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Letter to the editor Open Access

Triplophysa xuanweiensis sp. nov., a new blind loach species from a cave in China (Teleostei: Cypriniformes: Nemacheilidae)

Triplophysa xuanweiensis **sp. nov.**, a new blind loach species from the family Nemacheilidae, is described based on specimens collected from a subterranean river in Xuanwei City, Yunnan Province, China. The new species can be distinguished from all known congeners by a combination of the following characters: eyes absent, dorsal-fin origin anterior to pelvic-fin origin, tip of pelvic fin reaching anus, dorsal fin with 7–8 branched rays, caudal fin forked with 16–18 branched rays, body scaleless and colorless, lateral line complete, and posterior chamber of air bladder well developed and connected to anterior chamber by long, slender tube. Maximum-likelihood phylogenetic analysis based on the cytochrome *b* (cyt *b*) gene placed *Triplophysa xuanweiensis* **sp. nov.** in a well-supported clade with seven other species of the genus, and three specimens of the new species formed a monophyletic group, consistent with morphological comparisons.

Triplophysa Rendahl, 1933 is one of the largest genera in Cypriniformes, containing 147 valid species (Froese & Pauly, 2021). *Triplophysa* species are distributed from the Qinghai-Xizang Plateau into North China, Central Asia, and the Himalayas (Li, 2018). Most fish in the genus inhabit high plateau or mountainous areas, with some species also exhibiting cave-dwelling behavior (Li, 2018; Ma et al., 2019). *Triplophysa* is the largest genus of freshwater fish in China and includes 102 species (Zhang et al., 2020). Up to now, all identified cave-dwelling species of *Triplophysa* are reported from China (Ma et al., 2019).

Caves are considered extreme environments for animals due to the lack of sunlight. As such, cave-dwelling fish have evolved a suite of morphological adaptations to cope with their extreme environment. These troglomorphic traits include reduction or loss of eyes, pigmentation, scales, and swim bladders, as well as enhancement of mechano- and chemo-sensation and elongation of fin rays (Zhao et al., 2011). Cavefish can thus be assigned into two morphological types, i.e., stygobite/typical and stygophile/atypical cavefish, according to the presence of troglomorphic characters (Zhao et al., 2011).

China has more than 148 cavefish species, half of which are classified as stygobites (Ma et al., 2019). There are 198 stygobite species worldwide, which are found on every continent but Antarctica (Niemiller et al., 2019). Nearly 40% of the total number of stygobiotic species are found in China, more than any other country (Ma et al., 2019; Niemiller et al., 2019). These cavefish are mainly distributed in the South China Karst region, which is the largest continuous karst area and one of the most spectacular examples of humid tropical to subtropical karst landscapes in the world (Zhao et al., 2011). Caves are the dominant habitat in the region, harboring a substantial diversity of organisms adapted to life underground, including distinctive cavefish.

In April 2018, 16 specimens of an undescribed blind loach were collected from a cave in Xinle Village, Tangtang Town, Xuanwei City, Yunnan Province, China, which is interconnected with the Beipan River system, a tributary of the Hongshui River in the Pearl River drainage. After comparing the morphological characters to all known closely-related species, we determined the specimens to be a new species within the genus *Triplophysa*.

Detailed information on comparative material is provided in Supplementary Text. Counts and proportional measurements followed Li (2018). All measurements were taken point-to-point with digital calipers to 0.1 mm. Morphometric and meristic data were tabulated and analyzed using Microsoft Excel.

Total DNA was extracted from tissues using a TIANamp Marine Animals DNA Kit (Tiangen, China) according to the manufacturer's protocols. A fragment of the mitochondrial

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gene coding for cyt *b* was amplified following the method described by Huang et al. (2020). Polymerase chain reaction (PCR) mixtures contained 5 μL of PCR buffer (×10), 5 μL of 2 mmol/L deoxynucleotide triphosphate (dNTP), 2.5 μL of 2 μmol/L primers, 0.5 μL of Taq DNA polymerase, 1.0 μL of extracted DNA, and 36 μL of H_2O. The PCR thermal cycle profile included: 5 min at 94 °C, followed by 35 cycles of 45 s at 94 °C, 30 s at 54 °C, 1 min at 72 °C, and final extension of 7 min at 72 °C.

The mitochondrial cyt *b* gene sequences of 15 other species in the genus *Barbatula* Linck, 1790 and *Triplophysa* were obtained from GenBank (Supplementary Table S1). Alignment of the mitochondrial cyt *b* sequences was performed using the Clustal W algorithm in MEGA X (Kumar et al., 2018), along with manual checks for inconsistencies. Phylogenetic reconstructions were performed using maximum-likelihood (ML) with the General Time Reversible model (Nei & Kumar, 2000), with 1 000 bootstrap replicates.

Taxonomy

Triplophysa xuanweiensis Lu, Li, Mao *et* Zhao, **sp. nov**. (Figure 1A–F; Supplementary Table S2)

Holotype: ASIZB 223816, 68.6 mm standard length (SL), Xinle Village, Tangtang Town, Xuanwei City, Yunnan Province, China, Beipan River system, a tributary of the Hongshui River; N26°32′44″, E104°08′01″, collected by Z.M. Lu, W.N. Mao, W.J. Lü, G. Huang, T.K. Xu, April 2018.

Paratypes: ASIZB 223817-223831 (15 specimens), 21.7–85.5 mm SL; same collection information as holotype.

Diagnosis: The new species, along with *Triplophysa erythraea* Liu & Huang, 2019, *T. fengshanensis* Lan, 2013, *T. rosa* Chen & Yang, 2005, *T. xiangxiensis* (Yang, Yuan & Liao, 1986), *T. anshuiensis* Wu, Wei, Lan & Du, 2018, *T. gejiuensis* (Chu & Chen, 1979), *T. langpingensis* Yang, 2013, *T. shilinensis* (Chu & Yang, 1992), and *T. qiubeiensis* Li & Yang, 2008, differs from all other congeners by eyes absent (vs. eyes present). Among these blind loaches, the new species can be distinguished from *T. erythraea* and *T. fengshanensis* by dorsal-fin origin anterior to pelvic-fin origin (vs. dorsal-fin origin opposite to pelvic-fin origin), from *T. rosa* and *T. xiangxiensis* by pectoral-fin not reaching pelvic-fin origin (vs. pectoral-fin reaching or beyond pelvic-fin origin), from *T. anshuiensis* by 16–18 branched caudal-fin rays (vs. 14), from *T. gejiuensis* by pelvic fin reaching anus (vs. pelvic fin not reaching anus), from *T. langpingensis* by adipose crest absent (vs. adipose crest well developed), and from *T. shilinensis* and *T. qiubeiensis* by posterior chamber of air bladder well developed (vs. posterior chamber of air bladder degenerated).

Description: General body features in Figure 1A–D. Meristic and morphometric data for new species and comparative material are provided in Supplementary Table S2.

Body elongated and cylindrical, posterior portion gradually compressed from dorsal fin to caudal-fin base. Dorsal profile slightly convex from snout to dorsal-fin insertion, then straight from posterior portion of dorsal-fin origin to caudal-fin base. Ventral profile flat. Greatest body depth anterior of dorsal-fin origin.

Head slightly depressed and flattened, width greater than depth. Snout slightly pointed. Eyes absent. Anterior and posterior nostrils adjacently located, anterior nostril forming valve, end of valve elongated to form short barbel extending beyond posterior margin of posterior nostrils, posterior nostril round (Figure 1E). Mouth inferior and curved; mouth corner situated below anterior nostril, lips thick, upper lip smooth, lower lip with V-type median notch (Figure 1F). Three pairs of barbels; inner rostral barbel shortest, ~8.2%–29.4% of head length, outer rostral barbel longest, extending to mouth corner, maxillary barbel length ~8.2%–46.0% of head length.

Dorsal fin emarginated, origin closer to tip of snout than to caudal-fin base, anterior to vertical line at pelvic-fin origin; first branched ray longest, slightly shorter than head length. Anal-fin origin separated from anus by short distance, fin short with 5 branched rays. Pectoral fin moderately developed, tip reaching mid-point between pectoral and pelvic fin origins. Pelvic-fin origin posterior to dorsal-fin origin, vertically aligned with second branched ray of dorsal fin, pelvic-fin tip reaching to anus. Caudal fin forked, with 17–18 branched rays, upper lobe slightly longer than lower one, tips blunt.

Body entirely smooth and scaleless. Lateral line complete and straight, ending at caudal-fin base. Two air bladder chambers, posterior chamber of air bladder well developed and connected to anterior chamber by long, slender tube.

Coloration: Live fish semi-translucent and pale pink, without skin pigment, all fins hyaline (Figure 1D). Whole body yellowish white after fixation in alcohol, fins yellowish and translucent.

Distribution: The new species is known only from a cave located in Xinle Village, Tangtang Town, Xuanwei City, Beipan River system, a tributary of the Hongshui River (Figure 1H).

Etymology: The species name is derived from the city, Xuanwei, where the cave is located. The Chinese common name for this species is “宣威盲高原鳅”, which means Xuanwei blind high-plateau loach.

Genetic comparisons: ML phylogenetic analysis (Figure 1G) based on the mitochondrial cyt *b* gene showed that *Triplophysa xuanweiensis* **sp. nov.** and 10 other cave-dwelling species of *Triplophysa* formed a clade with 100% bootstrap support, which was sister to the group composed of four non-cave-dwelling species of *Triplophysa*. Three specimens of the new species formed a monophyletic group, consistent with morphological comparisons. Furthermore, the interspecific genetic distances for cyt *b* between *Triplophysa xuanweiensis* **sp. nov.** and its congeners were all greater than 12% (Supplementary Table S3). Therefore, we confirmed *Triplophysa xuanweiensis* **sp. nov.** as a new species of the genus *Triplophysa*.

Remarks: Li (2018) reviewed all nemacheilid cavefish from China and placed *Triplophysa dongganensis* Yang, 2013, *T. huanjiangensis* Yang, Wu & Lan, 2011, *T. jiarongensis* Lin, Li & Song, 2012, *T. lihuensis* Wu, Yang & Lan, 2012, *T. lingyunensis* (Liao, Wang & Luo, 1997), and *T. longibarbatus* (Chen, Yang, Sket & Algancic, 1998) into the genus *Troglonectes*, which was followed by Huang et al. (2020) and Zhao et al. (2021). With the new species described herein, *Triplophysa* currently contains 29 cave-dwelling species, 13 of which are classified as stygophile/atypical type as they do not show any troglomorphic traits, including *T. baotianensis* Li, Li,

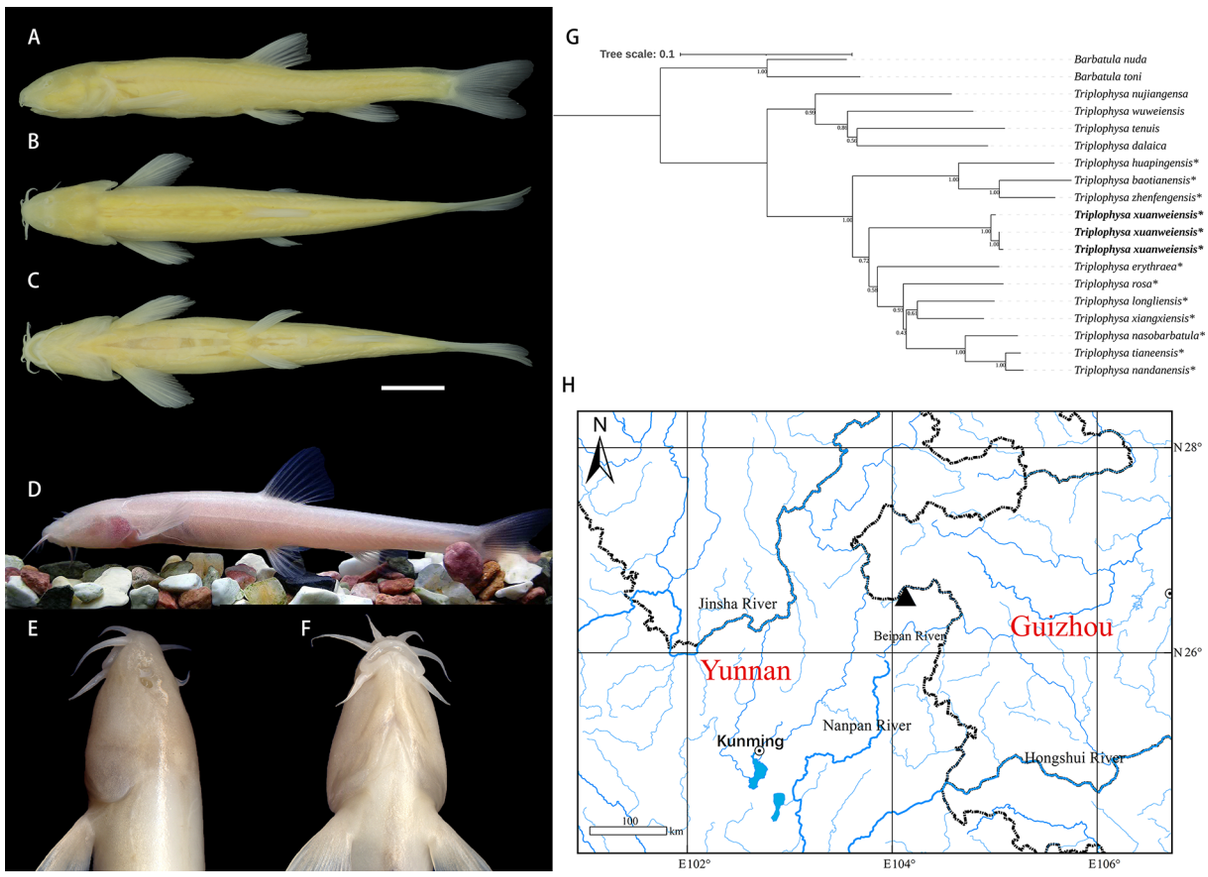

Figure 1 Photos, distribution, and phylogenetic position of *Triplophysa xuanweiensis* sp. nov.

A: Lateral view (holotype, 68.6 mm standard length). B: Ventral view. C: Dorsal view. D: Live fish. E: Lateral view of head. F: Dorsal view of head. G: ML phylogenetic tree of *Triplophys*a species and two outgroup species of *Barbatula* using mitochondrial cyt *b* gene sequences (“*”: Cave-dwelling species of *Triplophysa*). H: Type locality of *Triplophysa xuanweiensis* **sp. nov.** Photos by Xue-Jian Li (A–C, E, F) and Wei-Ning Mao (D).

Liu & Li, 2018, *T. guizhouensis* Wu, He, Yang & Du, 2018, *T. huapingensis* Zheng, Yang & Chen, 2012, *T. longipectoralis* Zheng, Du, Chen & Yang, 2009, *T. longliensis* Ren, Yang & Chen, 2012, *T. nandanensis* Lan, Yang & Chen, 1995, *T. nasobarbatula* Wang & Li, 2001, *T. sanduensis* Chen & Peng, 2019, *T. tianxingensis* Yang, Li & Chen, 2016, *T. wulongensis* Chen, Sheraliev, Shu & Peng, 2021, *T. xiangshuingensis* Li, 2004, *T. yunnanensis* Yang, 1990, and *T. zhenfengensis* Wang & Li, 2001. However, the new species can be assigned to the stygobite/typical type based on a series of troglomorphic characteristics, such as loss of eyes, pigmentation, and scales.

There are 16 stygobite species of *Triplophysa* (Supplementary Table S4): *Triplophysa xuanweiensis* **sp. nov.**, *T. aluensis* Li & Zhu, 2000, *T. anshuiensis*, *T. erythraea*, *T. fengshanensis*, *T. gejiuensis*, *T. langpingensis*, *T. luochengensis* Li, Lan Chen & Du, 2017, *T. macrocephala* Yang, Wu & Yang, 2012, *T. qiubeiensis*, *T. rosa*, *T. shilinensis*, *T. tianeensis* Li, Li, Lan & Du, 2004, *T. tianlinensis* Li, Li, Lan & Du, 2017, *T. xiangxiensis*, and *T. xichouensis* Liu, Pan, Yang & Chen, 2017. Among them, *T. aluensis*, *T. luochengensis*, *T. macrocephala*, *T. tianeensis*, *T. tianlinensis*, and *T. xichouensis* have small eyes or eye-dots. Therefore, *Triplophysa xuanweiensis* **sp. nov.** can be easily distinguished from the above species by its lack of eyes.

All remaining eyeless loaches, including the new species, share the following morphological characters: lateral line complete and body scaleless and colorless. However, *Triplophysa xuanweiensis* **sp. nov.** differs from *T. erythraea* and *T. fengshanensis* by dorsal-fin origin anterior to pelvic-fin origin (vs. dorsal-fin origin opposite to pelvic-fin origin), from *T. erythraea* by anal-fin origin separated from anus (vs. anal-fin origin close to anus), and from *T. fengshanensis* by smooth lips (vs. lips with shallow furrows). *Triplophysa xuanweiensis* **sp. nov.** can be distinguished from *T. rosa* and *T. xiangxiensis* by shorter pectoral fin (10.8%–21.7% of SL, not reaching pelvic-fin origin vs. 24.7%–31.1% of SL, reaching pelvic-fin origin, and 23.1%–35.5% of SL, extending beyond pelvic fin origin, respectively). The new species can be distinguished from *T. anshuiensis*, *T. gejiuensis*, *T. langpingensis*, and *T. shilinensis* by anterior nostril not forming barbel-like tube (vs. anterior nostril prolonged as barbel-like tube). The new species can be further distinguished from *T. anshuien*sis by 16–18 branched caudal-fin rays (vs. 14), from *T. gejiuensis* by compressed pelvic-fin tip reaching anus (vs. tip of compressed pelvic fin not reaching anus), from *T. langpingensis* by adipose crest absent (vs. adipose crests well developed on caudal peduncle), and from *T. shilinensis* by developed posterior air

bladder chamber (vs. posterior chamber degenerated).

Triplophysa qiubeiensis is the most similar species to *Triplophysa xuanweiensis* **sp. nov.**, and shares the following characteristics: body elongated, scaleless and colorless, eye absent, dorsal-fin origin anterior to pelvic-fin origin, tip of pelvic fin reaching anus, and anus separated from anal-fin origin by short distance. However, the new species differs from *T. qiubeiensis* by having smooth lips (vs. lips with mastoids), posterior chamber of air bladder well developed (vs. degenerated), 10–12 branched pectoral-fin rays (vs. 8–9), 6–8 branched pelvic-fin rays (vs. 5), and 16–18 branched caudal-fin rays (vs. 15).

NOMENCLATURAL ACTS REGISTRATION

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

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SCIENTIFIC FIELD SURVEY PERMISSION INFORMATION

All field collections followed the Implementation Rules of Fisheries Law of the People's Republic of China. All activities followed the Laboratory Animal Guidelines for the Ethical Review of Animal welfare (GB/T 35 892–2018).

SUPPLEMENTARY DATA

Supplementary data to this article can be found online.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

Y.H.Z. and W.N.M. designed the study. Z.M.L., W.N.M., W.J.L., G.H., and T.K.X. contributed to fieldwork and collected data. X.J.L. and J.Q.H. performed data analyses. X.G.L., Z.M.L., and Y.H.Z. wrote the manuscript with input from F.Q.Q., P.Y., and S.G.Y. Y.H.Z. revised the manuscript. All authors read and approved the final version of the manuscript.

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