

Letter to the editor

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A new loach species of *Troglonectes* (Teleostei: Nemacheilidae) from Guangxi, China

A new species of the genus *Troglonectes* is described from Guangxi Zhuang Autonomous Region, China. *Troglonectes hechiensis* sp. nov. can be easily distinguished from its congeners based on the following characters: eyes normal; whole body covered by scales except head, throat, and abdomen; lateral line incomplete; caudal fin concave; and color pattern present on body.

Nemacheilidae is ecologically important and taxonomically challenging family distributed in Asia, Europe, and northeast Africa (Fricke et al., 2021; Kottelat, 2012; Prokofiev, 2010; Zhang & Zhao, 2016). To date, 758 species belonging to 47 genera of Nemacheilidae have been considered as valid (Chen, 2013; Du et al., 2021; Eagderi et al., 2019; Freyhof & Geiger, 2021; Fricke et al., 2021; Gransee et al., 2019; Jiang et al., 2021; Kaya et al., 2020a, 2020b, 2021; Kottelat, 2012; Prokofiev, 2010; Yoğurtçuoğlu et al., 2020; Zhang & Zhao, 2016).

Du et al. (2008) divided *Oreonectes* Günther, 1868 into the *platycephalus* group with a rounded or truncated caudal fin and the *furcicaudalis* group with a forked caudal fin. Kottelat (2012) later suggested that some named species, including *O. elongatus* Tang, Zhao & Zhang, 2012, *O. furcicaudalis* Zhu & Cao, 1987, *O. macrolepis* Huang, Du, Chen & Yang, 2009, *O. microphthalmus* Du, Chen & Yang, 2008, and *O. translucens* Zhang, Zhao & Zhang, 2006, do not belong to *Oreonectes* due to their forked caudal fin and dorsal-fin origin being clearly anterior to the pelvic fin. Tang et al. (2012) also confirmed species within *Oreonectes* and suggested establishing the new genus of *Troglonectes* for all species with a forked caudal fin, dorsal fin origin anterior to the pelvic fin origin, and well-developed caudal crests. Subsequently, Zhang & Zhao (2016) described the genus *Troglonectes* with *O. furcicaudalis* as the type species and noted several diagnostic characters, including: nostrils narrowly separated; anterior nostril tube short, extending into short barbel; caudal fin forked; and dorsal

fin origin anterior to pelvic fin origin. Seven species were suggested for inclusion in *Troglonectes*, including *T. acridorsalis* (Lan, 2013), *T. barbatus* (Gan, 2013), *T. elongatus* (Tang, Zhao & Zhang, 2012), *T. macrolepis*, *T. microphthalmus*, *T. translucens*, and its type species (Zhang & Zhao, 2016). Based on mitochondrial (*COI* and cytochrome *b* (*cyt b*)) and nuclear (*IRBP*, *RAG1*, and *RH1*) genes, Yan (2017) stated that *Triplophysa dongganensis* Yang, 2013, *T. lihuensis* Wu, Yang & Lan, 2012, and *T. longibarbatatus* Chen, Yang, Sket & Algancic, 1998 should not be included in *Triplophysa*. Subsequently, Li (2018) placed 16 species and three synonym species in *Troglonectes* based on several morphological characters, i.e., nostrils narrowly separated, caudal fin forked, and well-developed caudal crests. The suggested species include *T. furcicaudalis*, *T. acridorsalis*, *T. barbatus*, *T. dongganensis*, *T. donglanensis*, *T. duanensis*, *T. huanjiangensis* (Yang, Wu & Lan, 2011, junior synonym to *T. elongatus* Tang, Zhao & Zhang, 2012), *T. jiarongensis*, *T. lihuensis*, *T. lingyunensis*, *T. longibarbatatus* (junior synonym to *Homatula posterodarsalus* (Li, Ran & Chen, 2006)), *T. macrolepis*, *T. maolanensis* (Li, Ran & Chen, 2006, junior synonym to *O. daqikongensis* Deng, Wen, Xiao & Zhou, 2016), *T. microphthalmus*, *T. nasobarbatula*, *T. shuilongensis* Deng, Xiao, Hou & Zhou, 2016, and *T. translucens* (Li, 2018). Huang et al. (2020) also suggested that *O. daqikongensis*, *O. shuilongensis* Deng, Xiao, Hou & Zhou, 2016, and *Triplophysa jiarongensis* belong to *Troglonectes* based on morphological characters and molecular analysis. At present, 17 species have been recorded in *Troglonectes*, including the type species.

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In January 2017, six specimens of *Troglonectes* were collected in Tongjin Village, Hechi City, Guangxi Zhuang Autonomous Region, China. Compared with other congeneric species, we observed several distinctive characteristics. We considered these specimens to represent a new species of the genus *Troglonectes*, which we describe herein.

Upon collection, the freshly caught fish were euthanized with eugenol. The pectoral fin from the right side was taken and preserved in ethanol for molecular analysis. Specimens used for morphological studies were preserved in formalin for three days, and then transferred to 75%–80% ethanol for long-term storage at the collection of the Kunming Natural History Museum of Zoology, Kunming Institute of Zoology (KIZ), Chinese Academy of Sciences (CAS).

Methods used for counting and measurement followed Tang et al. (2012). All measurements were taken point-to-point with dial calipers to the nearest 0.1 mm. A fragment of the mitochondrial gene coding for *cyt b* was amplified with primers designed by Šlechtová et al. (2006). DNA extraction and polymerase chain reaction (PCR) amplification using the traditional pheno-chloroform extraction method were conducted by the Tsingke Biological Technology Company, Kunming, China (Taggart et al., 1992). All new sequences were deposited in GenBank (Accession Nos.: MW495267–MW495269). To test the phylogenetic position of *T. hechiensis* sp. nov., Bayesian inference was performed in MRBAYES v3.2.6 (Ronquist et al., 2012) and *cyt b* sequences of another 29 nemacheilids from GenBank were included in the data. *Parabotia fasciata* Dabry de Thiersant, 1872 and *Leptobotia elongata* (Bleeker, 1870), two species of botiid, were used as outgroup. Two runs were performed simultaneously with four Markov chains starting from random tree. The chains were run for five million generations and sampled every 100 generations. The first 25% of the sampled tree was discarded as burn-in and the remaining trees were then used to create a consensus tree and to estimate Bayesian posterior probabilities (BPPs).

Taxonomic account

Troglonectes hechiensis sp. nov. (Figure 1C–E; Table 1)

Holotype: KIZ2021000001 (Kunming Natural History Museum of Zoology, KIZ, CAS), 50.9 mm standard length (SL), Tongjin Village, Hechi City, Guangxi Zhuang Autonomous Region, China, Xiaohuanjiang River, a tributary of Longjiang River; N24.7341°, E107.9956°, F.G. Luo, January 2017 (Figure 1B).

Paratypes: KIZ2021000002–6, 47.4–58.3 mm SL, same as holotype.

Etymology: The specific name of *hechiensis* is derived from Hechi City, where the type specimens were collected. We suggest the Chinese common name as “河池洞鲈”.

Diagnosis: *Troglonectes hechiensis* sp. nov. can be distinguished from its congeners by the following combination of characters: caudal fin concave (vs. forked except *T. shuilongensis*), eyes normal (vs. absent or degenerated a black pigment in *T. acridorsalis*, *T. barbatus*, *T. dongganensis*, *T. donglanensis*, *T. huanjiangensis*, *T. jiarongensis*, *T. lihuensis*, *T. lingyunensis*, *T. longibarbatulus*, *T. macrolepis*, *T. maolanensis*, *T. microphthalmus*, *T. nasobarbatula*, *T.*

shuilongensis, and *T. translucens*); whole body covered by scales (vs. scaleless in *T. acridorsalis*, *T. barbatus*, *T. dongganensis*, *T. donglanensis*, *T. huanjiangensis*, *T. jiarongensis*, *T. lihuensis*, *T. longibarbatulus*, *T. maolanensis*, *T. microphthalmus*, *T. nasobarbatula*, *T. shuilongensis*, and *T. translucens*, after dorsal fin origin covered by scales in *T. furcocaudalis*); lateral line incomplete (vs. complete in *T. jiarongensis* and *T. shuilongensis*, lateral line absent in *T. acridorsalis*, *T. barbatus*, *T. duanensis*, *T. huanjiangensis*, *T. lihuensis*, *T. longibarbatulus*, *T. maolanensis*, and *T. nasobarbatula*); with 13–14 branched rays (vs. 16 in *T. jiarongensis*, *T. nasobarbatula* and *T. translucens*).

Description: Morphometric data of type specimens of *Troglonectes hechiensis* sp. nov. are given in Supplementary Table 1. D, IV, 8; A, III, 5; P, I, 11; V, I, 5–6, C, 13–14; 14 inner gill raker on first gill arch ($n=2$). Cephalic lateral-line canals unclear. Lateral line incomplete, not exceeding tip of pectoral fin.

Body elongated and cylindrical, with deepest body depth anterior to dorsal fin origin, deepest body depth 15.0%–17.4% SL. Head slightly depressed and flattened, width greater than depth. Snout round, shorter than postorbital length. Mouth inferior, upper and lower lips smooth, lower lip with V-shaped median notch. Processus dentiformis absent. Three pairs of barbels are long: inner rostral extending to anterior margin of eye, outer rostral and maxillary barbels extending over posterior margin of eye. Nostrils narrowly separated. Anterior nostril tube short, with elongated short barbel-like tip. Eyes normal, diameter 17.7%–21.2% of lateral head length, lacking a suborbital groove in the male.

Dorsal fin distally truncate, origin opposite to pelvic-fin insertion, situated slightly posterior to midpoint between snout tip and caudal-fin base. Pectoral fins almost reaching midpoint between pectoral fin and pelvic fin origin. Tips of depressed pelvic fin not reaching anus, distance between tips of pelvic fin and anus 1.4–1.8 times eye diameter. No axillary lobe present at base of pelvic fin. Anal fin origin close to anus (44.2%–61.5% of eye diameter). Caudal fin concave. Caudal peduncle with adipose crests along both dorsal and ventral sides, dorsal adipose crest depth nearly 1/4 of caudal peduncle depth.

Except head, throat, and abdomen, body completely covered by scales. Intestine short and straight. Two air-bladder chambers, posterior chamber developed, filling body cavity, connected with anterior chamber by long, slender tube, tube length nearly 3/4 of posterior chamber length.

Coloration: Back and flank yellowish-brown, belly yellowish. Three longitudinal stripes comprised of spots on flank: middle longitudinal stripe comprised of nearly 20 round spots along lateral line from posterior of operculum to base of caudal fin; 15–17 spots from posterior head to base of caudal fin on upper flank; 14–16 spots from pectoral fin origin to anal fin origin on lower flank. One larger dark brown blotch on the caudal-fin base than the other blotches on the flank. There are numerous dark brown pigment cells on the fin rays, fin membrane hyaline.

Distribution: The new species is currently only found in Tongjin Village, Hechi City, Guangxi Zhuang Autonomous Region, China, Xiaohuanjiang River, a tributary of Longjiang

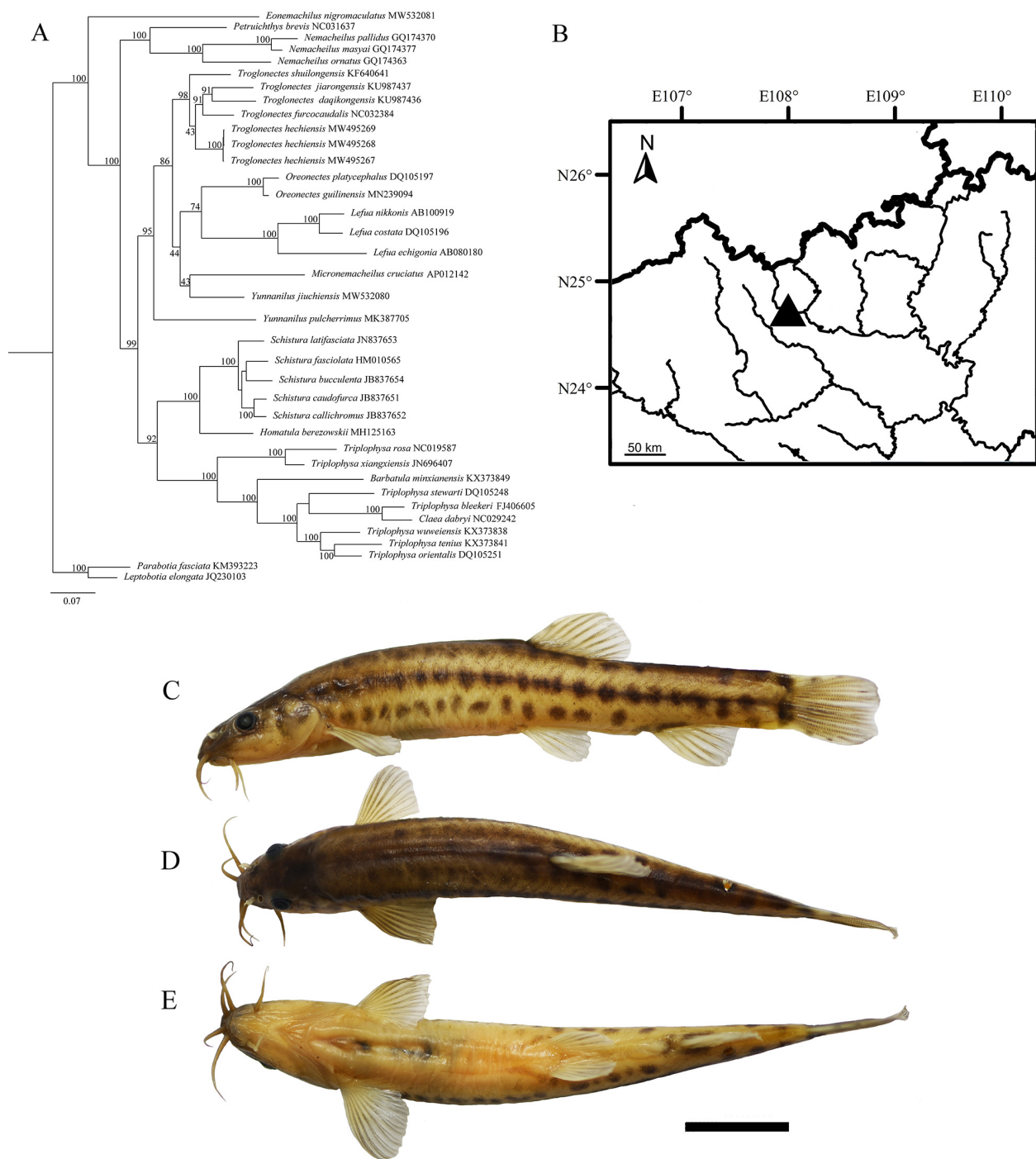


Figure 1 Phylogeny, collection sites and photos of *Troglonectes hechiensis* sp. nov. (KIZ2021000001)

A: Bayesian phylograms of nemachilids inferred from *cyt b* gene. B: Collection site of *Troglonectes hechiensis* sp. nov. (black triangle) in Guangxi, China. C–E: Lateral, dorsal and ventral views of *T. hechiensis* sp. nov. Scale bar: 1 cm.

River (N24.7341°, E107.9956°) (Figure 1B).

Genetic comparisons: Molecular phylogenies based on BI analyses (Figure 1A) show that *Troglonectes hechiensis* sp. nov. and the type species *T. furcocaudalis*, *T. daqikongensis*, *T. jiarongensis*, and *T. shuilongensis* form a monophyletic group with strong support (98% bootstrap), and sister to the clade including species of *Oreonectes*, *Lefua*,

Micronemacheilus, and *Yunnanilus*. On this basis, as well as morphological comparisons, we are confident in assigning the new species to the genus *Troglonectes* (Figure 1A).

Remarks: Zhang & Zhao (2016) established the genus *Troglonectes* based on various characters, including forked caudal fin, pelvic fin originating opposite or slightly posterior to dorsal fin origin, and caudal peduncle with adipose crests

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

	Holotype	Range (n=5)	Mean±SD
Total length (mm)	60.4	56.6–69.3	63.2±5.5
Standard length (mm)	50.9	47.4–58.3	53.0±4.8
Percent of standard length (%)			
Deepest body depth	16.1	15.0–17.4	16.0±0.8
Lateral head length	24.6	23.5–25.1	24.4±0.6
Predorsal length	56.9	54.4–59.3	56.7±1.6
Prepelvic length	56.7	55.9–60.4	57.5±1.6
Preanal length	79.2	78.2–80.9	79.7±1.2
Preanus length	77.0	75.3–78.0	77.0±0.9
Caudal peduncle length	12.7	12.0–13.6	12.7±0.5
Caudal peduncle depth	8.6	8.7–10.3	9.3±0.6
Head width	14.2	14.3–16.0	14.9±0.7
Percent of lateral head length (%)			
Eye diameter	18.3	17.7–21.4	19.2±1.4
Interorbital width	28.4	27.1–30.4	28.7±1.4
Snout length	35.3	32.4–36.2	34.6±1.7
Head width	57.9	59.2–64.4	1.3±2.6
Head depth	51.4	51.9–54.2	52.5±1.0
Percent of caudal peduncle length (%)			
Caudal peduncle depth	67.8	72.7–76.0	73.8±3.2
Dorsal-fin rays	IV, 8	IV, 8	8
Pectoral-fin rays	I, 11	I, 11	11
Pelvic-fin rays	I, 6	I, 5–6	5.7±0.5
Anal-fin rays	III, 5	III, 5	5
Caudal-fin branched rays	14	13–14	13.5±0.6

along both dorsal and ventral sides. Within the genus *Troglonectes*, *T. shuilongensis* and *Troglonectes hechiensis* **sp. nov.** both possess a concave caudal fin. However, *Troglonectes hechiensis* **sp. nov.** can be distinguished from *T. shuilongensis* by the following characters: eye normal (vs. absent); body covered by scales (vs. scaleless); lateral line incomplete (vs. complete); and color pattern present (vs. absent).

Troglonectes hechiensis **sp. nov.**, *T. donglanensis*, *T. duanensis*, *T. furcocaudalis*, *T. lingyunensis*, *T. macrolepis*, and *T. microphthalmus* have normal eyes or degenerated black pigment. However, the new species can be distinguished by caudal fin concave (vs. forked). *Troglonectes hechiensis* **sp. nov.** can be further distinguished from *T. donglanensis*, *T. furcocaudalis*, and *T. microphthalmus* by whole body covered by scales (vs. scaleless in *T. donglanensis* and *T. microphthalmus* and after dorsal fin origin in *T. furcocaudalis*) and lateral line incomplete (vs. lateral line absent in *T. duanensis*).

For all other congeneric species, including *T. acridorsalis*, *T. barbatus*, *T. dongganensis*, *T. huanjiangensis*, *T. jiarongensis*, *T. lihuensis*, *T. longibarbatulus*, *T. maolanensis*, *T. nasobarbatula*, and *T. translucens*, the new species can be easily distinguished by eyes normal (vs. absent) and body covered by scales (vs. scaleless).

NOMENCLATRURAL ACTS REGISTRATION

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SCIENTIFIC FIELD SURVEY PERMISSION INFORMATION

Field collections followed the rules of the Fisheries Law of the People's Republic of China. All activities conformed to the Laboratory Animal Guidelines for the Ethical Review of Animal welfare (GB/T 35892-2018).

SUPPLEMENTARY DATA

Supplementary data to this article can be found online.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

L.X.Z. and J.H.L. measured the specimens, analyzed the data, and performed the molecular experiments. L.N.D. conceived and designed the study and prepared the manuscript. F.G.L. conducted field surveys. All authors read and approved the final version of the manuscript.

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