Zoosyst. Evol. 100 (4) 2024, 1375–1385 | DOI 10.3897/zse.100.129520

PENSOFT

MUSEUM FÜR NATURKUNDE BERLIN

Description of two new species of the genus *Paranemachilus* (Cypriniformes, Nemacheilidae) from Guangxi, China

Hao-Lin Mo[1], Jian Yang[1], Peng Li[1], Li-Na Du[2,3]

1 *Key Laboratory of Wildlife Evolution and Conservation in Mountain Ecosystem of Guangxi, Nanning Normal University, Nanning, 530001, China*

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

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

https://zoobank.org/C676AE6A-A1BE-4017-B11F-516593F09A81

Corresponding authors: Jian Yang (yj1981yj@163.com); Li-Na Du (dulina@mailbox.gxnu.edu.cn)

Academic editor: Nicolas Hubert ♦ **Received** 12 June 2024 ♦ **Accepted** 18 September 2024 ♦ **Published** 14 October 2024

Abstract

This study describes two new species within the genus *Paranemachilus*. *Paranemachilus luegvetensis* **sp. nov.** and *Paranemachilus liui* **sp. nov.** can be distinguished from all recognized congeners by a combination of morphological characteristics and substantial genetic divergences. *Paranemachilus luegvetensis* **sp. nov.** is characterized by scaled cheeks; the whole body covered in scales, except for the head; 11–12 branched pectoral fin rays; 4–5 preoperculomandibular canal pores; body depth 13.5%–16.8% of standard length; and preanus length 68.3%–73.9% of standard length. *Paranemachilus liui* **sp. nov.** is characterized by scaled cheeks; the whole body covered in scales except for the head; 11–13 branched pectoral fin rays; 11–15 supraorbital canal pores at the base of the anterior nostrils; 7–9 preoperculomandibular canal pores; preanus length 67.4%–74.3% of standard length; snout length 21.4%–28.7% of lateral head length.

Key Words

cave loach, scaled cheeks, taxonomy, Xijiang River

Introduction

The genus *Paranemachilus* Zhu, 1983, is classified with the family Nemacheilidae of the order Cypriniformes. Despite its type species, *Paranemachilus genilepis* Zhu, 1983, being characterized by scaled cheeks (Zhu, 1983), later described species, such as *Paranemachilus pingguoensis* Gan, 2013, are noted for their scaleless cheeks (Lan et al. 2013). For a long time, no more species were added to the genus *Paranemachilus*. With the help of molecular biology, some species have been re-studied and classified into the genus *Paranemachilus*. Following taxonomic revisions of Yunnanilini using both morphological characteristics and molecular evidence, Du et al. (2021) placed *Yunnanilus jinxiensis* Zhu, Du, Chen & Yang, 2009 into *Paranemachilus*. Luo et al. (2023) subsequently suggested that *Heminoemacheilus* Zhu & Cao, 1987 is a synonym of *Paranemachilus*, placing the type species of *Heminoemacheilus*, i.e., *Heminoemacheilus zhengbaoshani* Zhu & Cao, 1987, into *Paranemachilus*, while *Heminoemacheilus hyalinus* Lan, Yang & Chen, 1996 and *Heminoemacheilus parvus* Zhu & Zhu, 2014 were transferred to the newly established genus *Karstsinnectes* Zhou, Luo, Wang, Zhou & Xiao, 2023. More recently, Du et al. (2023) revised the phylogenetic relationships among Chinese nemacheilids with tube-shaped anterior nostrils and undertook the description of a new species of *Paranemachilus*, *Paranemachilus chongzuo* Du et al., 2023, from Guangxi. Currently, five valid species of *Paranemachilus* are recognized, all of which inhabit underground rivers in Guangxi Province, China. The diagnostic characteristics of the genus include anterior

Copyright *Mo, H.-L. et al*. This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

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, lips without papillae, lateral line incomplete, disappearing behind vertical at end of pectoral fin, and air-bladder lying free in abdominal cavity (Zhu 1983; Zhu and Cao 1987; Lan et al. 2013; Du et al. 2023).

Fieldwork conducted in 2017 and 2023 resulted in the collection of two and nine loach specimens, respectively, in Liuzhou City, Guangxi Province, China. Additionally, in June 2023, eight loach specimens were collected from Wuming County, Nanning City, Guangxi, China. Based on morphological analysis and molecular evidence, these specimens represent two previously undescribed species of *Paranemachilus*, formally described herein as *Paranemachilus liui* sp. nov. and *Paranemachilus luegvetensis* sp. nov.

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). Collection and preservation of specimens followed Li and Gao (2016). Specimens of *Paranemachilus liui* sp. nov. and *Paranemachilus luegvetensis* sp. nov. were euthanized rapidly by an overdose of clove oil anesthetic. Tissue samples were taken from the fins and preserved in anhydrous ethanol. Complete mitochondrial genes of *Paranemachilus luegvetensis* sp. nov. were sequenced by the Nanjing Jisi Huiyuan Biotechnology Company (China) following standard Illumina procedures. The mitochondrial cytochrome b (cytb) gene of *Paranemachilus liui* sp. nov. was sequenced by the Kunming Branch of Beijing Genco Biotechnology Co., Ltd. (China). The polymerase chain reaction (PCR) primer sequence was (5′-GAC TTG AAA AAC CAC CGT TG-3′ and 5′-CTC CGA TCT CCG GAT TAC AAG AC-3′) (Xiao et al. 2001). The MUSCLE was used to align the gene sequence in MEGA v11 with default parameters (Edgar, 2004; Tamura et al. 2021) and subsequently concatenated. Reference sequences of 27 nemacheilid species were retrieved from GenBank to reconstruct phylogenetic trees. The trees were constructed based on Bayesian inference (BI) using MrBayes on XSEDE (v3.2.7a) and the CIPRES Science Gateway (Miller et al. 2010). The analysis involved the Markov Chain Monte Carlo (**MCMC**) method with three heated chains and one cold chain, running for 2000000 generations with sampling every 100 generations under a complex model (GTR+I+G). The first 5000 samples were discarded as burn-in, with the remaining samples then used to generate a majority rule consensus tree.

Mitochondrial cytochrome b gene (cytb) sequencing data for the two new species were submitted to GenBank (Accession No. PP566646, PP566647 for *Paranemachilus luegvetensis* sp. nov.; PP566648, PP566649, PP566650 for *Paranemachilus liui* sp. nov.).

Most counts and morphometric measurements followed Tang et al. (2012), except for dorsal fin length (measured at the last simple dorsal ray) and caudal-peduncle depth (measured at the posterior anal-fin base), taken from the left side of the specimen. Additionally, characteristics of the cephalic lateral line system followed Kottelat (1990). Measurements were made with digital calipers, and data were recorded to the nearest 0.1 mm. The morphometric and meristic data of the two new species in this study have been deposited in the ScienceDB repository (https://www.scidb.cn/s/6ZFzQ3, accessed on 19 May 2024). The type specimens were deposited at the Nanning Normal University (**NNNU**).

Results

Paranemachilus luegvetensis Mo, Yang, Li & Du, sp. nov.

https://zoobank.org/F28EAC7B-6706-4AFB-9071-EF94CEE578AF

Figs 1, 2A, C, Tables 1, 3

Materials. ***Holotype*.** • NNNU230611 (NNNU: Nanning Normal University), 41.2 mm standard length. China: Guangxi, Wuming County, Chuanqian Village, 23°13'25.08"N, 108°26'18.50"E, collected by H.L. Mo and J.H. Zhong in June 2023.

***Paratypes*.** • NNNU230603–230610, 8 ex., 26.1–33.4 mm standard length; same data as for holotype.

Etymology. The name luegvetensis originates from the Zhuang language of the Luoyue Ancient Kingdom, pronounced LOKWET. This kingdom, believed to be located in what is now the Wuming District of Nanning City, Guangxi, China, where the type specimens were collected, is considered the cradle of this ancient civilization. The Luoyue Ancient Kingdom was known for its rich cultural contributions, including rice cultivation, cotton textiles, cliff paintings, witchcraft, Longmu rituals, and jade carving. These cultural elements profoundly influenced not only Chinese and Southeast Asian civilizations but also had significant global impacts. We propose the common Chinese name “骆越异条鳅” (Luo Yue Yi Tiao Qiu).

Diagnosis. Comparative data between *Paranemachilus luegvetensis* sp. nov. and all five known species within the genus *Paranemachilus* are provided in Table 3. *Paranemachilus luegvetensis* sp. nov. differs from all other congeneric species of *Paranemachilus* by cheeks scaled (vs. scaleless in *P. chongzuo*, *P. pingguoensis*, and *P. zhengbaoshani*), 11–12 branched pectoral fin rays (vs. 10 in *P. chongzuo*), 4–5 preoperculomandibular canal pores (vs. zero in *P. chongzuo*, 11–12 in *P. pingguoensis* and *P. zhengbaoshani*, 10 in *P. genilepis*, and 10–11 in *P. jinxiensis*), body depth 13.5%–16.8% of standard length (vs. greater than 18% in *P. jinxiensis* and *P. pingguoensis*), and preanus length 68.3%–73.9% of standard length (vs. greater than 75.3% in *P. chongzuo*, *P. genilepis*, *P. jinxiensis*, *P. pingguoensis*, and *P. zhengbaoshani*).

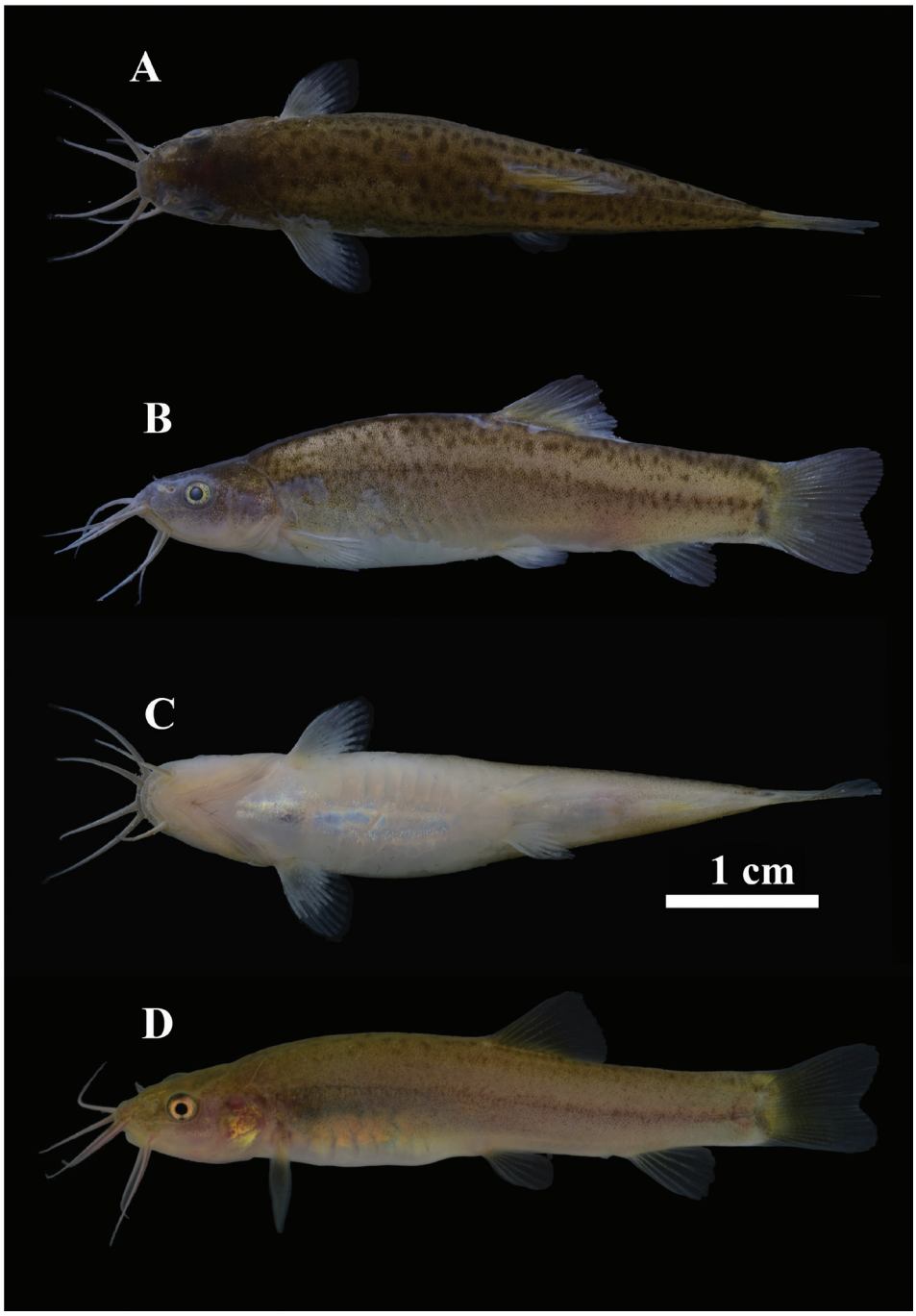

Figure 1. The dorsal (**A**), lateral (**B**), and ventral (**C**) views of *Paranemachilus luegvetensis* sp. nov. NNNU230611, holotype, 41.2 mm standard length. **D.** Living photo of *Paranemachilus luegvetensis* sp. nov., NNNU230611, holotype. Scale bar: 1 cm

Description. The morphometric data of the type specimen of *Paranemachilus luegvetensis* sp. nov. are given in Table 1. Body short, slightly bulging at back. From snout to dorsal-fin origin, body depth increases to maximum, 13.5%–16.8% of standard length, head slightly depressed, flattened, maximum head width greater than deepest head depth, 44.7%–56.5% of head length, snout length 24.5–32.1% of lateral head length, shorter than postorbital length. Mouth inferior, snout obtuse, lips developed and smooth, median of lower lip with V-shaped notch. Anterior and posterior nostrils adjacent; anterior nostrils tube-like, barbel-like elongation of anterior nostrils shorter than half the depth of nostril tube. Cheeks scaled, no reduction in eye size, eye diameter 19.3%–25.3% of head length. Three pairs of barbels: inner rostral barbel extending to posterior margin of eye, outer rostral barbel reaching or exceeding posterior margin of anterior opercular, above posterior operculum, and maxillary barbel above posterior margin of posterior operculum. Dorsal fin with three unbranched and 8–9 branched rays; distal margin cut; origin closer to caudal-fin base than to tip of snout. Pectoral fin with one unbranched and 11–12 branched rays; pectoral fin length 45.7%–61.4% of pelvic-fin insertion; pelvic fin with one unbranched and 6–7 branched rays, inserted below first or

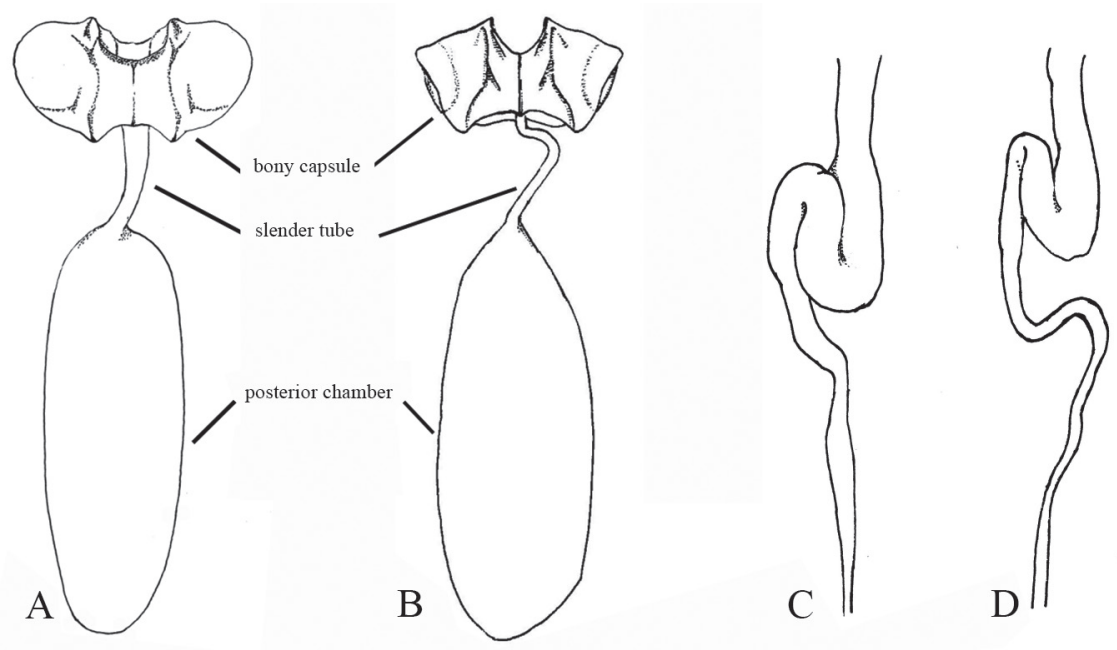

Figure 2. Swim bladder, stomach, and intestine of *Paranemachilus luegvetensis* sp. nov. (**A, C**) and *Paranemachilus liui* sp. nov. (**B, D**).

Table 1. Morphometric characters of *Paranemachilus luegvetensis* sp. nov. and *Paranemachilus liui* sp. nov.

	***Paranemachilus luegvetensis* sp. nov.**		***Paranemachilus liui* sp. nov.**	
	Holotype	**Paratypes (n = 8)**	**Holotype**	**Paratypes (n = 8)**
	NNNU230611	**NNNU230603–10**	**NNNU230710006**	**NNNU230710001–008**
Dorsal fin ray	3, 8	3, 8–9	3, 8	3, 8
Anal fin ray	3, 5	3, 6	3, 5	3, 5
Pectoral fin ray	1, 12	1, 11–12	1, 11	1, 11–13
Pelvic fin ray	1, 6	1, 6–7	1, 7	1, 6–7
Caudal fin ray	2, 17	2, 17–18	2, 17	2, 17
Standard length/mm	41.7	26.1–34.8 (31.6 ± 2.7)	51.4	47.6–61.6 (53.0 ± 4.3)
Body height	8.3	3.7–5.4 (4.7 ± 0.6)	10.0	9.8–12.2 (10.8 ± 1.1)
Maximum body height	9.4	3.8–5.7 (4.9 ± 0.7)	10.4	10.4–14.0 (11.6 ± 0.8)
Body width	6.6	2.2–3.8 (3.2 ± 0.5)	7.0	6.5–8.7 (7.2 ± 0.8)
Maximum body width	7.7	2.6–4.8 (3.8 ± 0.7)	8.7	6.7–9.8 (8.4 ± 1.0)
Body width at anus	4.7	1.3–2.8 (2.0 ± 0.4)	5.2	4.8–6.9 (5.5 ± 0.6)
Predorsal length	22.7	14.0–18.7 (16.9 ± 1.4)	28.9	27.2–33.0 (29.8 ± 1.9)
Dorsal fin length	5.0	3.3–5.9 (4.7 ± 1.0)	5.7	5.5–7.3 (6.2 ± 0.6)
Dorsal fin-base length	5.1	3.1–4.6 (4.0 ± 0.5)	5.9	5.4–7.6 (6.4 ± 0.7)
Prepelvic length	22.7	14.2–19.0 (17.4 ± 1.5)	29.0	26.4–34.8 (29.6 ± 2.4)
Pelvic fin length	5.0	3.6–4.9 (4.2 ± 0.4)	6.1	5.9–8.7 (6.8 ± 0.8)
Pelvic fin base length	1.1	0.6–1.1 (0.9 ± 0.1)	2.0	1.8–2.3 (2.0 ± 0.2)
Preanus length	29.2	17.8–24.6 (22.5 ± 2.1)	36.7	33.9–45.8 (37.4 ± 3.6)
Preanal length	32.2	18.6–26.0(24.0 ± 2.3)	39.2	36.7–48.6 (40.3 ± 3.6)
Anal fin length	5.2	4.3–6.3 (5.5 ± 0.6)	6.5	6.5–9.8 (7.8 ± 1.0)
Anal fin base length	3.1	2.3–2.9 (2.6 ± 0.2)	3.7	3.1–4.6 (3.8 ± 0.4)
Prepectoral length	9.7	7.0–8.6 (7.8 ± 0.6)	12.0	11.3–15.3 (12.4 ± 1.2)
Pectoral fin length	6.4	4.3–5.5 (5.2 ± 0.4)	8.1	7.0–9.8 (8.2 ± 0.9)
Pectoral fin base length	1.5	0.7–1.2 (1.0 ± 0.2)	1.9	1.4–3.0 (1.8 ± 0.5)
Caudal fin length	6.3	5.6–7.8 (6.8 ± 0.6)	9.2	9.1–11.1 (9.9 ± 0.7)
Caudal-peduncle length	5.2	4.5–5.5 (5.0 ± 0.3)	8.2	7.0–9.5 (8.6 ± 0.7)
Caudal-peduncle depth	5.3	2.3–4.0 (3.0 ± 0.6)	6.9	6.9–8.8 (7.8 ± 0.6)
Head length	9.7	7.2–8.7 (8.1 ± 0.6)	12.6	11.7–15.6 (12.9 ± 1.2)
Head height at eye	5.1	2.0–4.1 (3.0 ± 0.6)	5.2	4.5–6.7 (5.7 ± 0.7)
Head height at nape	5.8	3.4–4.4 (4.1 ± 0.3)	6.9	6.9–8.8 (7.5 ± 0.7)
Head width at eye	6.4	3.2–4.8 (4.1 ± 0.5)	8.0	6.1–9.6 (7.4 ± 1.0)
Maximum head width	7.1	3.9–5.7 (5.0 ± 0.5)	9.4	7.3–11.6 (8.9 ± 1.2)
Snout length	2.4	1.8–2.8 (2.3 ± 0.3)	2.7	2.7–4.5 (3.4 ± 0.5)

second unbranched dorsal-fin ray; tip of pelvic fin far from anus. Anal fin with three unbranched and 5–6 branched rays with truncate distal margins; origin halfway between pelvic-fin insertion and caudal-fin base. Caudal fin with 17–18 branched rays; caudal fin forked, upper and lower lobes equivalent.

Cephalic lateral-line canals developed, with 7+18–19 infraorbital canal pores, 15–17 supraorbital canal pores at base of anterior nostrils, 2–4 supratemporal canal pores, and 4–5 preoperculomandibular canal pores. Lateral line incomplete, with 2–6 lateral line pores present before midpoint of pectoral fin, 15–16 inner gill rakers on first gill arch (two specimens).

Stomach "U"-shaped, intestines slightly to back of stomach, curved. Two air-bladder chambers: anterior chamber encased in bony capsule, posterior chamber filling body cavity, and anterior and posterior chambers connected by short, thin, and curved tubes (Fig. 2).

Coloration. Photograph of *Paranemachilus luegvetensis* sp. nov. alive is provided in Fig. 1D. Sides of head and trunk brownish yellow; abaxial surface dark brown; ventral and lateral surfaces of head untextured. Obscure brown longitudinal stripe extending along lateral line to base of caudal fin. Body sides light yellow, except along lateral line; no spots on entire body at capture. Post-feeding, some individuals developed small black spots on side and back above lateral line, with darkened longitudinal stripes on side of body, though basic body color remained unchanged. No spots on fin, fin membrane hyaline. Specimens preserved in 10% formalin appear grayish-white.

Distribution and habitat. The species is known only from a karst cave (108°26'18.4973"N, 23°13'25.0813"E) near Nonghu Tun, Chuanqian Village, Wuming District, Nanning City (Fig. 3). The cave entrance has been artificially enlarged, with the underground river serving as an important source of drinking water and irrigation for local residents. *Paranemachilus luegvetensis* sp. nov. inhabits this silt-based underground river, co-occurring with *Caridina* sp. and *Silurus* sp. The underground river was not swollen at the time of collection, so the specimens were obtained deep within the cave.

Remarks. All collected individuals were initially extremely thin and weak. The holotype, raised in the same tank as other cavefish species for 91 days in an artificial environment. It was fed once every two days and sacrificed on day 91, with prepared specimens showing obvious fat accumulation on its back and sides. The species often migrates from the cave to feed in flooded mountain swales and farmlands during the rainy season, a behavior frequently observed by working locals. In the dry season, the water temperature of the pool is 18–20 °C, and in the rainy season, the temperature of the pool formed outside the outflow of the river can reach 26 °C. Despite its water habitats being contaminated with fertilizers and pesticides, *Paranemachilus luegvetensis* sp. nov. appears to have adapted to these environmental conditions.

Paranemachilus liui Mo, Yang, Li & Du, sp. nov.

https://zoobank.org/62C3991C-CF91-4DF5-B446-029287E5E852

Figs 2B, D, 4, Tables 1, 3

Materials. ***Holotype*.** • NNNU230710006, 51.4 mm standard length, China: Guangxi, Liuzhou County, Youshan Village, 24°15'03.9813"N, 109°25'42.8397"E, collected by H.L. Mo and J.J. Zhou in July 2023.

***Paratypes*.** • NNNU230710001–008, 47.59–61.63 mm standard length; same data as for holotype.

Etymology. The new species is named in honor of Zongyuan Liu, a distinguished official, literary author, and thinker. During his tenure in Liuzhou, Liu significantly promoted the economic and cultural landscape of the region, thereby elevating the quality of life for its residents and earning him deep admiration and respect from the local community. We propose the common Chinese name "柳氏异条鳅" (Liu Shi Yi Tiao Qiu).

Figure 3. Pool or a cave from which *Paranemachilus luegvetensis* sp. nov. type species was collected in Nonghu Tun, Chuanqian Village, Wuming District, Nanning City. Left (15 October 2023), right (10 June 2023).

Diagnosis. Comparative data among *Paranemachilus liui* sp. nov. and all five known species within the genus *Paranemachilus* are provided in Table 3. *Paranemachilus liui* sp. nov. can be distinguished from all other congeneric species of the genus *Paranemachilus* by cheeks scaled (vs. scaleless in *P. chongzuo*, *P. pingguoensis*, and *P. zhengbaoshani*), 11–13 branched pectoral fin rays (vs. 10 in *P. chongzuo*), 7–9 preoperculomandibular canal pores (vs. zero in *P. chongzuo*, 11–12 in *P. pingguoensis* and *P. zhengbaoshani*), preanus length 67.4%–74.3% of standard length (vs. greater than 75.3% in *P. chongzuo*, *P. genilepis*, *P. jinxiensis*, *P. pingguoensis*, and *P. zhengbaoshani*), snout length 21.4%–28.7% of lateral head length (vs. greater than 30.0% in *P. chongzuo*, *P. genilepis*, *P. jinxiensis*, *P. pingguoensis*, and *P. zhengbaoshani*), and 11–15 supraorbital canal pores at base of anterior nostrils (vs. 3 in *P. chongzuo*, 9 in *P. jinxiensis*, and 9–10 in *P. zhengbaoshani* and *P. pingguoensis*).

Description. The morphometric data of the type specimen of *Paranemachilus liui* sp. nov. are given in Table 1. Body elongated, large in size. Maximum body depth occurs before insertion of dorsal fin, body depth 18.7%–23.2% of standard length, head slightly depressed, flattened, maximum head width greater than deepest head depth, head width 49.9%–63.7% head length, snout length 21.4%–28.7% of lateral head length, shorter than postorbital length. Anterior and posterior nostrils adjacent; anterior nostrils tube-like; tip of anterior nostrils with distinct triangular elongation, shorter than half of tube depth. Cheeks scaled, no reduction in eye size, eye diameter 17.3%–21.9% of head length. Mouth inferior, snout obtuse, lips developed and smooth; in most specimens, median of lower lip with V-shaped notch; missing in specimens such as NNNU230710006 (holotype). Three pairs of barbels: inner rostral extending to posterior margin of posterior opercular, outer rostral, and maxillary barbel reaching or exceeding posterior margin of head. Dorsal fin with three unbranched and eight branched rays; distal margin straight; origin closer to caudal-fin base than to tip of snout. Pectoral fin with one unbranched and 11–13 branched rays; pectoral fin length 49.5%–51.8% of pelvic-fin insertion; pelvic fin with one unbranched and 6–7 branched rays, inserted below first or second unbranched dorsal-fin ray; tip of pelvic fin far from anus. Anal fin with three unbranched and five branched rays with truncate distal margins; origin closer to pelvic-fin insertion than to caudal-fin base. Caudal fin with 17 branched rays; caudal fin forked, upper and lower lobes equivalent.

Body completely covered by tiny scales. Cephalic lateral-line canals developed, with 4–6+13–15 infraorbital canal pores, 11–15 supraorbital canal pores at base of anterior nostrils, four supratemporal canal pores, and 7–9 preoperculomandibular canal pores. Lateral line incomplete, with 8–14 lateral line pores present before tip of pectoral fin, 14–16 inner gill rakers on first gill arch (two specimens).

Stomach "U"-shaped, intestines slightly to back of stomach, curved. Two air-bladder chambers: anterior chamber encased in bony capsule, posterior chamber filling body cavity, and anterior and posterior chambers connected by short, thin, and curved tubes (Fig. 2).

Coloration. Photograph of *Paranemachilus liui* sp. nov. specimen in life is provided in Fig. 4D. Dorsal surface dark brown, head and trunk of body yellowish brown, head untextured. Obscure brown longitudinal stripe extending along lateral line to base of caudal fin. Whole body untextured at capture. Post-feeding, some samples developed small black spots on dorsal surface. Fin rays faint yellow, fin membrane hyaline.

Distribution and habitat. Specimens of *Paranemachilus liui* sp. nov. were collected from several caves around Youshan Village, Yifeng District, Liuzhou City, Guangxi Province, China. The type specimen was collected from an underground river in Keyuan Cave (Fig. 5), which sustains a long, narrow, perennially water-filled pool. Near the cave entrance, some water sections are exposed to sunlight, with an average water temperature ranging from 20 to 23 °C. In the same location, we also collected *Yunnanilus bailianensis*, *Typhlocaridina semityphlata*, and an unidentified catfish species. Notably, the vicinity of Keyuan Cave is heavily utilized for farmlands and duck farms, resulting in a significant population from fertilizers and feed in the water where the specimens were found. Despite this, *Paranemachilus liui* sp. nov. appears to have adapted to these environmental conditions.

Phylogenetic analysis. The BI phylogenetic tree (Fig. 6) revealed that the *Paranemachilus* species formed a monophyletic group, sister to the genus *Troglonectes*. *Paranemachilus luegvetensis* sp. nov. and *Paranemachilus liui* sp. nov. formed a well-supported monophyletic group (bootstrap support = 98 and Bayesian posterior probability = 1) with other *Paranemachilus* species, including the type species *P. genilepis*. All samples from Wumin District, Nanning City, Guangxi Zhuang Autonomous Region clustered together in a sister clade to (*P. genilepis* + *Paranemachilus liui* sp. nov.). All samples from Yüfeng District, Liuzhou City, and Guangxi Zhuang Autonomous Region clustered together in a sister clade to *P. genilepis*. The tree topologies are similar to those reported in previous studies [2, 13], with highly consistent and strong support of the monophyly of the genus *Paranemachilus*. Analysis also indicated that the genus could be divided into two major clades, i.e., cheek-scaled group and cheek-scaleless group. The cheek-scaled group contained *P. genilepis*, *P. jinxiensis*, *Paranemachilus liui* sp. nov. and *Paranemachilus luegvetensis* sp. nov., while the cheek-scaleless group contained *P. chongzuo*, *P. pingguoensis*, and *P. zhengbaoshani*.

Mitochondrial differentiation. Comparative analysis of the cytb gene revealed that the two new species could be distinguished from all known *Paranemachilus* species based on distinct molecular differences. The minimum p-distances were 7.92% between *Paranemachilus luegvetensis* sp. nov. and *Paranemachilus liui* sp. nov. and 6.16% between *Paranemachilus liui* sp. nov. and *P. genilepis* (Table 2).

Table 2. Uncorrected pairwise distances among *Paranemachilus* species based on cytb gene.

		1	2	3	4	5	6	7	8	9
1	*Paranemachilus genilepis*									
2	*Paranemachilus jinxiensis*	6.98%								
3	*Paranemachilus pingguoensis*	7.26%	6.70%							
4	NNNU20230710001Liuzhou	6.21%	7.69%	7.10%						
5	NNNU20230710002Liuzhou	6.16%	7.92%	7.04%	0.29%					
6	NNNU20230710003Liuzhou	6.73%	8.19%	7.31%	0.30%	0.88%				
7	Paranemachilus *zhengbaoshani*	9.22%	8.38%	3.63%	7.69%	7.62%	7.89%			
8	NNNU230601.Wuming	9.22%	10.06%	8.94%	8.58%	8.50%	8.77%	8.94%		
9	NNNU230602.Wuming	8.66%	9.50%	8.38%	7.99%	7.92%	8.19%	8.94%	0.56%	
10	*Paranemachilus chongzuo*	6.98%	6.42%	1.40%	6.21%	6.16%	6.43%	3.35%	8.66%	8.10%

Table 3. Comparison of identifying characteristics between the two new species and its congeners of the genus *Paranemachilus*.

	Cheek scales	Pectoral-fin rays	body depth of standard length (%)	Preoperculomandibular canal pores	Supraorbital canal pores
Paranemachilus luegvetensis sp. nov.	Present	i, 11–12	13.5–16.8	4–5	15–17
Paranemachilus liui sp. nov.	Present	i, 11–13	18.7–23.2	7–9	11–15
P. jinxiensis	Present	i, 13–14	21.7–25.6	10–11	9
P. genilepis	Present	i, 11–13	17.8–21.0	10	11–12
P. chongzuo	Absent	i, 10	16.6–17.6	Absent	3
P. zhengbaoshani	Absent	i, 11–12	17.0–20.1	11–12	9–10
P. pingguoensis	Absent	i, 12–13	18.2–21.2	11–12	9–10
	Preanus length of standard length (%)		**Snout length of head length (%)**		**Reference**
Paranemachilus luegvetensis sp. nov.	68.3–73.9		24.5–32.1		This study
Paranemachilus liui sp. nov.	67.4–74.3		21.4–28.7		This study
P. jinxiensis	/		37–41.7		This study
P. genilepis	76–78.5		30–33.5		This study
P. chongzuo	76.7–78		32.5–37		This study
P. zhengbaoshani	75.3–75.8		33–34.6		This study
P. pingguoensis	75.5–78.2		29.1–36.7		This study

Identification Key to Species of *Paranemachilus*

1 Cheeks scaled 2
– Cheeks scaleless 5
2 Body depth 13.5%–16.8% of standard length ..... *Paranemachilus luegvetensis* sp. nov.
– Body depth exceeds 17.8% of standard length 3
3 Snout length 21.4%–28.7% of lateral head length ..... *Paranemachilus liui* sp. nov.
– Snout length exceeds 30.0% of lateral head length 4
4 Caudal peduncle length 88.1%–97.7%, 18 inner gill rakers on first gill arch ..... *P. genilepis*
– Caudal peduncle length 100.0%–130.0%, 14 inner gill rakers on first gill arch ..... *P. jinxiensis*
5 Preoperculomandibular canal pores absent ..... *P. chongzuo*
– Preoperculomandibular canal pores present 6
6 Processus dentiformis on upper lip ..... *P. zhengbaoshani*
– No processus dentiformis on upper lip ..... *P. pingguoensis*

Discussion

The genus *Paranemachilus* can be diagnosed by the following combination of characteristics: 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, lips without papillae, lateral line incomplete, disappearing behind vertical at the end of the pectoral fin, and anterior part of the air-bladder lying free in the abdominal cavity (Zhu 1983; Zhu and Cao 1987; Lan et al. 2013; Du et al. 2023). These two new species were assigned to the genus *Paranemachilus* based on the following additional characters: body completely covered by tiny scales, lateral line incomplete,

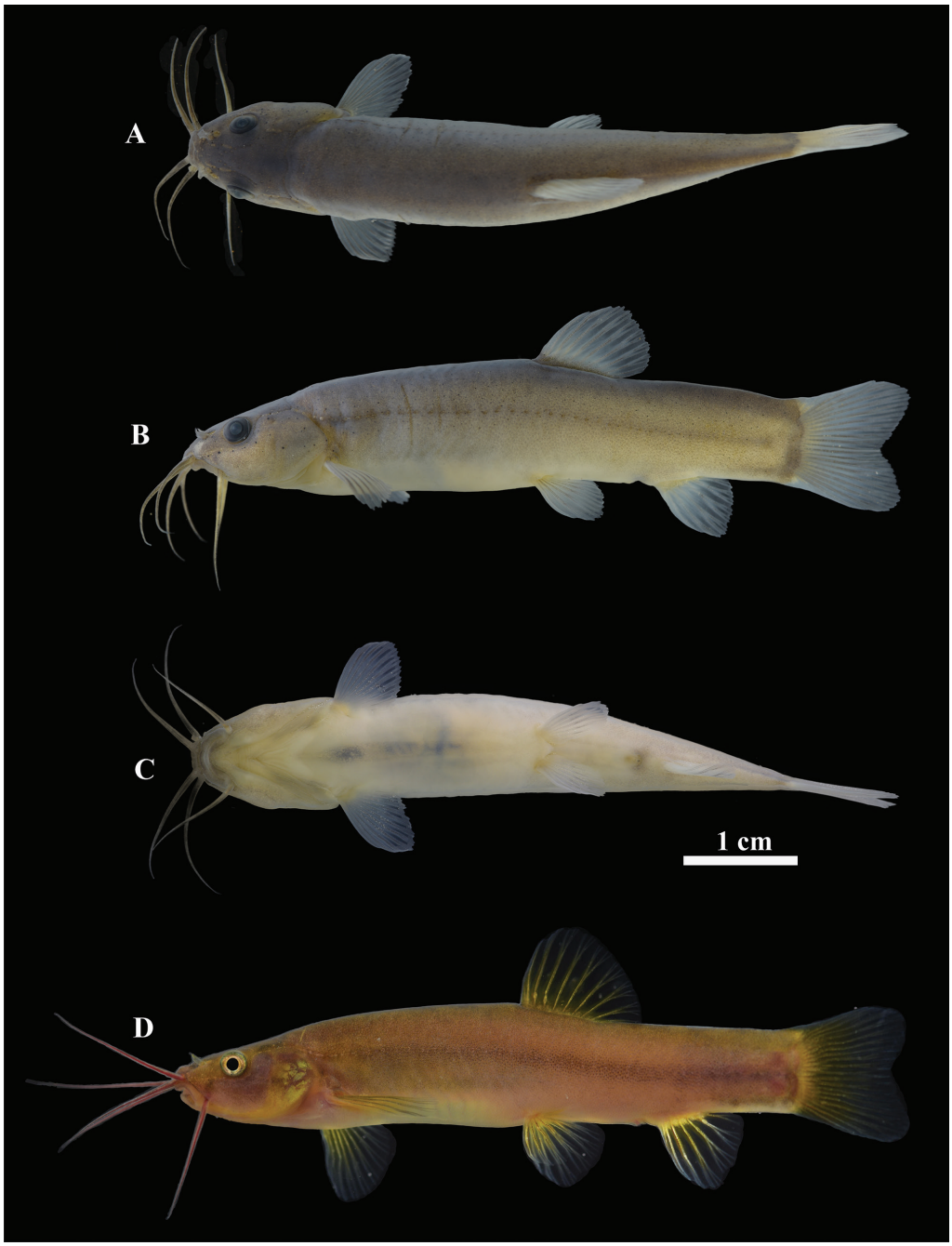

Figure 4. A–C. dorsal, lateral, and ventral views of *Paranemachilus liui* sp. nov. NNNU230710006, holotype, 51.39 mm standard length. **D** *Paranemachilus liui* sp. nov. NNNU230710006, holotype, live, photo taken on 11 June 2023. Scale bar: 1 cm.

present before the mid-point of the pectoral fin, mouth inferior, snout obtuse, lips developed and smooth, median of lower lip with V-shaped notch, anterior and posterior nostrils adjacent, anterior nostrils tube-like, tip of anterior nostrils with triangular-like elongation, shorter than half of the depth of the nostril tube, stomach "U"-shaped, intestines slightly to the back of the stomach, curved, two air-bladder chambers: anterior chamber encased in a bony capsule, posterior chamber filling the body cavity, and anterior and posterior chamber connected by a slender tube.

The genus *Paranemachilus* Zhu, 1983, was established with *P. genilepis* as the type species (Zhu, 1983), while *Heminoemacheilus* Zhu & Cao, 1987, was established with *H. zhengbaoshani* Zhu & Cao, 1987, as the type species (Zhu and Cao 1987). *Heminoemacheilus zhengbaoshani* differs from *P. genilepis* by having scaleless cheeks (vs. scaled) and an absent postcleithrum (vs. present). *Yunnanilus jinxiensis* Zhu, Du, Chen & Yang, 2009 and *H. zhengbaoshani* were later reclassified as *P. jinxiensis* and *P. zhengbaoshani* in *Paranemachilus* (Du et al. 2021; Luo et al. 2023), with *P. jinxiensis* having scaled cheeks (Du et al. 2023). Similarly, the two new species, *Paranemachilus luegvetensis* sp. nov. and *Paranemachilus liui* sp. nov., also feature scales on their cheeks. Both *P. pingguoensis* and *P. chongzuo* were placed in *Paranemachilus* based on shared characteristics, notably anterior and posterior nostrils adjacent, anterior nostrils tube-like, lateral line incomplete, disappearing behind the vertical at the end of

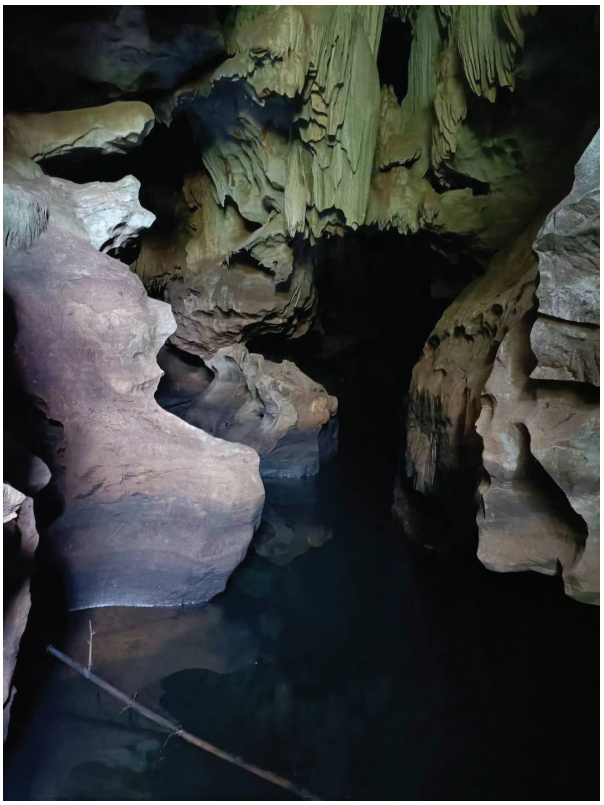

Figure 5. Pool and a cave from which type *Paranemachilus luegvetensis* sp. nov. was collected in Youshan Village, Yufeng District, Liuzhou County.

the pectoral fin, and anterior part of the air-bladder lying free in the abdominal cavity (Zhu 1983; Zhu and Cao 1987; Lan et al. 2013; Du et al. 2023). These classifications are supported by morphological and molecular evidence (Luo et al. 2023). The BI phylogenetic tree constructed during this study (Fig. 6) revealed that *Paranemachilus luegvetensis* sp. nov. and *Paranemachilus liui* sp. nov. clustered together in a sister clade to (*P. genilepis* + *P. jinxiensis*), while *P. pingguoensis*, *P. zhengbaoshani*, and *P. chongzuo* clustered together in another sister clade. These findings suggest that *Paranemachilus* could be divided into two species groups: the cheek-scaled group, including *P. genilepis*, *P. jinxiensis*, *Paranemachilus luegvetensis* sp. nov., and *Paranemachilus liui* sp. nov., and the cheek-scaleless group, including *P. chongzuo*, *P. pingguoensis*, and *P. zhengbaoshani*. However, apart from cheek scales, no additional distinguishing traits have been identified between the two groups. In this study, the phylogenetic tree was constructed based solely on the cytb gene, as only this gene was analyzed. The genus *Heminoemacheilus* is characterized by the absence of a postcleithrum (Zhu and Cao 1987), a feature that has not yet been examined in *P. jinxiensis* and the two new species. Thus, further research is necessary to determine the validity of the *Heminoemacheilus* genus. In this work, we do not consider *Heminoemacheilus* valid; the group with scaleless cheeks was still regarded as *Paranemachilus* when making the identification key.

Freshwater fish continues to be undervalued and overlooked—and thousands of species are heading towards extinction. Due to their exclusive confinement to cave ecosystems, cavefish are highly vulnerable to threats such as habitat degradation, hydrological changes, environmental pollution, resource overexploitation, and non-native species introduction (Lan et al. 2013; Ma, Zhao & Yang, 2019). As noted in Ma et al. (2019), cavefish typically possess morphological adaptations to the extreme conditions of cave environments, such as eye degeneration or loss, reduced pigmentation, and diminished scales.

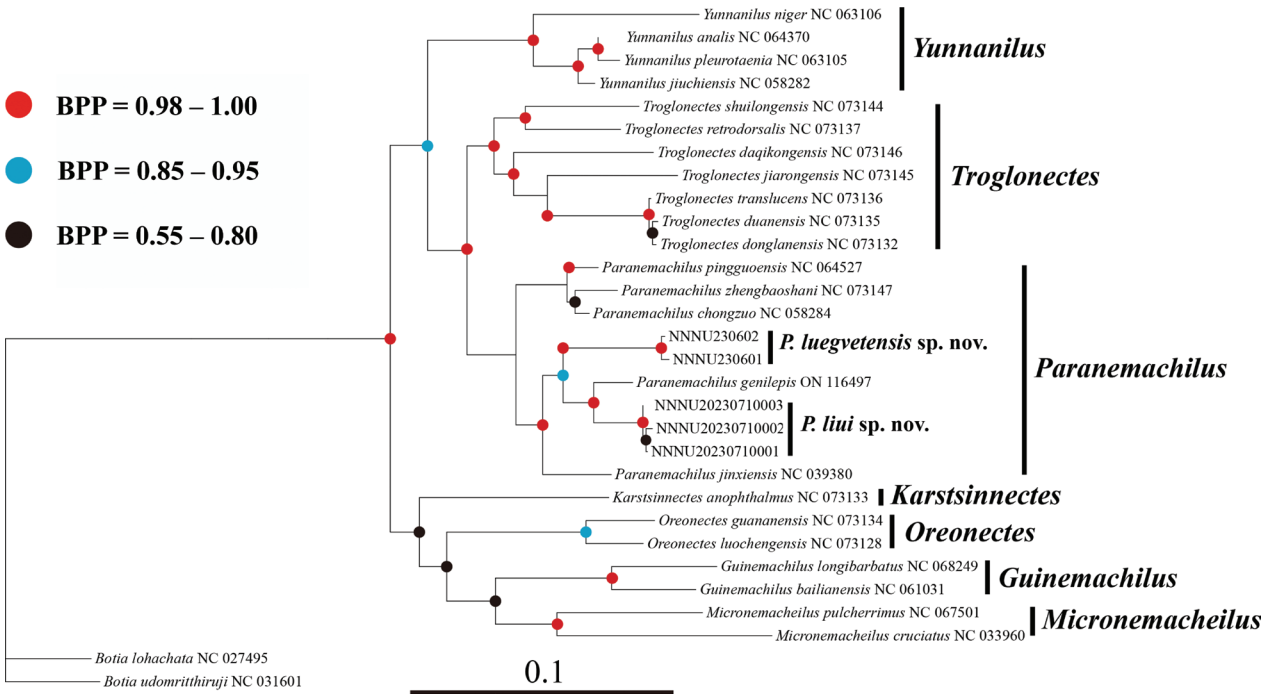

Figure 6. Bayesian phylogeny of cytb lineages of species within the family Nemacheilidae. *Botia* genus was used as an outgroup. Numbers on internode branches are Bayesian posterior probabilities.

These unique features endow cavefish with a distinctive exotic appearance, which often attracts the attention of fish collectors. In contrast, *Paranemachilus liui* sp. nov. and *Paranemachilus luegvetensis* sp. nov. do not exhibit these distinctive phenotypes but demonstrate remarkable adaptability. For instance, *Paranemachilus liui* sp. nov. annually migrates to a cave pond that floods during the rainy season for breeding purposes, despite the pond often being heavily polluted by effluent from nearby sugar factories and duck farms. Furthermore, the cave inhabited by *Paranemachilus luegvetensis* sp. nov. serves as the sole water source for irrigation in the area, subject to unregulated exploitation and intrusion by non-cave species like *Metzia* sp. and *Labeo rohita* due to human activities. Nevertheless, no decline in the populations of the two newly described species has been observed. Notably, the currently known species of *Paranemachilus* found are distributed in scattered areas in western and central Guangxi (Fig. 7), with recorded sites being relatively distant from each other. In Guangxi, where karst landforms are well-developed and underground rivers suitable for *Paranemachilus* survival are widely distributed, it is plausible that additional habitats for this genus exist. This suggests the likely presence of more undescribed *Paranemachilus* species in the region. In our view, the diversity of *Paranemachilus* species in Guangxi may be underestimated, warranting comprehensive and systematic exploration.

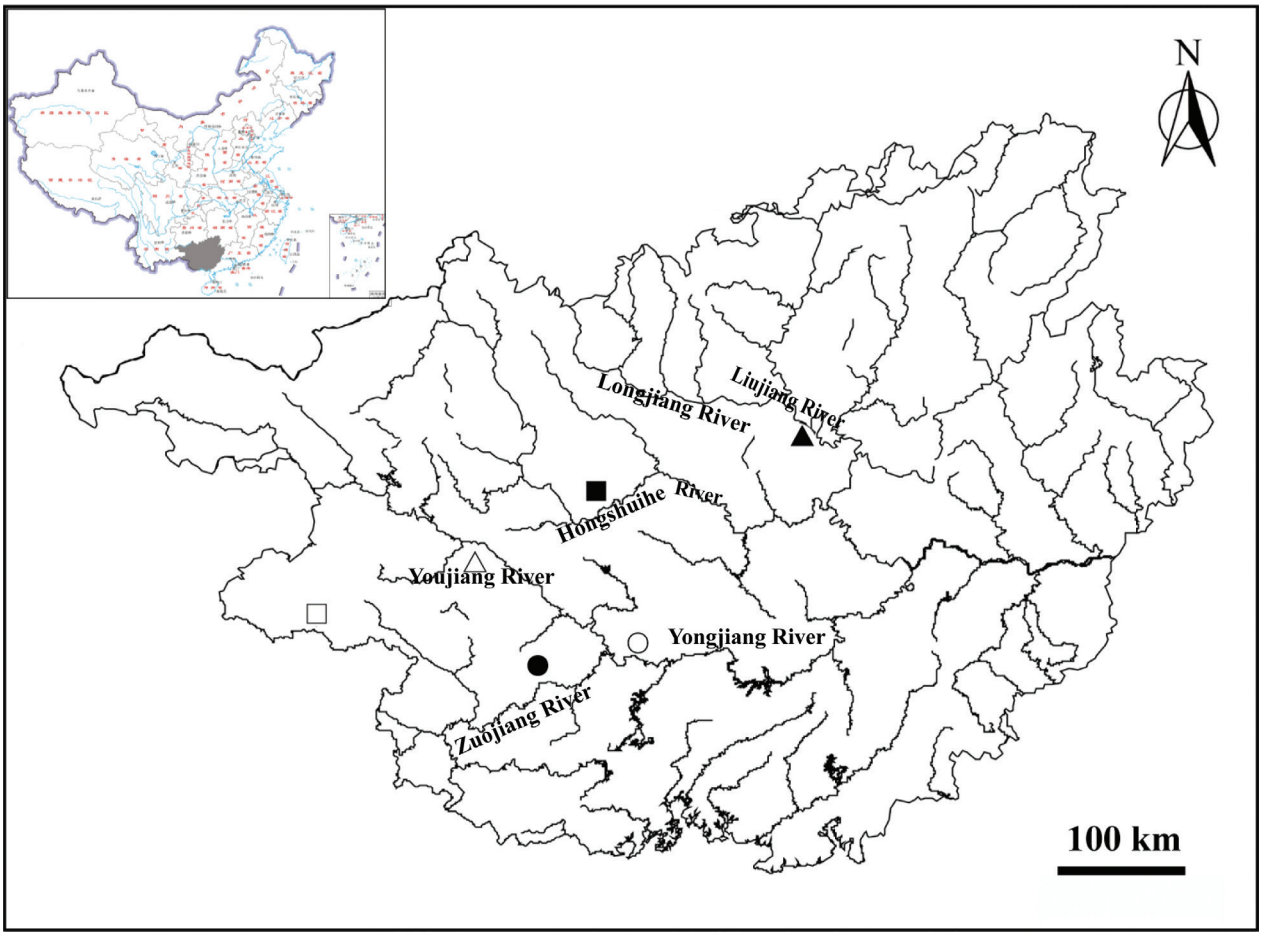

Figure 7. Map showing distribution of *Paranemachilus* species in Guangxi Zhuang Autonomous Region. *P. zhengbaoshani* (black square), *P. jinxiensis* (white square), *P. genilepis* (black dott), *Paranemachilus luegvetensis* sp. nov. (white dott), *Paranemachilus liui* sp. nov. (black triangle), and *P. pingguoensis* (white triangle).

Acknowledgments

We are grateful to J.H. Zhong and D.D. Huang from Nanning Normal University and J.J. Zhou from the Zhejiang Forest Resource Monitoring Center for their help in specimen collection. We are also grateful to Y.J. Wei from the Agricultural Bank of China, Ltd., Liuzhou Branch, for providing part of the funds to support our field research, and C. Watts for English corrections and suggestions. This work was supported by the Project of Financial Funds of the Ministry of Agriculture and Rural Affairs: Investigation of Fishery Resources and Habitat in the Pearl River Basin, Innovation and Entrepreneurship Training Program for College Students (202427060300203), and Guangxi Natural Science Foundation Project (2022GXNSFAA035563).

References

Du LN, Yang J, Min R, Chen XY, Yang JX (2021) A review of the Cypriniform tribe Yunnanilini Prokofiev, 2010 from China, with an emphasis on five genera based on morphologies and complete mitochondrial genomes of some species. Zoological Research 42: 310–334. https://doi.org/10.24272/j.issn.2095-8137.2020.229

Du LN, Li SJ, Xu F, Luo T, Luo FG, Yu GH, Zhou J (2023) Clarification of Phylogenetic Relationships among Chinese Nemacheilids with Tube–Shaped Anterior Nostrils, with a Description of a New Genus and Two New Species. Journal of Zoological Systematics and Evolutionary Research 2023: 3600085. https://doi.org/10.1155/2023/3600085

Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32: 1792–1797. https://doi.org/10.1093/nar/gkh340

Kottelat M (1990) Indochinese nemacheilines: A revision of nemacheiline loaches (Pisces, Cypriniformes) of Thailand, Burma, Laos, Cambodia and southern VietNam. Verlag Dr. Friedrich Pfeil, München, 262 pp.

Lan JH, Gan X, Wu TJ, Yang J (2013) Cave Fishes of Guangxi, China; Science Press: Beijing, China, 266 pp.

Li DY, Gao BG (2016) Collection and Preparation of Biological Specimens. Chemical Industry Press, Beijing, 178 pp.

Luo T, Yang Q, Wu Li, Wang YL, Zhou JJ, Deng HQ, Xiao N, Zhou J (2023) Phylogenetic relationships of Nemacheilidae cavefish (Heminoemacheilus, Oreonectes, Yunnanilus, Paranemachilus, and Troglonectes) revealed by analysis of mitochondrial genome and seven nuclear genes. Zoological Research 44: 693–697. https://doi.org/10.24272/j.issn.2095-8137.2022.266

Ma L, Zhao YH, Yang JX (2019) Chapter 28 - Cavefish of China. In: White WB, Culver DC, Pipan T (Eds) Encyclopedia of Caves. 3rd edn. Waltham: Academic Press, 237–254. https://doi.org/10.1016/B978-0-12-814124-3.00027-3

Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In 2010 Gateway Computing Environments Workshop (GCE), 8 pp., New Orleans, LA, USA, November 2010. https://doi.org/10.1109/GCE.2010.5676129

Tamura K, Stecher G, Kumar S (2021) MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. Molecular Biology and Evolution 38: 3022–3027. https://doi.org/10.1093/molbev/msab120

Tang L, Zhao YH, Zhang CG (2012) A new blind loach, *Oreonectes elongatus* sp. nov. (Cypriniformes, Balitoridae) from Guangxi, China. Environmental Biology of Fishes 93(4): 483–449. https://doi.org/10.1007/s10641-011-9943-7

Xiao WH, Zhang YP, Liu HZ (2001) Molecular systematics of Xenocyprinae (Teleostei, Cyprinidae): taxonomy, biogeography, and coevolution of a special group restricted in East Asia. Molecular Phylogenetics and Evolution 18: 163–173. https://doi.org/10.1006/mpev.2000.0879

Zhu SQ (1983) A new genus and species of Nemachilinae (Pisces, Cobitidae) from China. Acta Zootaxonomica Sina 12: 311–313.

Zhu SQ, Cao WX (1987) The Noemacheiline fishes form Guangdong and Guangxi with descriptions of a new genus and three new species. Acta Zootaxonomica Sina 12: 323–331.

Zhu Y, Du LN, Chen XY, Yang JX (2009) A new nemacheiline loach of genus *Yunnanilus* (Balitoridae) from Guangxi, China—*Yunnanilus jinxiensis*. Zoological Research 30: 195–198. https://doi.org/10.3724/SP.J.1141.2009.02195