper lip smooth with wrinkle but no papillae; lower lip with median incision, two lobes on each side. Processus dentiformis absent. Three pairs of barbels; inner rostral barbel reaching to posterior nostril; outer rostral barbel reaching to anterior margin of eye; maxillary barbel reaching to posterior margin of eye.

Dorsal-fin rays iii, 8–9; anal-fin rays iii, 5–6; pectoral-fin rays i, 11–12; pelvic-fin rays i, 7–8; branched caudal-fin rays 16; outer gill rakers absent, inner gill with 10–11 rakers on first gill arch. Lateral line incomplete, extending through to vertical at end of pectoral-fin but not to insertion of dorsal fin.

Dorsal fin truncate, insertion slightly anterior to vertical through pelvic fin insertion, closer to caudal-fin base than to snout tip. Anal fin truncate, tip close to caudal-fin base. Pectoral-fin length half of distance between pectoral- and pelvic-fin origins when adpressed. Pelvic-fin origin slightly posterior to vertical through dorsal-fin origin, closer to anal-fin origin than pectoral-fin origin. Tip of pelvic fin not reaching anus when adpressed. Anus closer to anal-fin origin than posterior insertion of pelvic fin. Caudal peduncle length slightly greater than depth. Caudal fin emarginated.

Body trunk covered with scarce scales, except on ventral area between pectoral and pelvic fins. Air bladder with two chambers; anterior chamber enclosed in bony capsule, posterior chamber large and oval, filling body cavity freely to pelvic-fin origin; anterior and posterior chamber contacted by slender tube, about half length of posterior chamber length. Stomach "U" shaped; intestine straight.

Coloration: In life: both sexes, body brown to grayish yellow, with large brown blotches irregularly distributed on dorsal and lateral body, connected to longitudinal black stripe on lateral line (more distinct in males than females, Figure 1A, C). In

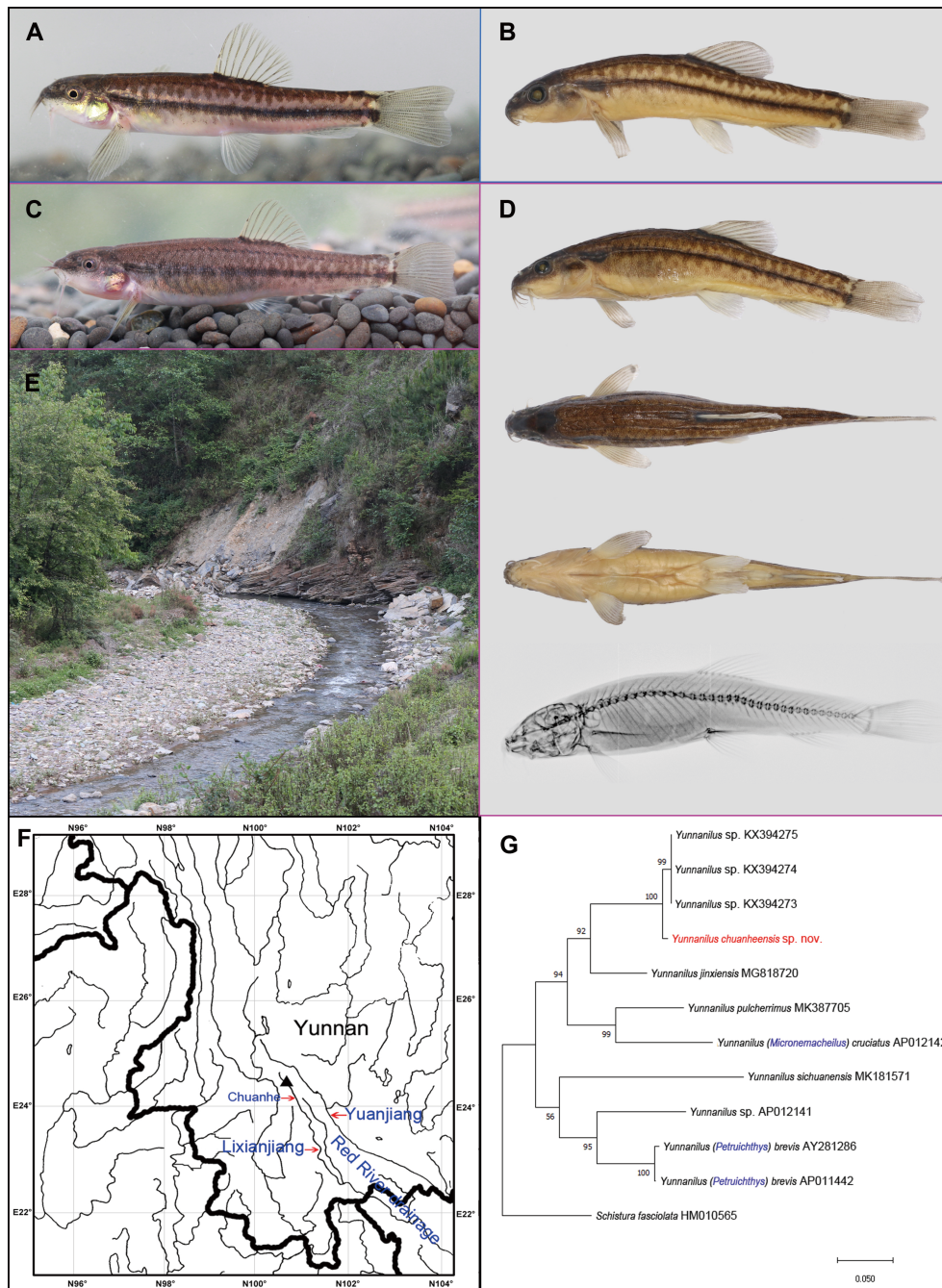


Figure 1 Type photo, habitat, distribution, and phylogenetic position of *Yunnanilus chuanheensis* sp. nov.

Pictures show living (A) and preserved paratype (B, male, KIZ 2016007380); living (C) and lateral, dorsal, ventral, and X-ray views of preserved holotype (D, female, KIZ 2016007379); habitat photo of type locality at time of collection (E); distribution map (F); and phylogenetic position of *Yunnanilus chuanheensis* sp. nov. based on all known sequences of *Yunnanilus* from NCBI (G).

breeding season, males usually with elongated and subacute pectoral fins and tubercles on caudal peduncle (Figure 1A). Ventral surfaces of head and body light flesh white. Fins hyaline. In 75% ethanol: lateral stripe somewhat faded, brown blotches maintained, body generally light yellow (Figure 1B, D).

Distribution and habitat: Specimens were collected from Chuanhe in the upper reaches of the Lixianjiang River, a tributary of the Red River basin (Figure 1F). The type locality at the time of capture (dry season) consisted of clear and slow-flowing water, with a sand- and cobble-covered bottom and water depth mostly less than 1 m. The banks were fully

Table 1 Morphometric and meristic data of *Yunnanilus chuanheensis* sp. nov. Range, mean, and standard deviation (Mean±SD) include holotype values

	Holotype	Range (n=12)	Mean±SD
Total length (mm)	57.8	44.3–60.3	53±5.7
Standard length (mm)	46.8	35.4–49.4	42.4±5.2
Percent of standard length			
Body depth	18.9	14.4–20	17.9±1.5
Lateral head length	24.6	22.8–26.3	24.2±1.2
Predorsal length	55.1	52.1–59.4	55±2.1
Prepelvic length	55.6	53.1–58.6	55.2±1.5
Preanal length	76.3	76.2–80.3	78.1±1.7
Preanus length	74.5	73.8–78	76.2±1.5
Caudal peduncle length	12.4	11–13.7	12.2±0.8
Caudal peduncle depth	10.3	10.1–11.8	11±0.5
Head width	13.6	11.8–14.8	13.1±0.9
Percent of lateral head length			
Eye diameter	20.1	19.1–28.3	22.7±2.8
Interorbital width	35.3	26.7–40.8	34.8±3.9
Snout length	35.4	33–41.3	37.8±2.3
Head width	55.1	47–60.7	54.1±4.8
Head depth	60.4	54.7–70.1	63.2±4.4
Percent of caudal peduncle length			
Caudal peduncle depth	83.1	83.1–99.3	90.6±5.8
Dorsal-fin rays	iii,9	iii,8–9	
Pectoral-fin rays	i,12	i,11–12	
Pelvic-fin rays	i,7	i,7–8	
Anal-fin rays	iii,6	iii,5–6	
Caudal-fin branched rays	16	16	

covered with secondary forest, mostly shrubs (Figure 1E). Another Nemacheiline loach, *Schistura fasciolata*, was the only species collected syntopically with this new species.

Etymology: The name of the new species, *chuanheensis*, is derived from the Chinese name of the locality river, Chuanhe (川河), and therefore the Chinese name of this new species is "川河云南鳅".

Phylogenetic relationships within *Yunnanilus*: So far, only 11 nonredundant *cyt b* sequences are available from NCBI with names attributable to *Yunnanilus*. We downloaded these sequences (some extracted from mitochondrial genomes) and reconstructed a preliminary guide tree using *S. fasciolata* as the outgroup. The pairwise genetic distances between *Yunnanilus chuanheensis* sp. nov. and other nominal *Yunnanilus* species ranged from 1.3% to 22.8%. The phylogenetic tree further confirmed *Yunnanilus chuanheensis* sp. nov. as a member of the genus *Yunnanilus* (Figure 1G).

Remarks: Although the division of *Yunnanilus* into the *Y. nigromaculatus* and *Y. pleurotaenia* species groups by Yang & Chen (1995) has facilitated taxonomic studies, this classification system has not yet been tested due to very limited molecular information. Kottelat (2012) assumed that *Yunnanilus* is not a monophyletic group, and placed *Y. longidorsalis*, *Y. nigromaculatus*, and *Y. yangzonghaiensis*

into *Eonemacheilus* based on their terminal mouths and color patterns. Kottelat (2012, 2013) also placed *Y. altus*, *Y. analis*, *Y. caohaiensis*, *Y. niger*, and *Y. obtusirostris* into *Heminoemacheilus*, *Y. brevis* into *Petruichthys*, *Y. pulcherrimus* and *Y. chui* into *Micronemacheilus*, and *Y. cruciatus* into *Micronemacheilus* but without providing any substantial arguments for these classifications.

The phylogenetic tree (Figure 1G) reconstructed in this study, although preliminary, reflects that the inner relationships of *Yunnanilus* are indeed complicated. For instance, the genetic distances between *Yunnanilus chuanheensis* sp. nov. and other nominal *Yunnanilus* species fell within a very wide range, from 1.3% to 22.8%. Interestingly, both the minimum-distanced (*Y. sp.* KX-2017, 1.3%) and maximum-distanced species (*Y. sichuanensis*, 22.8%) from *Yunnanilus chuanheensis* sp. nov. were from Sichuan Province. Additionally, the other genera removed from *Yunnanilus* by Kottelat (2012), such as *Y. (Micronemacheilus) cruciatus* and *Y. (Petruichthys) brevis*, were mixed within the remaining *Yunnanilus* according to the present tree. This further suggests that a comprehensive molecular phylogenetic tree is urgently required to uncover the inner relationships of the *Yunnanilus* group. However, this is beyond the scope of the current study.

NOMENCLATURAL ACTS REGISTRATION

The electronic version of this article in portable document format will represent a published work according to the International Commission on Zoological Nomenclature (ICZN), and hence the new names contained in the electronic version are effectively published under that Code from 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, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) 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/>.

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SCIENTIFIC FIELD SURVEY PERMISSION INFORMATION

The collection of fish used in this study complied with the Wildlife Protection Act of China. Permission for field survey in Baohua Town was approved by the Nanjian Bureau of Wuliangshan National Nature Reserve.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

W.S.J. and Y.P.Z. collected the fish. W.S.J. and M.W. designed the study. W.S.J., Y.P.Z., and L.N.D. examined the fish. Y.P.Z. and L.N.D. performed morphological comparisons. W.S.J. conducted molecular analysis. W.S.J., Y.P.Z., and M.W. wrote the draft. All authors read and approved the final version of the manuscript.

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Wan-Sheng Jiang^{1,2,#,*}, Ya-Peng Zhao^{3,#}, Li-Na Du^{4,5,#},
Mo Wang^{3,*}

¹ Hunan Engineering Laboratory for Chinese Giant Salamander's Resource Protection and Comprehensive Utilization, and Key Laboratory of Hunan Forest and Chemical Industry Engineering,

Jishou University, Zhangjiajie, Hunan 427000, China

² Yunnan Key Laboratory of Plateau Fish Breeding, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan 650223, China

³ Key Laboratory for Conserving Wildlife with Small Populations in Yunnan, and Faculty of Biodiversity Conservation, Southwest Forestry University, Kunming, Yunnan 650224, China

⁴ Key Laboratory of Ecology of Rare and Endangered Species and Environmental Protection (Guangxi Normal University), Ministry of Education, Guilin, Guangxi 541004, China

⁵ Guangxi Key Laboratory of Rare and Endangered Animal Ecology, College of Life Science, Guangxi Normal University, Guilin, Guangxi 541004, China

*Authors contributed equally to this work

*Corresponding authors, E-mail: jiangwschina@163.com;
wangmo.139@163.com

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