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Taxonomic revision of the cavefish genus *Karstsinnectes* (Cypriniformes, Nemacheilidae), with a description of a new species from Guangxi Province, China

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Abstract

The blind cavefish genus *Karstsinnectes*, established in 2023, is the subject of taxonomic revision in the present study. Five valid species are recognized, including one new species, *Karstsinnectes longzhouensis* **sp. nov.**, described from Guangxi, China, based on a combination of morphological evidence. *Karstsinnectes longzhouensis* **sp. nov.** can be distinguished from all other congeners based on the presence of a lateral line, 11–12 branched pectoral fin rays, and five branched pelvic fin rays. Additionally, due to the loss of the type specimens, a neotype is designated for *K. parvus*. The lateral line of *K. hyalinus* is revised as lacking in this study. A key to all valid *Karstsinnectes* species is provided. Blind cavefish serve as a valuable natural framework for investigating convergent and adaptive evolutionary processes. The survival of cavefish is under significant threat due to human activities, climate change, water pollution, and invasive species. Thus, to preserve these valuable species, it is crucial to implement various conservation measures, such as habitat protection, artificial breeding, and fundamental research.

Key Words

Blind cavefish, complete mitochondrial genome, morphology, Taxonomy, Xijiang River

Introduction

Karst caves and subterranean streams represent dominant geomorphological features in the Guangxi, Guizhou, Sichuan, and Yunnan provinces and Chongqing City of China. These regions are renowned for harboring many unique cave-dwelling fish species (Zhao and Zhang 2009). Ma et al. (2019) recorded 148 hypogean species in China, including 65 nemacheilids in *Heminoemacheilus*, *Oreonectes*, *Protocobitis*, *Paranemachilus*, *Schistura*, *Troglonectes*, *Triplophysa*, and *Yunnanilus*. Luo et al. (2023) described a new genus, *Karstsinnectes* Zhou, Luo, Wang, Zhou & Xiao, 2023, with *Oreonectes anophthalmus* Zheng, 1981, as the type specimen, based on morpho-

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logical characters and molecular evidence. The diagnostic characters of *Karstsinnectes* include body scaleless and colorless, anterior and posterior nostrils separated, the base of the anterior nostril tube-shaped, a tip not elongated to barbel-like, adipose crests present on the caudal peduncle, lateral line and cephalic lateral line canals present, and the body capsule of the swim bladder open posteriorly (Luo et al. 2023). Four species within the genus *Karstsinnectes* have been recorded, including *K. acridorsalis* (Lan, 2013), *K. anophthalmus* (Zheng, 1981), *K. hyalinus* (Lan, Yang & Chen, 1996), and *K. parvus* (Zhu & Zhu, 2014).

In December 2022, four *Karstsinnectes* specimens were collected from a cave in Longzhou County, Chongzuo City, Guangxi, China. Morphological characters and molecular analyses indicated that these specimens represented an undescribed species of *Karstsinnectes*. Additionally, a taxonomic revision of *K. acridorsalis* and *K. hyalinus* is provided, and a neotype is designated for *K. parvus* herein.

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 of *Karstsinnectes longzhouensis* sp. nov. were rapidly euthanized by an overdose of anesthetic clove oil. The right-side pectoral fin and pelvic fin were excised and preserved in 99% ethanol. Specimens for morphological study were initially stored in 10% formalin, then transferred to 75% alcohol for long-term preservation at the Kunming Natural History Museum of Zoology, Kunming Institute of Zoology (KIZ), Chinese Academy of Sciences (CAS).

Counts and measurements followed Du et al. (2021) and Luo et al. (2023). Data were initially processed using Microsoft Excel software for preliminary statistical analysis. The original data were converted to percentages of standard length (SL) or lateral head length (HL), followed by logarithmic conversion (log10) to remove the effects of allometry. Comparative morphometry was examined using principal component analysis (PCA) in IBM SPSS Statistics v20.0. Non-parametric analysis of variables with principal component loadings greater than 60% was used to determine the degree of differences between subjects, and the scores of each principal component were used to generate scatter plots for analysis.

Complete mitochondrial genome sequencing data were submitted to GenBank under Accession No. OR947935. The mitogenome of OR947935 was sequenced on Illumina Novaseq 6000 (Origingene Bio-pharm Technology Co. Ltd., Shanghai, China). The quality of sequencing raw data was evaluated by Fastqc (v.0.11.8) and trimmed using Cutatapt (v.4.8) software (Martin 2011). Obtained Illumina reads were de novo assembled using the NOVOPlasty software (Dierckxsens et al. 2017), and the assembled genome was annotated by Mitos2 (Bernt et al. 2013). Relative synonymous codon usage (RSCU) in protein coding sequences and simple sequence repeats (SSRs) of OR947935 were determined in CodonW (v.1.4.2) and MISA, respectively (Peden 1999; Beier et al. 2017). To test the phylogenetic position of *Karstsinnectes longzhouensis* sp. nov., Bayesian inference (BI) was performed using MrBayes in XSEDE (v3.2.7a) by CIPRES Science Gateway (Miller et al. 2010). The entire sequence of the mitochondrial genomes was used as a single partition. The substitution model GTR + I + G was selected as the best model using jModelTest v.2.1.10 (Darriba et al. 2012). Likelihood Model parameters were set as number of substitution types allow all rates to be different, subject to the constraint of time-reversibility (Nst = 6), nucleotide substitution model. Nucmodel was standard model of DNA substitution in which there are only four states (Nucmodel = 4×4), the rate at a site is drawn from a gamma distribution, and all site patterns had the possibility of being sampled (Coding = all). Forty complete mitochondrial genomes obtained from GenBank were included in the data. *Parabotia fasciata* Dabry de Thiersant, 1872 and *Leptobotia elongata* (Bleeker, 1870), two botiid species, were used as outgroups. 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). Uncorrected pairwise distances between species of Karstsinnectes were calculated in MEGA v11.

Results

Genus *Karstsinnectes* Zhou, Luo, Wang, Zhou & Xiao, 2023

Karstsinnectes Zhou, Luo, Wang, Zhou & Xiao, 2023, 696 original descriptions.

Type species. *Oreonectes anophthalmus* Zheng, 1981.

Diagnosis. Base of anterior nostril tube-like and tip not elongated to barbel-like; anterior and posterior nostrils adjacent; lips with furrows; caudal peduncle with adipose crests; bony capsule of swim bladder opens posteriorly.

Remarks. In the family Nemacheilidae, the genera *Eonemachilus* Berg, 1938; *Karstsinnectes*; *Micronemacheilus* Rendahl, 1944; *Protonemacheilus* Yang & Chu, 1990; *Traccatichthys* Freyhof & Serov, 2001; and *Yunnanilus* Nichols, 1925, are characterized by a tube-like anterior nostril with tip not elongated to barbel-like structure (Du et al. 2021, 2023). However, *Karstsinnectes* can be distinguished from *Eonemachilus*, *Protonemacheilus*, *Traccatichthys*, and *Yunnanilus* by anterior and posterior nostrils adjacent (vs. separated in *Eonemachilus* and *Yunnanilus*, closely set in *Protonemacheilus* and *Traccatichthys*) and from *Micronemacheilus* and *Traccatichthys* by lips with furrows (vs. with papillae).

Species included. *Karstsinnectes acridorsalis* (Lan, 2013), *K. anophthalmus* (Zheng, 1981), *K. hyalinus* (Lan, Yang & Chen, 1996), *K. longzhouensis* sp. nov., and *K. parvus* (Zhu & Zhu, 2014).

Key to species of *Karstsinnectes*

1	Caudal fin truncated	*K. anophthalmus*
–	Caudal fin forked	2
2	Body covered by scales	*K. hyalinus*
–	Body scaleless	3
3	Lateral line present	4
–	Lateral line absent	*K. acridorsalis*
4	Pectoral fin with 10 branched rays, five branched pelvic fin rays	*K. parvus*
–	Pectoral fin with 11 or 12 branched rays, six branched pelvic fin rays	*Karstsinnectes longzhouensis* sp. nov.

Karstsinnectes acridorsalis (Lan, 2013)

Figs 1A, 2; Table 1

Oreonectes acridorsalis Lan, 2013: 68, fig. 50 (Bamu Town, Tian'e County, Hechi City, Guangxi).

Troglonectes acridorsalis Xiao & Lan, 2023: 33 (Bamu Town, Tian'e County, Hechi City, Guangxi).

Karstsinnectes acridorsalis Luo et al., 2023, 696 (Bamu Town, Tian'e County, Guangxi).

Material examined. *Paratypes*. 2 ex. China; Guangxi, Hechi City, Tian'e County, Bamu Town, 22.8754°N, 107.1947°E, 284 m a.s.l. CLJH 1202001, CLJH 04100607, 37.9–38.1 mm SL, deposited in the Fishery and Animal Husbandry Bureau of Du'an, Guangxi, China.

Diagnosis. *Karstsinnectes acridorsalis* differs from *K. anophthalmus* by caudal fin forked (vs. truncated), 14 branched caudal-fin rays (vs. 12), longer and lower head (length 29.9%–32.7% of SL vs. 22.7%–24.6%, height 34.6%–39.6% of head length vs. 41.0%–44.0%), longer pectoral fin (61.6%–67.7% of distance between pectoral-fin origin and pelvic-fin origin vs. 35.9%–48.9%); from *K. hyalinus* by scaleless (vs. scaled), five branched anal-fin rays (vs. four), 14 branched caudal-fin rays (vs. 11 or 12); from *K. parvus* and *K. longzhouensis* sp. nov. by lateral line absent (vs. present), lower body (body depth 13.9%–15.5% of SL vs. 17.1%–18.4% and 16.1%–19.4%, respectively), shorter caudal peduncle (length 15.7%–15.9% of SL vs. 18.4%–22.3% and 17.3%–19.5%, respectively).

Description. Body elongated, head depressed, snout depressed, forehead raised, head height at nostril 46.1%–67.9% of maximum head height. Body trunk compressed, with maximum body depth anterior to dorsal-fin origin, deepest body depth 13.9%–15.5% of SL. Dorsal profile of head and predorsal profile slightly convex, clearly concave from dorsal-fin origin to tip of dorsal fin, and gradually convex from tip of dorsal fin to anterior quarter of caudal fin due to caudal adipose keel on upper edge of caudal peduncle. Ventral profile of head straight, nearly straight from pectoral-fin insertion to anal-fin origin, convex from posterior margin of anal-fin base to anterior quarter of caudal fin due to caudal adipose keel on lower edge of caudal peduncle.

Anterior and posterior nostrils adjacent, distance less than posterior nostril diameter, base of anterior nostril tube-shaped and tip not elongated to barbel-like. Eyes absent. Mouth inferior, snout rounded, upper and lower lips smooth, lower lip with V-shaped median notch. Three pairs of barbels, inner and outer rostral barbels reaching mouth corner, and maxillary barbel reaching anterior margin of interopercle. Inner gill rakers on first gill arch nine (one specimen).

Dorsal fin with three unbranched and eight branched rays, distal margin straight, origin posterior to pelvic-fin origin, predorsal length 54.3%–55.5% of SL. Pectoral fin with one unbranched and 10 branched rays, pectoral-fin length 61.6%–67.7% of distance between pectoral-fin origin and pelvic-fin origin. Pelvic fin with one unbranched and five branched rays, tip of pelvic fin not reaching anus. Anal fin with two unbranched and five branched rays, distal margin straight. Anus abutting anal-fin base. Caudal fin forked. High caudal adipose keels on upper and lower edges of caudal peduncle, height at most of upper adipose keel nearly 1/2 caudal peduncle depth. Caudal peduncle depth 46.1%–51.9% of its length (containing adipose keels). Lateral line and cephalic sensory pores absent. Body scaleless.

Coloration. Whole body translucent, without color pattern. Fin membrane hyaline.

Distribution and habitat. Only known from the type locality. *Karstsinnectes acridorsalis* inhabits a subterranean river, 22.8754°N, 107.1947°E, 284 m a.s.l. Co-inhabitants of the stream include *Triplophysa tianeensis* Chen, Cui & Yang, 2004, *Sinocyclocheilus furcodorsalis* Chen, Yang & Lan, 1997, and *Hongshuia megalophthalmus* (Chen, Yang & Cui, 2006).

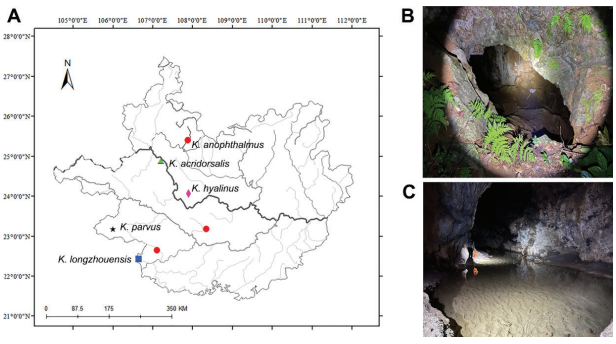

Figure 1. Collection sites (**A**), *K. anophthalmus* (red round), *K. acridorsalis* (green triangle), *K. hyalinus* (purple rhombus), *K. longzhouensis* sp. nov. (blue square), *K. parvus* (black star), and habitat of *Karstsinnectes longzhouensis* sp. nov. (**B**) and *K. parvus* (**C**).

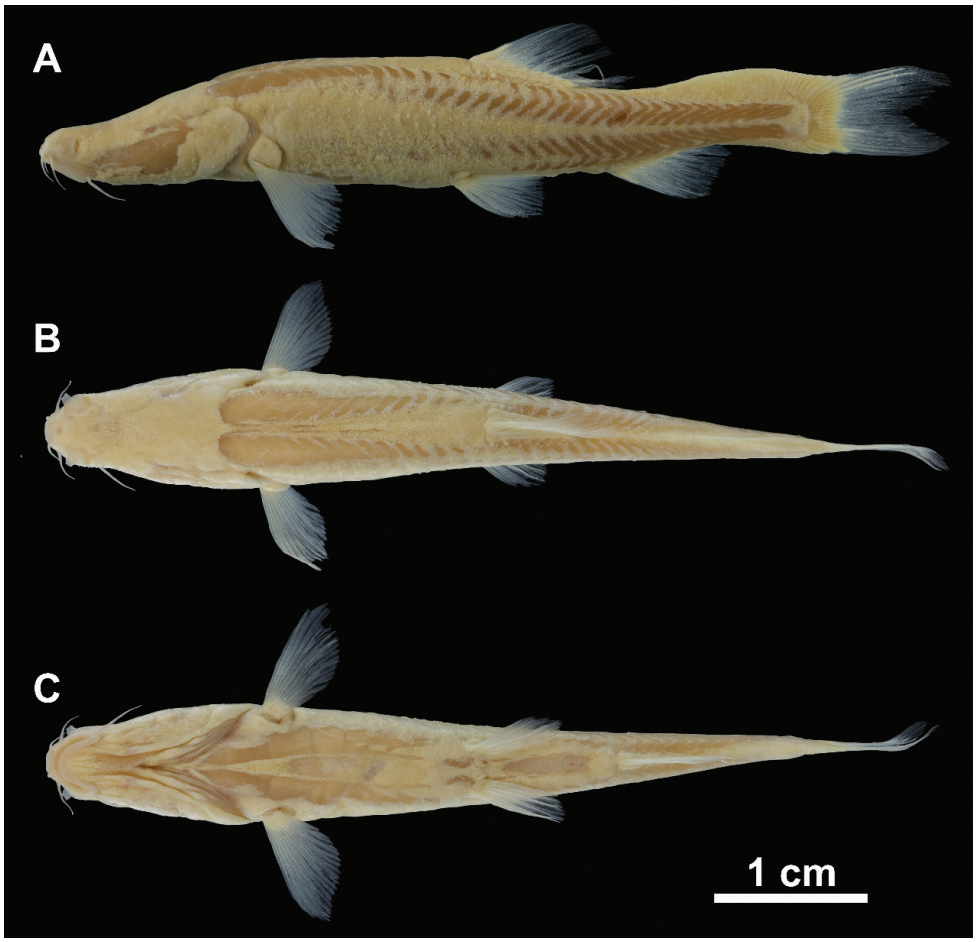

Figure 2. Lateral, dorsal, and ventral views of *Karstsinnectes acridorsalis*, holotype, CLJH04100608 (photograph from J.H. Lan). Scale bar: 1 cm.

Remarks. The population of *K. acridorsalis* is small. Initially described by Lan et al. (2013) based on three specimens, subsequent collections yielded only one specimen, collected in 2019 by J.J. Zhou.

Karstsinnectes hyalinus (Lan, Yang & Chen, 1996)

Figs 1A, 3; Table 1

Heminoemacheilus hyalinus Lan, Yang & Chen, 1996, 109–112 (Bao'an Township, Du'an County, Hechi City, Guangxi).

Karstsinnectes hyalinus Luo et al., 2023: 696 (Bao'an Township, Du'an County, Hechi City, Guangxi).

Material examined. 3 ex. China; Guangxi, Hechi City, Du'an County, Bao'an Township, 24.0709°N, 107.9027°E, 283 m a.s.l. GXNU 94098009–94098011, 38.9–40.2 mm SL.

Diagnosis. *Karstsinnectes hyalinus* can be distinguished from other members of *Karstsinnectes* based on body-scaled (vs. scaleless). It can be further differentiated from *K. anophthalmus* by caudal fin forked (vs. truncated); from *K. acridorsalis*, *Karstsinnectes longzhouensis* sp. nov., and *K. parvus* by seven branched dorsal-fin rays (vs. eight in *K. acridorsalis* and nine in *Karstsinnectes longzhouensis* sp. nov. and *K. parvus*).

Description. Body elongated, head depressed. Body trunk compressed, dorsal profile of head and predorsal profile gradually convex, with maximum body depth in middle of pectoral-fin origin and pelvic-fin origin. After dorsal-fin base, dorsal profile of caudal peduncle convex due to developed caudal adipose keel on upper edge of caudal peduncle. Ventral profile of head straight, slightly convex from pectoral-fin origin to pelvic-fin origin, straight from pelvic-fin origin to caudal-fin base.

Anterior and posterior nostrils adjacent, distance less than posterior nostril diameter, base of anterior nostril tube-shaped and tip not clearly elongated to barbel-like. Eyes absent. Mouth inferior, snout obtuse, upper and lower lips smooth. Three pairs of barbels, inner rostral barbel reaching mouth corner, outer rostral and maxillary barbels reaching anterior margin of interopercle. One specimen with one outer and 12 inner gill rakers on first gill arch.

Dorsal fin with two branched and seven branched rays, distal margin straight, origin slightly anterior to pelvic-fin origin. Pectoral fin with one unbranched and 11 branched rays, pectoral-fin length 66.1%–74.8% of distance between pectoral-fin origin and pelvic-fin origin. Pelvic fin with one unbranched and five branched rays, tip of pelvic fin reaching anus. Anal fin with two unbranched and four branched rays, distal margin straight.

Table 1. Morphometric and meristic data of the genus *Karstsinnectes*. Range, mean, and standard deviation (mean ± standard deviation (SD)).

	***K. acridorsalis* (N = 2)**	***K. anophthalmus* (N = 4)**	***K. hyalinus* (N = 3)**	***Karstsinnectes longzhouensis* sp. nov. (N = 4)**	***K. parvus* (N = 3)**
Total length (mm)	44.8–45.9 (45.3 ± 0.8)	30.5–42.8 (36.4 ± 5.1)	47.4–47.5 (47.4 ± 0.1)	57.8–73.3 (64.6 ± 6.8)	32.3–34.8 (33.4 ± 1.3)
Standard length (mm)	37.9–38.1 (38.0 ± 0.1)	25.3–36.9 (31.4 ± 4.8)	38.9–40.2 (39.5 ± 1.0)	49.4–60.1 (53.6 ± 4.8)	26.5–28.3 (27.1 ± 1.1)
Percentage of standard length (%)					
Deepest body depth	13.9–15.5 (14.7 ± 1.1)	11.0–14.4 (13.2 ± 1.6)	19.9–23.6 (21.6 ± 1.8)	16.1–16.4 (17.8 ± 1.4)	17.1–18.4 (17.9 ± 0.7)
Lateral head length	29.9–32.7 (31.3 ± 2.0)	22.7–24.6 (23.5 ± 0.8)	27.1–29.2 (28.0 ± 1.1)	30.2–34.0 (32.2 ± 1.6)	32.5–33.0 (32.7 ± 0.2)
Prodorsal length	54.4–55.5 (54.9 ± 0.8)	59.5–62.6 (60.5 ± 1.4)	55.9–59.0 (57.7 ± 1.6)	55.1–57.3 (56.2 ± 1.1)	52.9–56.3 (54.8 ± 1.8)
Prepelvic length	52.9–54.6 (53.7 ± 1.2)	57.4–58.3 (57.8 ± 0.5)	52.2–56.8 (55.1 ± 2.6)	55.6–58.3 (57.4 ± 1.3)	53.5–58.1 (56.0 ± 2.3)
Preanal length	74.2–75.1 (74.7 ± 0.6)	68.0–76.3 (73.7 ± 3.9)	74.4–79.8 (77.8 ± 2.9)	74.7–76.0 (75.4 ± 0.6)	71.8–76.1 (74.6 ± 2.4)
Preanus length	67.5–70.8 (69.1 ± 2.3)	-	70.6–74.0 (72.5 ± 1.7)	69.4–71.8 (70.2 ± 1.1)	61.7–74.1 (69.2 ± 6.6)
Caudal peduncle length	15.7–15.9 (15.8 ± 0.1)	13.2–17.0 (15.4 ± 1.7)	15.1–16.2 (15.7 ± 0.5)	17.3–19.5 (18.1 ± 1.0)	18.4–22.3 (19.9 ± 2.1)
Caudal peduncle depth (containing caudal adipose keels)	7.3–8.3 (7.8 ± 0.7)	5.7–8.7 (6.9 ± 1.3)	13.0–14.7 (13.8 ± 0.9)	9.2–11.7 (10.5 ± 1.1)	8.4–9.8 (9.2 ± 0.7)
Head width	11.7–12.3 (12.0 ± 0.4)	14.1–16.8 (15.3 ± 1.2)	16.1–17.6 (16.7 ± 0.8)	17.7–21.5 (19.4 ± 1.6)	14.9–18.0 (16.8 ± 1.7)
Percentage of lateral head length (%)					
Head depth	34.6–39.6 (37.1 ± 3.5)	41.0–44.0 (42.6 ± 1.3)	45.1–48.5 (46.9 ± 1.7)	42.3–49.1 (45.4 ± 3.0)	40.8–44.6 (42.7 ± 1.9)
Head width	37.5–39.2 (38.4 ± 1.2)	60.0–73.4 (65.5 ± 6.0)	59.5–60.3 (59.8 ± 0.4)	52.1–71.1 (60.4 ± 8.2)	45.8–54.5 (51.3 ± 4.8)
Percentage of caudal-peduncle length (%)					
Caudal peduncle depth (containing caudal adipose keels)	46.1–51.9 (49.0 ± 4.1)	33.6–57.2 (45.4 ± 9.9)	81.3–97.4 (87.8 ± 8.5)	47.0–66.1 (58.5 ± 8.1)	37.7–53.1 (46.8 ± 8.1)
Percentage of distance from pectoral-fin origin to pelvic-fin origin					
Pectoral-fin length	61.6–67.7 (64.6 ± 4.3)	35.9–48.9 (40.5 ± 6.1)	66.1–74.8 (70.8 ± 4.4)	72.4–85.9 (77.9 ± 5.8)	67.5–76.8 (73.3 ± 5.0)
Dorsal-fin rays	3, 8–9	3, 7	3, 7	3, 9	3, 9
Pectoral-fin rays	1, 10	1, 10	1, 11	1, 11–12	1, 10
Pelvic-fin rays	1, 5	1, 4	1, 5	1, 5	1, 6
Anal-fin rays	3, 5	3, 5	3, 4	3, 5	3, 5
Caudal-fin branched rays	14	12	11–12	13–14	12–13

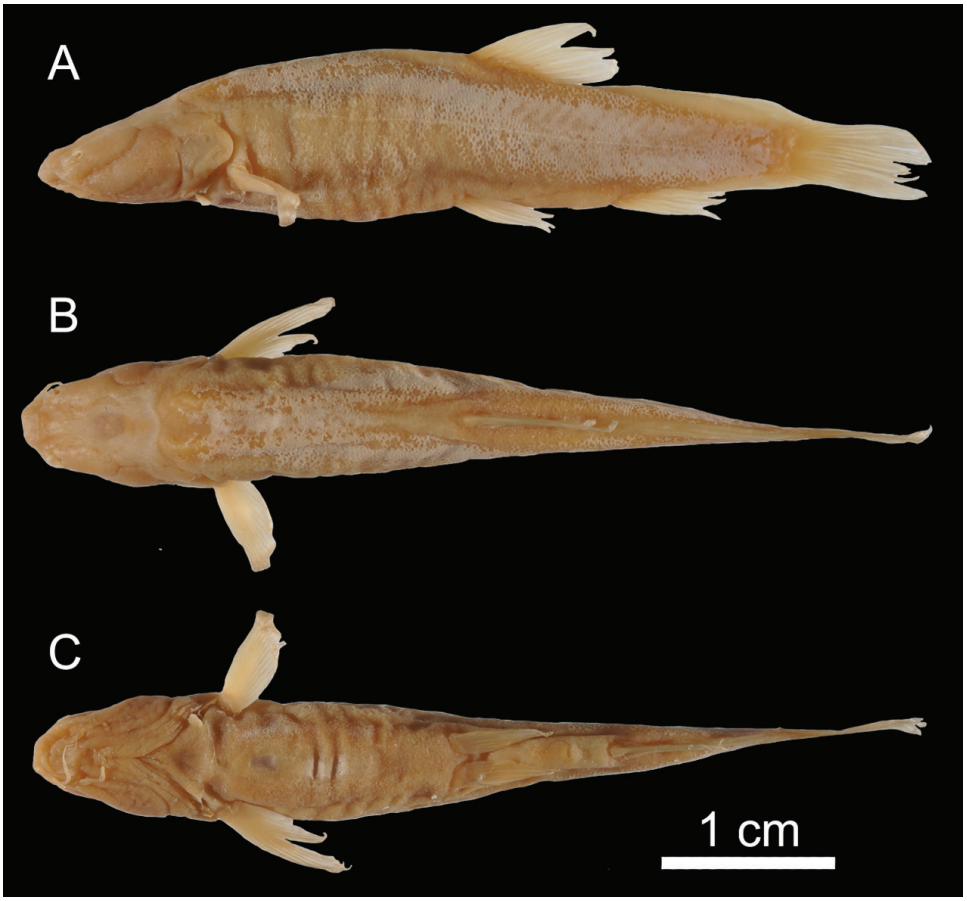

Figure 3. Lateral, dorsal, and ventral views of *K. hyalinus*, holotype KIZ1994000011 (photograph from R. Min). Scale bar: 1 cm.

Anus abutting anal-fin base. Caudal fin forked, with 11 or 12 branched rays. High caudal adipose keels on upper and lower edges of caudal peduncle, height at most of upper adipose keel nearly 1/2 caudal peduncle depth. Caudal peduncle length 102.7%–123.1% of its depth (containing adipose keels). Lateral line and cephalic sensory pores absent. Body covered by scales, except head and thorax.

Coloration. Whole body translucent, without color pattern. Fin membrane hyaline.

Distribution and habitat. Known only from the type locality, China; Guangxi, Hechi City, Du'an County, Bao'an, 24.0709°N, 107.9027°E, 283 m a.s.l. The cave water in which this species resides serves as the only source of drinking water for nearby residents. The decline in its population is primarily attributed to habitat alterations resulting from the extraction of cave water for domestic purposes (Zhang and Cao 2021).

Remarks. The population of *K. hyalinus* is extremely small, with its presence currently known only through type specimens collected in 1994, with no additional specimens gathered since. Although the lateral line of *K. hyalinus* was mentioned in the original description, a re-examination of all type specimens by R. Min, the administrator of the Fish Collection Room, Kunming Natural History Museum of Zoology, Kunming Institute of Zoology, revealed no obvious lateral line pores. Hence, *K. hyalinus* is described as lacking a lateral line in this study.

Karstsinnectes longzhouensis Ge, Du & Zhou, sp. nov.

https://zoobank.org/7D4659FE-C737-4F36-A56F-7DB474246352

Figs 1A, B, 4; Table 1

Type materials. ***Holotype.*** China (permanent whole specimen in 75% alcohol); Guangxi, Chongzuo City, Longzhou County, Xiadong Town; 22.4222°N, 106.6385°E, 170 m a.s.l.; collected by Z.Q. Nong and J.J. Zhou, 29 December 2022. Kunming Natural History Museum of Zoology, KIZ 2023000001, 50.6 mm standard length (SL).

Paratypes. China (permanent whole specimens in 75% alcohol); same collection data as for holotype, collected by Z.Q. Nong and J.J. Zhou, 29 December 2022; KIZ 2023000002–04, 3 ex., 49.4–60.1 mm SL.

Diagnosis. *Karstsinnectes longzhouensis* sp. nov. can be distinguished from all other members of *Karstsinnectes* based on the combination characteristics of the lateral line present, 11 or 12 branched pectoral-fin rays, and five branched pelvic-fin rays. It can be further distinguished from *K. anophthalmus* by caudal fin forked (vs. truncated), five branched pelvic-fin rays (vs. four), nine branched dorsal-fin rays (vs. seven), 13 or 14 branched caudal-fin rays (vs. 12); from *K. acridorsalis* by nine branched dorsal-fin rays (vs. eight), body depth 16.1%–19.4% of SL (vs. 13.9%–15.5%); from *K. hyalinus* by body scaleless (vs. scaled), lateral line present (vs. absent), nine branched dorsal-fin rays (vs. seven), five branched anal-fin rays (vs. four); from *K. parvus* by 11 or 12 branched pectoral-fin rays (vs. 10), five branched pelvic-fin rays (vs. six), and uncorrected *p* distance is 3.9%.

Description. Morphometric data of the type specimens of *Karstsinnectes longzhouensis* sp. nov. are given in Table 1. Body elongated, head depressed, forehead raised, head height at nostril 60.8%–71.0% of maximum head height. Body trunk compressed, with maximum body depth in middle of pectoral-fin origin and pelvic-fin origin, deepest body depth 16.1%–19.4% of SL. Dorsal profile of forehead and predorsal profile convex, concave from dorsal-fin origin to anterior margin of upper caudal adipose keel. Caudal adipose keel on upper edge of caudal of peduncle slightly convex. Ventral profile of head straight, slightly convex from pectoral-fin origin to pelvic-fin origin, straight between pelvic-fin and anal-fin origin, and gradually concave from anal-fin base to anterior quarter of caudal fin due to caudal adipose keel on lower edge of caudal peduncle.

Anterior and posterior nostrils adjacent, distance less than posterior nostril diameter, base of anterior nostril tube-shaped and tip not elongated to barbel-like. Eyes absent. Mouth inferior, snout rounded, upper and lower lips smooth, lower lip with V-shaped median notch. Three pairs of barbels, inner rostral barbel reaching anterior nostril, outer rostral barbel reaching posterior margin of posterior nostril, and maxillary barbel reaching anterior margin of interopercle. Two specimens with 11–12 inner gill rakers on first gill arch.

Dorsal fin with three unbranched and nine branched rays, distal margin of dorsal fin straight, origin anterior to pelvic-fin origin, predorsal length 55.1%–57.3% of SL. Pectoral fin with one unbranched and 10 branched rays, pectoral-fin length 72.4%–85.9% of distance between pectoral-fin origin and pelvic-fin origin. One unbranched and five branched pelvic-fin rays, tip of pelvic fin reaching, but not exceeding anus. Anus abutting anal-fin base. Caudal fin forked, with 13 or 14 branched caudal-fin rays. High caudal adipose keels on upper and lower edges of caudal peduncle, height at most of upper adipose keel less than 1/2 caudal peduncle depth. Caudal peduncle length 151.2%–212.9% of its depth (containing adipose keels). Lateral line and head sensory pores absent. Body scaleless.

Coloration. Dorsal and trunk of body yellowish, abdomen gray and translucent, stomach and intestine visible from outside. Without color pattern. Fin membrane hyaline.

Distribution and habitat. *Karstsinnectes longzhouensis* sp. nov. inhabits karst caves located in the Guangxi Qinglongshan provincial natural reserve, specifically in Xiadong Town, Longzhou County, Chongzuo City, Guangxi, China (22.4222°N, 106.6385°E, 171 m a.s.l.). The species was observed in a subterranean pool accessed through an oval cave entrance and a narrow passage. The pool water depth exceeded 1 m and was characterized by a substratum of mud and cobblestones.

Etymology. The specific name "*longzhouensis*" is derived from the Chinese name of the type locality in Longzhou County. Therefore, the Chinese and English common names for this new species are "龙州中华喀鳅" and "Longzhou Chinese Karst Loach," respectively.

Figure 4. Lateral, dorsal, and ventral views of *Karstsinnectes longzhouensis* sp. nov., holotype KIZ2023000001. Scale bar: 1 cm.

Remarks. On 29 December 2022, Z.Q. Nong collected type specimens within a karst cave. By May 2023, the cave showed significant signs of drying. In another cave located 300 m away, J.J. Zhou collected a deteriorated specimen. Decreased precipitation and the removal of domestic water from the cave appear to have negatively influenced the viability of cavefish during the dry season.

Karstsinnectes parvus (Zhu & Zhu, 2014)

Figs 1A, C, 5; Table 1

Heminoemacheilus parva Zhu & Zhu, 2014: 18–21 (Ande Town, Napo County, Guangxi).

Karstsinnectes parvus Luo et al., 2023, 696 (Ande Town, Napo County, Guangxi).

Neotype designation. Both holotype and paratypes were originally deposited at the Guangxi Fisheries and Animal Husbandry School under registration numbers 2011006–2011009 (Zhu and Zhu 2014) but were broken and lost three years ago (Y. Zhu, pers. comm.). Conforming with Article 75.3 of the Code (ICZN 1999), a neotype from the type locality is herein designated (Fig. 5).

Neotype. China; Guangxi, Baise City, Napo County, Nongma Village, 23.1803°N, 106.0020°E, 934 m a.s.l., collected by J.J. Zhou, J.Q. Luo, X.M. Luo, and Z.X. Qin on 1 May 2023; KIZ 2023000005 (Fig. 5), 26.5 mm SL.

Non-type material. 2 ex. China; same collected with neotype, collected by J.J. Zhou, J.Q. Luo, X.M. Luo, and Z.X. Qin on 1 May 2023; GXNU 20230501001, GXNU 20230501003, 26.5–28.3 mm SL.

Diagnosis. *Karstsinnectes parvus* can be distinguished from *K. acridorsalis* by lateral line present (vs. absent), nine branched dorsal-fin rays (vs. eight), six branched pelvic-fin rays (vs. five), 12 or 13 branched caudal-fin rays (vs. 14); from *K. anophthalmus* by caudal fin forked (vs. truncated), lateral line present (vs. absent), nine branched dorsal-fin rays (vs. seven), six branched pelvic-fin rays (vs. four); from *K. hyalinus* by body scaleless (vs. scaled), lateral line present (vs. absent), nine branched dorsal-fin rays (vs. seven), five branched anal-fin rays (vs. four); from *Karstsinnectes longzhouensis* sp. nov. by 10 branched pectoral-fin rays (vs. 11 or 12), six branched pelvic-fin rays (vs. five).

Description. Body elongated, slightly flattened in front, strongly compressed in back. Maximum body depth anterior to dorsal-fin origin, deepest body depth 17.1%–18.4% of SL. Head depressed and flattened, maximum width greater than maximum depth. Anterior and posterior nostrils adjacent, distance less than posterior nostril diameter, base of anterior nostril tube-shaped and tip not elongated to barbel-like. Eyes absent. Mouth inferior, snout rounded, upper and lower lips smooth, lower lip with V-shaped median notch. Three pairs of barbels, inner rostral barbel reaching anterior nostril, outer rostral barbel reaching posterior margin of posterior nostril, and maxillary barbel reaching anterior margin of interopercle. One specimen with 11 inner gill rakers on first gill arch.

Dorsal fin with three unbranched and nine branched rays, distal margin of dorsal fin straight, origin anterior to pelvic-fin origin, predorsal length 52.9%–56.3% of SL. Pectoral fin with one unbranched and 10 branched

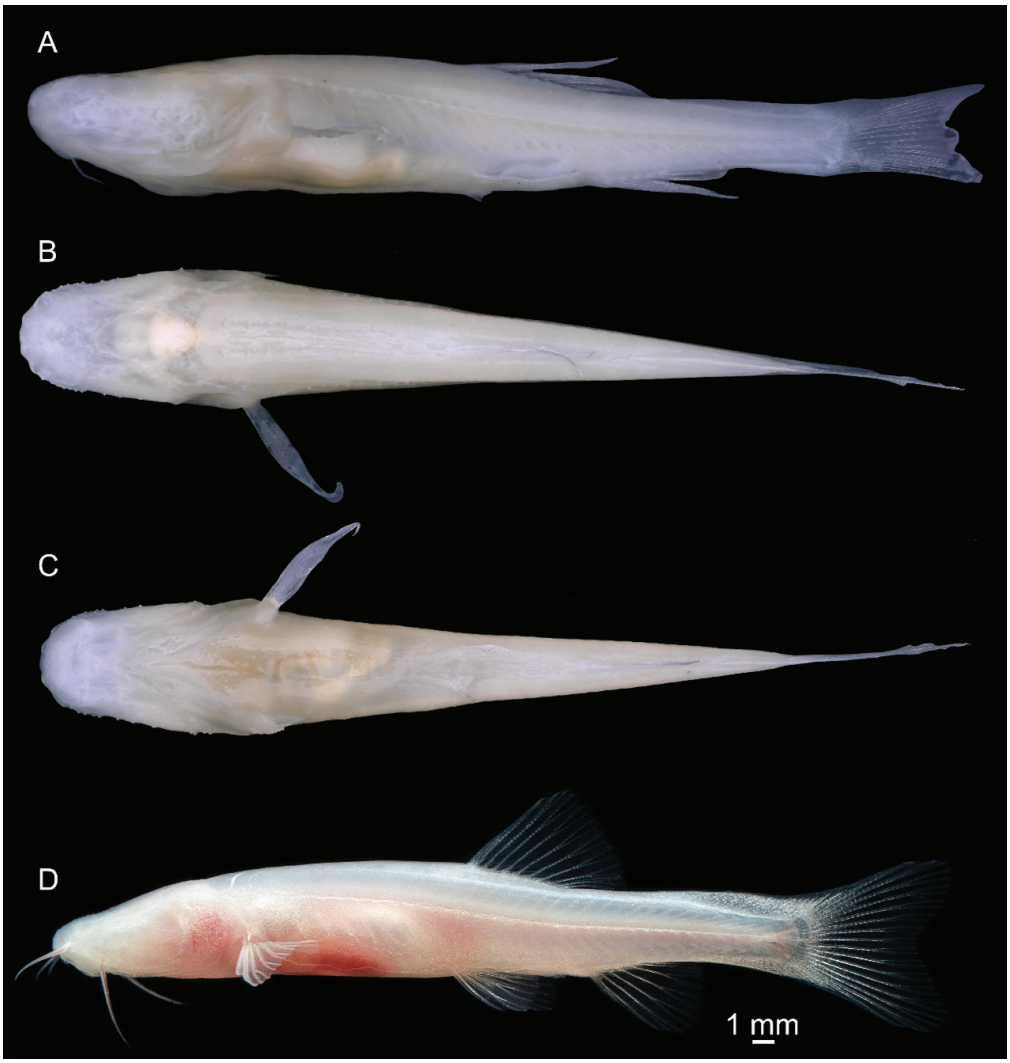

Figure 5. Lateral, dorsal, ventral views, and living photo of *K. parvus*, neotype KIZ2023000005 (photograph from M. Liang). Scale bar: 1 mm.

rays, pectoral-fin length 67.5%–76.8% of distance between pectoral-fin origin and pelvic-fin origin. Pelvic fin with one unbranched and six branched rays, tip of pelvic fin exceeding anus. Anal fin with three unbranched and five branched rays, distal margin straight. Anus abutting anal-fin base. Caudal fin forked, with 12 or 13 branched rays. High caudal adipose keels on upper and lower edges of caudal peduncle, height at most of upper adipose keel less than 1/2 caudal peduncle depth. Caudal peduncle length 188.4%–265.6% of its depth (containing adipose keels). Lateral line present. Body scaleless.

Coloration. Dorsal and trunk of body gray and translucent, stomach and intestine visible from outside. Without color pattern. Fin membrane hyaline.

Distribution and habitat. *Karstsinnectes parvus* inhabits a karst cave in Nongma Village, Napo County, Baise City, Guangxi, China; 23.1803°N, 106.0020°E, 934 m a.s.l., in a small and shallow river (approximately 300 m long, depths of less than 20 cm), characterized by substrata composed of mud and cobblestones. Five to six specimens were caught in each survey in 2021.

Remarks. Given the loss of the type specimens three years ago (Y. Zhu, pers. comm.), three specimens of *K. parvus* were newly collected from the type locality. These specimens conformed to the original description in all aspects except for the caudal fin count. The caudal fin of the holotype was damaged in the original account, preventing verification of the fin ray count from the holotype photograph in the initial description. Lan et al. collected this species from the type locality in 2021 and noted 13 branched rays of the caudal fin (Xiao and Lan 2023). This observation suggests that the unbranched rays of the caudal fin may have been included in the count of branched rays in the original description.

Genetic comparisons

Based on BI analyses, molecular phylogenies demonstrated that species of *Karstsinnectes* constituted a monophyletic group with robust support (100% bootstraps). Furthermore, they were sister to the clade com-

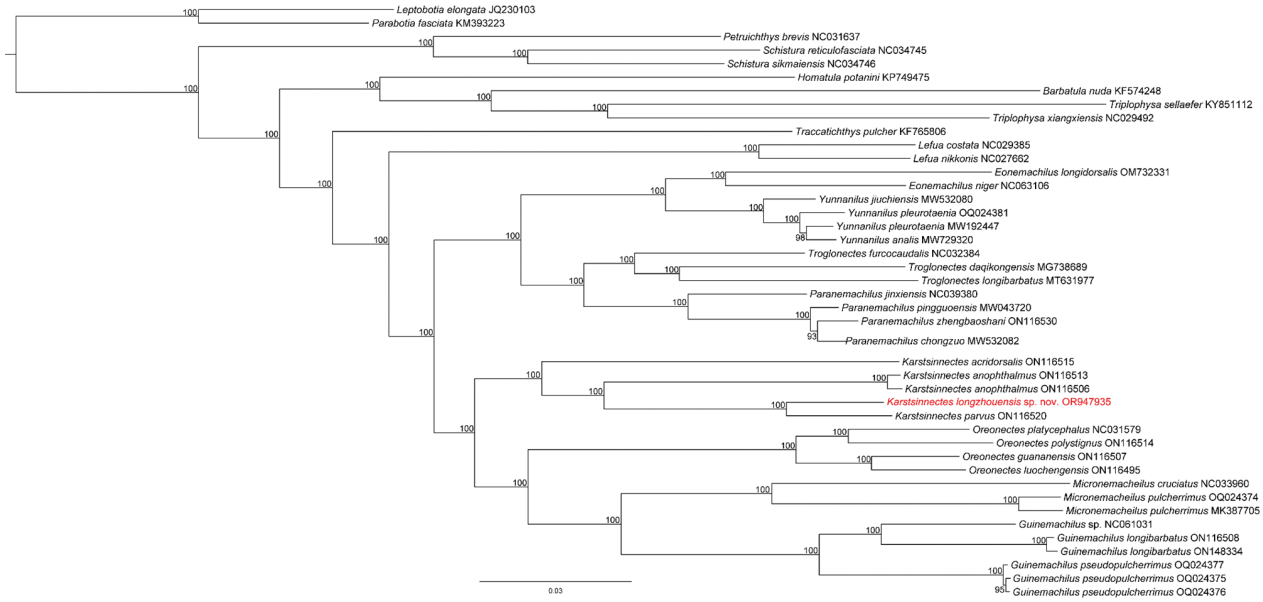

Figure 6. Bayesian phylogram of *Karstsinnectes* based on the mitochondrial genomes of 40 nemacheilid and two botiid species (outgroups). The numbers above the branches are Bayesian posterior probabilities (BPP%).

prised of *Oreonectes*, *Micronemacheilus*, and *Guinemachilus* species. *Karstsinnectes longzhouensis* sp. nov. was determined to be a sister group to *K. parvus* and further sister to *K. anophthalmus* and *K. acridorsalis* (Fig. 6). Additionally, pairwise comparisons of complete mitochondrial genomes revealed that the average uncorrected *p* distance between species of *Karstsinnectes* ranged from 3.96% to 11.38% (average 9.65%). The minimum uncorrected *p* distance is between *K. longzhouensis* sp. nov. and *K. parvus* (3.96%), and the maximum uncorrected *p* distance is both between *K. acridorsalis* and *K. anophthalmus* (11.38%) and between *K. acridorsalis* and *K. parvus* (11.38%) (Table 3). In consideration of both molecular and morphological comparisons, we confidently assign the new species to the genus *Karstsinnectes*.

Principal component analysis (PCA)

The first two principal components (PCs) explained 71.1% of the variance (Table 2). The first principal component (PC1) accounted for 46.7% of the morphological variation and distinguished variables such as body depth/SL, head lateral length/SL, caudal peduncle depth/SL, pectoral-fin length/SL, and pelvic-fin length/SL. Additionally, it separated pectoral-fin length relative to the distance between pectoral- and pelvic-fin origins and pelvic-fin length relative to the distance between pelvic- and anal-fin origins. Predorsal length/SL demonstrated a positive correlation with PC1 scores, while predorsal length/SL exhibited a negative correlation, with factor loadings exceeding 0.60. The second factor (PC2) accounted for 24.4% of the morphological variation, distinguishing variables such as head width to SL, head depth to head lateral length, and head width to head lateral length, which all showed a positive correlation with PC2 scores. Conversely, caudal peduncle length to caudal peduncle depth was negatively correlated with PC2 scores. Scatter plot analysis revealed that species within the genus *Karstsinnectes* could be differentiated based on their morphometric traits (Fig. 7).

Table 2. Loadings of the first three PCs for the morphometric characters of *Karstsinnectes*. * loadings > 60%.

Character	PC1	PC2	PC3
Body depth/SL	0.871*	0.271	-0.126
Head lateral length/SL	0.798*	-0.445	0.221
Prodorsal length/SL	-0.704*	0.592	0.099
Propelvic length/SL	-0.314	0.387	0.577
Preanal/SL	0.392	0.343	-0.310
CPL/SL	0.489	-0.415	0.690*
CPD/SL	0.830*	0.455	-0.242
Head width/SL	0.462	0.662*	0.498
Pectoral-fin length/SL	0.912*	0.157	0.146
Pelvic-fin length/SL	0.883*	-0.195	-0.247
Head depth/head lateral length	0.404	0.779*	0.248
Head width/head lateral length	-0.206	0.896*	0.257
Pectoral-fin length/distance between pectoral-fin and pelvic-fin origin	0.912*	-0.193	0.083
Pelvic-fin length/distance between pelvic-fin and anal-fin origin	0.858*	-0.274	0.075
CPL/CPD	-0.579	-0.607*	0.522
Prp. Tot1	**46.70%**	**24.40%**	**11.90%**

Table 3. Uncorrected pairwise distances between species of Karstsinnectes based on complete mitochondrial genomes.

		1	2	3
1	*K. anophthalmus*			
2	*K. acridorsalis*	0.1138		
3	*K. longzhouensis*	0.1008	0.1071	
4	*K. parvus*	0.1036	0.1138	0.0396

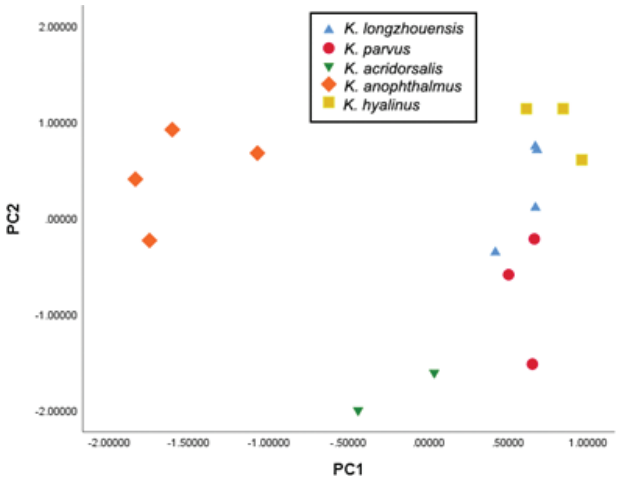

Figure 7. Scatter plots of first and second PCs of pooled morphometric data of *Karstsinnectes*.

Discussion

While Luo et al. (2023) established the genus *Karstsinnectes*, its diagnosis remains unclear. Du et al. (2023) indicated that the location of the anterior and posterior nostrils serves as an important characteristic for the generic diagnosis of Chinese nemacheilids with tube-shaped anterior nostrils and can be categorized into three types based on their location, namely separated, adjacent, and closed-set (Du et al. 2023). The anterior nostrils of *Karstsinnectes* are tube-shaped and located adjacent to the posterior nostrils, with the intervening distance shorter than the diameter of the posterior nostril. Additionally, the tip of the anterior nostril does not extend in a barbel-like manner. Therefore, the primary diagnostic features of *Karstsinnectes* include anterior nostril tube-shaped, adjacent to posterior nostril, anterior nostril tip not elongated to barbel-like, lips with furrows, caudal peduncle with adipose crests, and bony capsule of the swim bladder open posteriorly.

In morphology, *K. anophthalmus* possesses distinct characteristics from other congeneric species, including an obtuse snout, shorter pectoral and pelvic fins, a pectoral-fin length less than half the distance from its origin to the pelvic-fin origin, and a pelvic fin tip that does not reach the anus. Scatter plots of the first and second PCs of pooled morphometric data of *Karstsinnectes* also indicated the morphological difference of *K. anophthalmus*. The morphological difference could indicate that *K. anophthalmus* represents a new genus. However, the molecular analysis is inconsistent with the morphological evidence. In the phylogenetic tree, *K. longzhouensis* sp. nov. was determined to be a sister group to *K. parvus* and further sister to *K. anophthalmus* and *K. acridorsalis*. According to Luo et al. (2023), the specimens collected from Daxin County, Chongzuo City, Guangxi, China, were identified as *K. anophthalmus*. However, J.H. Lan and J. Yang observed that the morphology of the specimens from Daxin differed from those found in the type locality. However, the samples of ON116513 and ON116506 were from Daixin County; the specimens were not sequenced from the type locality. Therefore, further investigations are required to determine the taxonomic status of these specimens.

Blind cavefish represent a powerful natural model for studying adaptations in extreme environments. Cave habitats, characterized by limited food resources, low oxygen levels, and perpetual darkness (Zhao and Zhang 2009), present considerable challenges. To adapt and thrive in such environments, cavefish have undergone a series of evolutionary changes, including eye and pigmentation loss, enhancement of non-visual senses, development of specialized jaws for feeding, increased number of taste buds and barbels, and enhanced ability to store adipose tissue (Yang et al. 2016; Ma et al. 2019, 20233). Blind cavefish within the genus *Karstsinnectes* exhibit a suite of troglomorphic traits, including scaleless and colorless bodies, absence of eyes, elongated snouts (except *K. anophthalmus*), and caudal peduncles with well developed adipose crests. Current studies suggest that surface-dwelling species, upon colonizing cave environments and being granted sufficient time, undergo analogous transformations. Consequently, cave-dwelling organisms serve as a valuable natural framework for investigating convergent and adaptive evolutionary processes.

The survival of cavefish is significantly threatened by human activities, climate change, water pollution, and invasive species. As of 2021, the Red List of Biodiversity in China includes 13 cavefish species from the Nemacheilidae family (Zhang and Cao 2021). Zhang and Cao (2021) stated that the population of *K. anophthalmus* is very small, with only one or two specimens caught in each survey in 1996, 2003, and 2010, respectively. Major threats include road infrastructure construction, tourism activities, and groundwater contamination from agricultural operations in the recharge areas of the cave (Zhang and Cao 2021). Additionally, Shu et al. (2013) highlighted the impact of droughts and human extraction of water from cave pools on cave biodiversity in southwestern China. Thus, to conserve these valuable cavefish species, the implementation of conservation measures, such as habitat protection, artificial breeding, and fundamental research, is crucial.

Conclusions

In this paper, a new blind species is described from Guangxi, China. The phylogenetic tree indicated that the new species belongs to the genus *Karstsinnectes*. Additionally, the genus diagnosis is redefined, a neotype for *K. parvus* is designated due to the type specimens lost, and the morphology character of *K. hyalinus* is revised. The population of these species is quite small and sensitive to human activity. The protection of cave fish is often neglected due to the difficulty of cave exploration. The findings of this study improve our understanding of the species diversity of the genus *Karstsinnectes* and provide the basis for cavefish protection.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

J.Y.G. and L.N.D. measured the specimens, analyzed the data, and prepared the manuscript. L.N.D. and J.Y. conceived and designed the study, analyzed the molecular data, constructed the phylogenetic tree, and provided funding for complete mitochondrial genomes and field surveys. Z.Q.N. and J.J.Z. conducted the field survey. All authors read and approved the final version of the manuscript.

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