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RESEARCH PAPER

Two new hypogean species of *Triplophysa* (Cypriniformes: Nemacheilidae) from the River Yangtze drainage in Guizhou, China

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Abstract. Two hypogean species of genus *Triplophysa* are herein described from two subterranean tributaries of the River Yangtze drainage in Guiyang City, Guizhou Province, China. *Triplophysa wudangensis*, new species, can be distinguished from its congeners by the combination of the following characters: eye reduced, with diameter 5.1-6.5% HL; interorbital width 33.1-35.8% HL; body scaleless; lateral line complete; posterior chamber of air bladder degenerated; anterior nostril with elongated barbel-like tip; distal margin of dorsal fin truncate; dorsal fin with 7, anal fin with 5, and caudal fin with 14 branched fin rays; vertebrae 4 + 34. *Triplophysa qingzhenensis*, new species, can be distinguished from its congeners by the combination of the following characters: eye reduced, with diameter 2.1-4.4% HL; interorbital width 25.1-30.4% HL; body scaleless; lateral line complete; posterior chamber of air bladder degenerated; anterior nostril with elongated barbel-like tip; distal margin of dorsal fin truncate; dorsal fin with 7-8, anal fin with 5, and caudal fin with 14 branched fin rays; vertebrae 4 + 36. Molecular phylogenetic analysis supported the validity of these two new species and indicated their close relationship with *Triplophysa rosa*.

Key words: cavefish, morphology, phylogenetic analysis

Introduction

The genus *Triplophysa* Rendahl is a large group of loaches in the family Nemacheilidae of order Cypriniformes, which comprises over 180 valid species or subspecies distributed in the Qinghai-Tibet Plateau and adjacent regions (Zhu 1989, Eschmeyer et al. 2022). Species of *Triplophysa* are further subdivided into two groups based on their living habits and life-history traits: the epigean group and the hypogean group. Till now, 33 hypogean species of *Triplophysa* have been described, mainly found in the limestone caves or underground rivers of karst areas in southwestern China (Lan et al. 2013, Zhang et al. 2020, Chen et al. 2021, Deng et al. 2022). Meanwhile, the monophyly of both ecological groups of *Triplophysa* was also supported by recent phylogenetic analyses (Chen & Peng 2019, Chen et al. 2021).

Guizhou Province is located in southwestern China and has been recognised as a hotspot for cavefishes (Zhao & Zhang 2009). Nine hypogean species related to

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Triplophysa have been described in Guizhou Province, of which six are now valid, namely *T. nasobarbatula* Wang & Li, 2001 and *T. zhenfengensis* Wang & Li, 2001, *T. longliensis* Ren, Yang & Chen, 2012, *T. guizhouensis* Wu, He, Yang & Du, 2018, *T. baotianensis* Li, Liu, Li & Li, 2018, *T. sanduensis* Chen & Peng, 2019. Notably, all of the known *Triplophysa* species from Guizhou were captured from the River Pearl drainage. In addition, a recent ichthyological survey yielded two hypogean species of *Triplophysa* from the River Wujiang, a tributary of the upper River Yangtze in Guizhou Province, which could not be assigned to any of the other recorded species and are herein described as new species.

Material and Methods

Fish specimens were collected by dipnet. After anaesthesia, a small piece of muscle tissue was dissected from the right side of the dorsum and preserved in 95% ethanol for DNA extraction. The specimens were fixed in 10% formalin and later transferred to 5% formalin for long-term preservation. Morphological measurements were made point-to-point with a dial calliper and recorded to the nearest 0.1 mm from the left side of the specimens whenever possible. All measurements, counts and terminology follow Kottelat (1984). Internostril width was regarded as the distance between both anterior nostrils. One specimen of each species was dissected to examine the intestine shape, the chambers of the air bladder, and the inner gill rakers. Vertebrae counts were conducted using images of micro-computed tomography (Siemens Somatom Definition X-ray machine) reconstructed in CTvox software. Comparative specimens of this study were deposited at the Institute of Hydrobiology, Chinese Academy of Sciences (IHB) in Wuhan, Kunming Institute of Zoology, Chinese Academy of Sciences (KIZ) in Kunming, Guizhou Normal University (GZNU) in Guiyang, and Southwest University (SWU) in Chongqing.

Genomic DNA was extracted from ethanol-preserved muscle tissues using a modified salt-extraction method (Tang et al. 2008). Mitochondrial cytochrome *b* (cyt*b*) gene sequences were amplified by polymerase chain reaction (PCR) with the primers L14724 (5′-GACTTGAAAAACCACCGTTG-3′) and H15915 (5′-CTCCGATCTCCGGATTACAAGAC-3′) (Xiao et al. 2001). Amplifications were performed in a 30 μL reaction volume, containing 3 μL 10 × PCR buffer, 30-50 ng DNA template, 1 μL each primer (each 10 μM), 1.5 μL dNTPs (each 2.5 mM), and 2.5 U Taq DNA polymerase. Sterile water was added to reach the final volume. PCR procedures followed Tang et al. (2008). The amplified products were sent to a commercial sequencing company for purifying and sequenced in both directions with the same primers mentioned above.

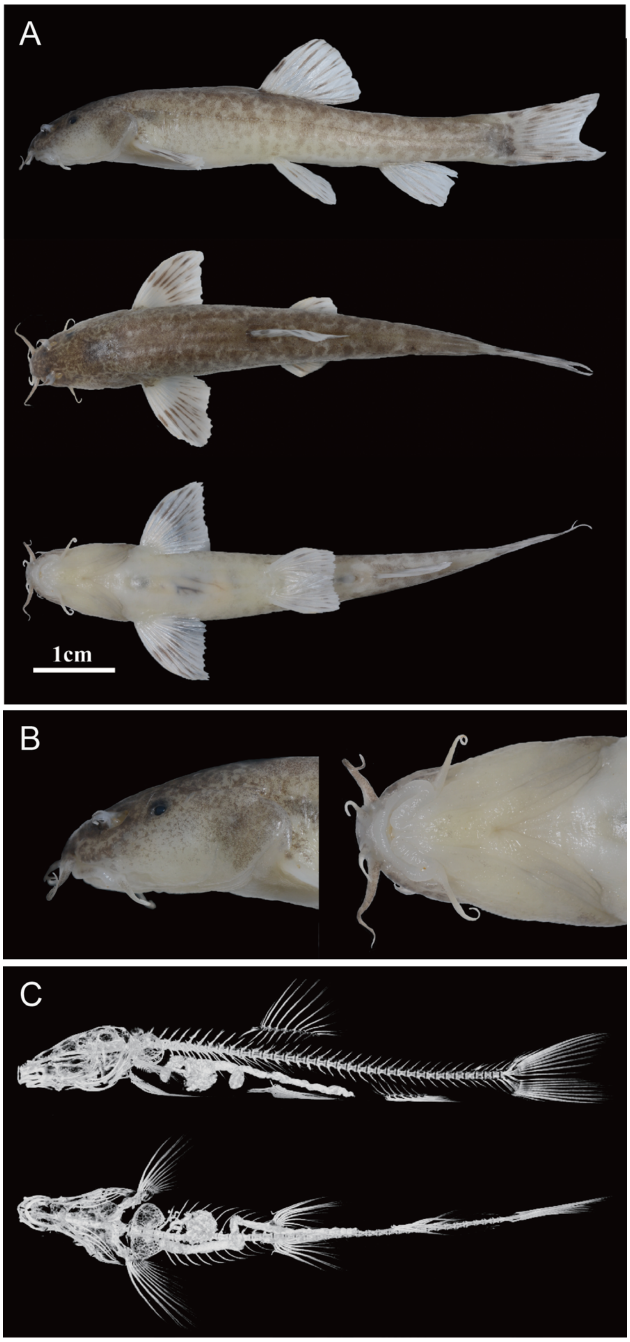

Fig. 1. *Triplophysa wudangensis*, holotype, IHB 2019080901 53.4 mm SL; from a subterranean tributary of the River Wujiang in the River Yangtze drainage in Wudang Distinct, Guiyang City, Guizhou Province, China. A) lateral, dorsal and ventral views; B) lateral and dorsal views of the head; C) lateral and ventral views of the micro-CT graph.

Phylogenetic analysis was performed based on four newly generated cyt*b* sequences of the two new species and already published data from NCBI GenBank for additional 27 sequences, including

24 formally described *Triplophysa* species and two species of *Homatula* (outgroup) (Table 1). The sequences were aligned with ClustalX v2 (Larkin et al. 2007) and revised manually with SEAVIEW (Galtier et al. 1996). The Maximum Likelihood (ML) method was employed to reconstruct the phylogenetic relationship in IQ-TREE v1 (Nguyen et al. 2015), with optimal nucleotide substitution model (GTR + F + I + G4) based on Bayesian inference criterion; nodal support values were estimated from 1,000 nonparametric bootstrap replicates. Calculation of pairwise genetic distances based on the Kimura-2-parameter (K2P) model (Kimura 1980) was conducted in MEGA v7 (Kumar et al. 2016).

Results

Triplophysa wudangensis Liu F., Zeng Z.-X. & Gong Z., new species (Figs. 1, 2A; Table 2)

Type series: Holotype: IHB 201908090001, 53.4 mm SL; a subterranean tributary of the River Wujiang in the

Table 1. GenBank accession numbers of included species in the molecular phylogenetic analysis.

Taxon	Locality	Drainage	Accession number
Triplophysa wudangensis 1 (IHB 201908090003)	Guizhou, China	The River Yangtze	MT700460
Triplophysa wudangensis 2 (IHB 201908090004)	Guizhou, China	The River Yangtze	MT700461
Triplophysa qingzhenensis 1 (IHB 201911150004)	Guizhou, China	The River Yangtze	MT700458
Triplophysa qingzhenensis 2 (IHB 201911150005)	Guizhou, China	The River Yangtze	MT700459
Triplophysa rosa 1	Chongqing, China	The River Yangtze	MG697587
Triplophysa rosa 2	Chongqing, China	The River Yangtze	JF268621
Triplophysa qini	Chongqing, China	The River Yangtze	ON528184
Triplophysa wulongensis	Chongqing, China	The River Yangtze	MW582823
Triplophysa xiangxiensis	Hunan, China	The River Yangtze	KT751089
Triplophysa erythraea	Hunan, China	The River Yangtze	MG967615
Triplophysa xuanweiensis	Yunnan, China	The River Pearl	OL675198
Triplophysa nandanensis	Guangxi, China	The River Pearl	MG697588
Triplophysa huapingensis	Guangxi, China	The River Pearl	MG697589
Triplophysa tianeensis	Guangxi, China	The River Pearl	MW582826
Triplophysa nasobarbatula	Guizhou, China	The River Pearl	MK610357
Triplophysa baotianensis	Guizhou, China	The River Pearl	MK610353
Triplophysa longliensis	Guizhou, China	The River Pearl	MW582825
Triplophysa sanduensis	Guizhou, China	The River Pearl	MW582822
Triplophysa siluroides	Gansu, China	The River Yellow	KJ781206
Triplophysa bleekeri	Chongqing, China	The River Yellow	JX135578
Triplophysa anterodorsalis	Sichuan, China	The River Yellow	KJ739868
Triplophysa lixianensis	Sichuan, China	The River Yellow	KT966735
Triplophysa robusta	Unknown	Unknown	KM406486
Triplophysa tibetana	Xizang, China	The River Brahmaputra	KT122845
Triplophysa venusta	Unknown	The River Yangtze	KT008666
Triplophysa stoliczkai	Unknown	Unknown	JQ663847
Triplophysa dorsalis	Xinjiang, China	The River Irtysh	KT241024
Triplophysa strauchii	Xinjiang, China	The River Irtysh	KP297875
Triplophysa zhenfengensis	Guizhou, China	The River Pearl	MK610360
Homatula pycnolepis	Yunnan, China	The River Lantsang	MT783421
Homatula potanini	Yunnan, China	The River Yangtze	KM017732

Table 2. Morphometric characters of *Triplophysa wudangensis*, *T. qingzhenensis* and *T. rosa*. Ranges include values of holotype and paratypes. SD – standard deviation.

	Triplophysa wudangensis		*Triplophysa qingzhenensis*		*Triplophysa rosa*
	Holotype	Range (Mean, SD) (n = 4)	Holotype	Range (Mean, SD) (n = 5)	Holotype
Standard length (SL, mm)	53.4	46.8-58.6	103.9	60.2-104.6	56.4
Head length (HL, mm)	14.3	11.6-14.3	23.7	14.7-25.9	14.1
As percentage of SL (%)					
Head length	26.8	24.2-26.8 (25.1, 1.2)	22.8	22.6-24.8 (23.5, 1.0)	24.9
Body depth	15.9	13.6-16.4 (15.3, 1.2)	15.0	13.0-15.6 (14.6, 1.0)	12.3
Body width	14.4	12.3-15.1 (13.9, 1.2)	12.5	11.8-13.8 (12.6, 0.7)	11.1
Pre-dorsal length	47.6	47.6-52.5 (49.8, 2.1)	47.8	46.4-50.6 (48.0, 1.6)	52.8
Pre-pectoral length	24.5	23.3-26.3 (24.8, 1.2)	24.1	22.8-25.3 (24.0, 0.9)	25.2
Pre-pelvic length	48.6	48.2-51.8 (49.7, 1.6)	49.5	47.3-50.6 (49.3, 1.2)	55.5
Pre-anal length	71.0	68.8-75.3 (71.4, 2.8)	71.6	69.0-73.2 (71.4, 1.5)	71.8
Pre-anus length	67.9	63.7-72.0 (67.6, 3.4)	67.6	65.3-68.6 (66.9, 1.3)	67.3
Dorsal fin length	20.1	20.1-23.7 (21.5, 1.5)	18.8	18.8-20.8 (19.6, 1.0)	22.4
Pectoral fin length	19.3	18.6-19.7 (19.3, 0.5)	17.6	17.1-19.3 (17.7, 0.9)	25.5
Pelvic fin length	15.9	15.1-17.7 (16.6, 1.2)	14.6	13.8-15.1 (14.6, 0.6)	18.1
Anal fin length	14.5	14.5-17.5 (16.3, 1.3)	14.4	14.1-15.7 (14.7, 0.6)	13.3
Caudal peduncle length	20.0	20.0-21.4 (20.7, 0.6)	18.2	16.9-20.7 (18.4, 1.4)	17.6
Caudal peduncle depth	9.0	7.0-9.0 (8.1, 0.8)	8.1	7.2-8.1 (7.9, 0.4)	7.3
In percentage of HL (%)					
Head depth	46.3	46.3-55.6 (51.3, 3.8)	49.4	46.3-49.4 (47.6, 1.3)	48.3
Head width	55.3	55.3-65.6 (59.8, 4.3)	57.8	50.6-62.2 (56.4, 4.2)	58.6
Snout length	34.8	34.8-48.5 (42.4, 5.7)	43.6	35.9-43.6 (41.4, 3.2)	41.9
Mouth width	24.2	23.0-25.5 (24.3, 1.0)	25.0	21.8-27.2 (24.7, 2.2)	22.5
Eye diameter	5.5	5.1-6.5 (5.8, 0.6)	2.5	2.1-4.4 (3.0, 1.0)	0.0
Interorbital width	33.1	33.1-35.8 (34.3, 1.1)	29.6	25.1-30.4 (28.1, 2.5)	26.8
Inter-nostril width	21.8	20.1-23.8 (22.0, 1.5)	21.2	18.8-22.8 (20.3, 1.7)	14.2
Inner rostral barbel length	17.6	17.6-22.2 (19.1, 2.1)	15.9	15.9-24.7 (20.6, 3.2)	19.9
Outer rostral barbel length	30.8	29.9-41.8 (33.7, 5.5)	40.7	26.2-41.6 (37.5, 6.4)	40.7
Maxillary barbel length	20.8	20.8-30.3 (26.4, 4.3)	34.2	24.6-35.0 (30.7, 4.2)	37.4

River Yangtze drainage in Wudang District, Guiyang City, Guizhou Province, China (26°39′30″ N, 106°46′0″ E; 1,105 m elevation); August 2019.

Paratypes: IHB 201908090002-201908090004, 3, 46.8-58.6 mm SL; other data same as holotype.

Diagnosis: Triplophysa wudangensis differs from its congeners by the combination of the following characters: eye reduced, with diameter 5.1-6.5% HL; interorbital width 33.1-35.8% HL; body scaleless; lateral line complete; posterior chamber of air bladder degenerated; anterior nostril with elongated barbel-like tip; distal margin of dorsal fin truncate; dorsal fin with 7, anal fin with 5, and caudal fin with 14 branched fin rays; vertebrae 4 + 34. The primary diagnostic characters for cave species of *Triplophysa* were summarised in Table 3.

Description: Morphometric characters of four type specimens are shown in Table 2. Body elongated, anterior part subcylindrical and posterior part laterally compressed. The dorsal profile of the head is near sloping, and that of the body convex. The ventral profile from snout tip to anal-fin origin is almost straight, and from anal-fin origin to caudal-fin base is slightly concave. Body depth increases to its maximum before dorsal-fin origin, slightly declining towards the caudal fin base. Body smooth and scaleless, lateral line complete. Cephalic lateral-line system with 3 + 8 infraorbital, 6 supraorbital, 3 supratemporal and 10 preoperculo-mandibular pores.

Fig. 2. Live photo of A) *Triplophysa wudangensis*; B) *Triplophysa qingzhenensis*.

Head moderately depressed, head width slightly greater than head depth. Snout slightly blunt, snout length 34.8-48.5% of HL. Eye reduced, diameter 5.1-6.5% HL, located in the upper part of head laterally. Both anterior and posterior nostrils are closely situated, the anterior one in a short tube with an elongated barbel-like tip, the tip of the nostril appendage not reaching to anterior margin of the eye. Mouth inferior and curved. Lip thick, with shallow furrows on its surface. The upper lip is complete and connected with the lower lip at the corners of the mouth. Lower lip with median interruption and well-marked V-shaped median incision. Upper jaw without processus dentiformis and lower jaw without a median notch. Three pairs of barbels, inner rostral barbel reaching to corner of the mouth, outer rostral barbel longest, extending beyond posterior margin of eye, maxillary barbel surpassing eye and not reaching to the anterior edge of the opercula.

Dorsal fin with 3 unbranched and 7 branched fin rays, origin at mid-point between snout tip and caudal-fin base and close to the insertion of the pelvic fin origin, distal margin truncate, length greater than body depth. Pelvic fin with 1 unbranched and 5 branched fin rays, origin closer to the anal fin origin than the pectoral fin origin, tip not reaching the anus. Pectoral fin with 1 unbranched and 8 branched fin rays, extending beyond the halfway to pectoral-fin origin. Anal fin with 3 unbranched and 5 branched fin rays, origin closer to pelvic-fin insertion than to caudal-fin base. The caudal fin forked, with 14 branched fin rays, with the upper lobe slightly longer than the lower.

Fig. 3. Habitat of *Triplophysa wudangensis*.

Intestine short, with a zigzag-shaped bend behind the stomach. The bony capsule of the air bladder is dumbbell-shaped (Fig. 1C), posterior chamber of the air bladder degenerated. Inner gill rakers on the first gill arch 7. Vertebrae 4 + 34 (holotype).

Colouration: In live specimens, the body is yellowish, densely distributed with small black pigments laterally and dorsally on the head and body, and each fin with 2-3 interrupted black stripes (Fig. 2A). In 10% formalin-preserved specimens, the body greyish, with black pigments on head, body and fins (Figs. 1A, 1B).

Sexual dimorphism: No sexual dimorphism is observed based on the present type specimens of *T. wudangensis*.

Distribution and habitat: This new species is presently only known from the outlet of a subterranean tributary of the River Wujiang in the River Yangtze drainage in Wudang District, Guiyang City, Guizhou Province, China (Figs. 3, 4). The water temperature was 15.0 °C during the survey period in August 2019. The species co-occurred with some endemic fishes of Guizhou Province, including *Sinocyclocheilus multipunctatus* and *Linichthys laticeps* in its type locality.

Etymology: The specific epithet is derived from its type locality, Wudang District.

Triplophysa qingzhenensis Liu F., Zeng Z.-X. & Gong Z., new species (Figs. 2B, 5; Table 2)

Type series: Holotype: IHB 201911150001, 103.9 mm SL; a subterranean tributary of the River Wujiang in the River Yangtze drainage in Qingzhen County, Guiyang City, Guizhou Province, China (26°47′0″ N, 106°16′0″ E; 1,258 m elevation); November 2019.

Paratypes: IHB 201911150002-201911150005, 4, 60.2-104.6 mm SL; other data same as holotype.

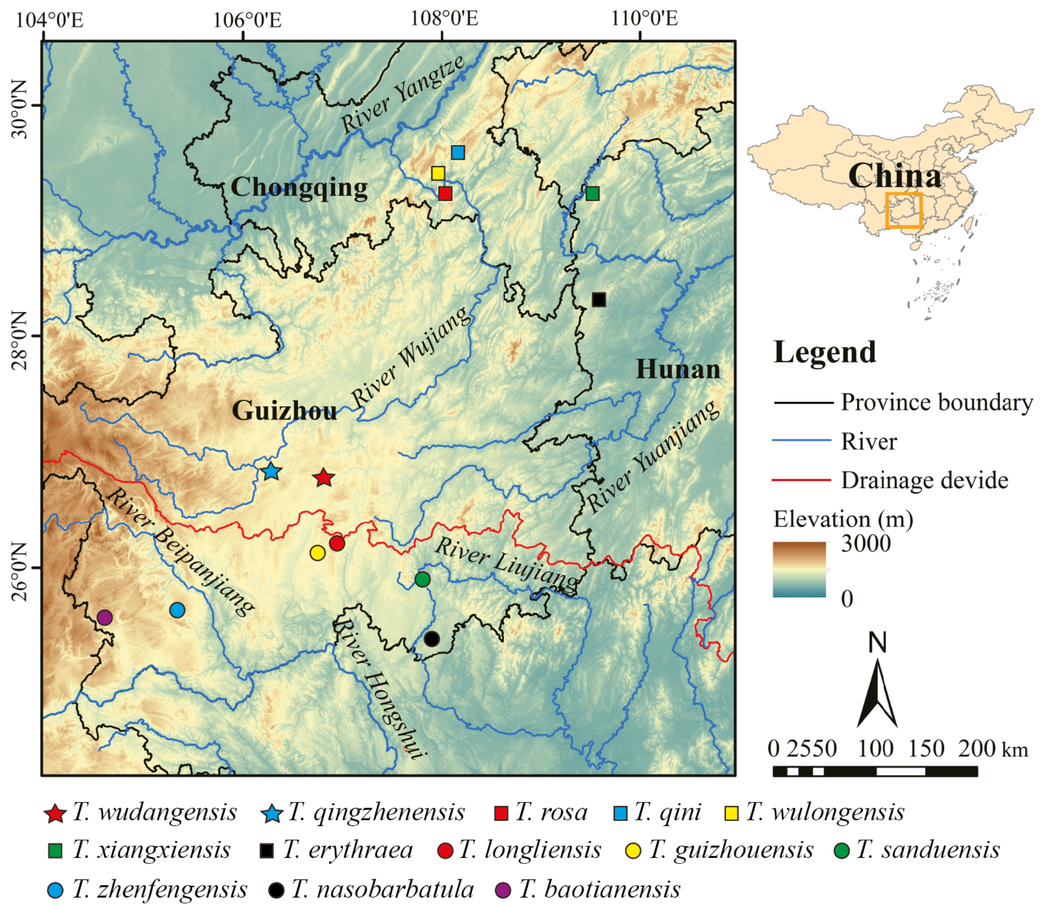

Fig. 4. Type localities of hypogean species of *Triplophysa* in Guizhou Province and the River Yangtze drainage.

Diagnosis: Triplophysa qingzhenensis can be distinguished from all of its congeners by the combination of the following characters: eye reduced, with diameter 2.1-4.4% HL; interorbital width 25.1-30.4% HL; body scaleless; lateral line complete; posterior chamber of air bladder degenerated; anterior nostril with elongated barbel-like tip; distal margin of dorsal fin truncate; dorsal fin with 7-8, anal fin with 5, and caudal fin with 14 branched fin rays; vertebrae 4 + 34. The primary diagnostic characters for cave species of *Triplophysa* were summarised in Table 3.

Description: Morphometric characters of five type specimens are shown in Table 2. Body elongated, anterior part subcylindrical and posterior part laterally compressed. Dorsal profile of head near flat, and that of the body convex. The ventral profile from the snout tip to the anal fin origin is almost straight; it is slightly concave from the anal fin origin. Body depth increases to its maximum before dorsal-fin origin, slightly declining towards the caudal fin base. Body smooth and scaleless, lateral line complete. Cephalic lateral-line system with 3 + 8 infraorbital, 6 supraorbital, 3 supratemporal and 10 preoperculo-mandibular pores. Head moderately depressed, with its width slightly greater than depth. Snout pointed, snout length 35.9-43.6% of HL. Eye reduced, diameter 2.1-4.4% of HL, located in the upper part of head laterally. Both anterior and posterior nostrils are closely situated, the anterior one in a short tube with an elongated barbel-like tip, the tip of the nostril appendage not reaching the anterior margin of the eye. Mouth inferior and curved. Lip thick, with shallow furrows on its surface. The upper lip is complete and connected with the lower lip at the corners of the mouth. Lower lip with median interruption and well-marked V-shaped median incision. Upper jaw without processus dentiformis and lower jaw without a median notch. Three pairs of barbels, inner rostral barbel reaching to corner of the mouth, outer rostral barbel longest, extending beyond posterior margin of eye, maxillary barbel surpassing eye and not reaching to the anterior edge of the opercula.

Dorsal fin with 3 unbranched and 7 (n = 1) or 8 (n = 4) branched fin rays, origin at mid-point between snout tip and caudal-fin base and anterior to the pelvic fin origin, distal margin truncate, length greater than body depth. Pelvic fin with 1 unbranched

Table 3. Major diagnostic characters for hypogean species of *Triplophysa*. % HL – percentage of head length; P – pectoral fin; D – dorsal fin; V – ventral fin (pelvic fin); A – anal fin; C – caudal fin; n/a – not available.

Species	Eye diameter (% HL)	Interorbital width (% HL)	Scales	Lateral line	Posterior chamber of air bladder	P tip reaching to V origin	V tip reaching to anus	D distal margin	Branched fin rays			Anterior nostril with barbel-like tip	Vertebrae
									D	A	C		
T. aluensis	5.6	22.2	Absent	Complete	Degenerated	No	No	Truncate	7	5	13	Yes	n/a
T. anshuiensis	Absent	n/a	Absent	Complete	Developed	No	Yes	Truncate	7-8	6	14	Yes	n/a
T. baotianensis	6.7-7.1	21.9-29.4	Absent	Complete	Degenerated	No	No	Truncate	6-7	4-5	11-13	Yes	n/a
T. erythraea	Absent	n/a	Absent	Complete	Developed	No	Yes	Truncate	8	6	17	No	n/a
T. fengshanensis	Absent	n/a	Absent	Complete	n/a	No	No	Truncate	8	6	16	Yes	n/a
T. flavicorpus	14.7-19.6	19.2-27.0	Present	Complete	Degenerated	No	Yes	Concave	10	6-7	16	No	4 + 34
T. gejiuensis	Absent	n/a	Absent	Complete	Developed	No	Yes	Truncate	7-8	4-6	14-15	Yes	n/a
T. guizhouensis	9.4-12.1	20.3-24.3	Present	Complete	Developed	No	No	Truncate	8	6	14	Yes	n/a
T. huapingensis	10.4-14.3	27.6-30.8	Present	Complete	Degenerated	No	No	Truncate	8	5	16	No	n/a
T. langpingensis	2.7-5.9	30.6-34.5	Absent	Incomplete	n/a	No	Yes	Truncate	7-8	5-6	14	Yes	n/a
T. longipectoralis	11.8-16.4	21.2-25.3	Present	Complete	Degenerated	Yes	Yes	Concave	8	5-6	16	Yes	4 + 35
T. longliensis	9.5-11.5	31.4-37.5	Absent	Complete	Developed	No	Yes	Concave	8	5	15-16	Yes	4 + 38
T. luochengensis	7.5-8.6	18.4-21.3	Present	Complete	Degenerated	No	No	Truncate	8	6	16-17	Yes	4 + 33-34
T. macrocephala	3.6-8.0	22.9-25.8	Absent	Complete	Degenerated	No	No	Truncate	8	5	16	Yes	n/a
T. nandanensis	11.1-21.3	24.4-27.8	Absent	Complete	Degenerated	No	No	Concave	8	5	15-16	Yes	4 + 36
T. nanpanjiangensis	12.0-16.5	30.3-34.5	Absent	Complete	Degenerated	No	No	Truncate	7-8	5	13-16	Yes	4 + 38
T. nasobarbatula	9.1-13.3	27.0-33.3	Present	Complete	Degenerated	No	No	Truncate	8	5	15	Yes	4 + 36
T. posterodorsalus	Absent	n/a	Absent	Complete	n/a	No	No	Truncate	6	4	15	Yes	n/a
T. qingzhenensis	2.1-4.4	25.1-30.4	Absent	Complete	Degenerated	No	No	Truncate	7-8	5	14	Yes	4 + 36
T. qini	Absent	n/a	Absent	Complete	n/a	No	Yes	Concave	8	5	14-16	No	4 + 34-35
T. qiubeiensis	Absent	17.4-24.0	Absent	Complete	Degenerated	No	Yes	Concave	7	5	14-15	No	4 + 35
T. rosa	Absent	26.8	Absent	Complete	n/a	No	Yes	Concave	9	6	14	Yes	n/a
T. sanduensis	11.9-15.4	31.2-40.2	Present	Complete	Degenerated	No	No	Concave	8-9	5	17-18	Yes	4 + 37
T. shilinensis	Absent	n/a	Absent	Complete	Degenerated	No	Yes	Truncate	7	5	14	Yes	n/a
T. tianeensis	3.0-5.9	21.3-25.6	Absent	Complete	Degenerated	No	No	Truncate	7	5	16	Yes	4 + 35
T. tianlinensis	n/a	n/a	Absent	Complete	Degenerated	No	Yes	Truncate	8-9	6	15-16	Yes	n/a
T. tianxingensis	4.2-6.7	17.4-24.0	Absent	Complete	Developed	No	No	Truncate	8	5	16	No	4 + 38
T. wudangensis	5.1-6.5	33.1-35.8	Absent	Complete	Degenerated	No	No	Truncate	7	5	14	Yes	4 + 34
T. wulongensis	11.1-19.1	38.5-43.1	Absent	Complete	Degenerated	No	No	Concave	8-9	5-6	18	Yes	4 + 38-39
T. xiangshuingensis	7.5	32.3	Absent	Complete	Degenerated	No	No	Concave	6	5	14	Yes	n/a
T. xiangxiensis	Absent	n/a	Absent	Complete	Developed	Yes	Yes	Concave	8	6	16	Yes	n/a
T. xichouensis	n/a	n/a	Absent	Complete	Developed	No	Yes	Truncate	8	6	16	Yes	4 + 36
T. xuanweiensis	Absent	n/a	Absent	Complete	Developed	No	Yes	Concave	7-8	5	17-18	Yes	n/a
T. yunnanensis	7.2-8.3	27.0-27.8	Present	Complete	Degenerated	No	No	Concave	7	5	15-16	Yes	n/a
T. zhenfengensis	7.1-16.7	22.2-34.5	Present	Complete	Degenerated	No	No	Truncate	7	5	14-15	Yes	4 + 36

and 5 branched fin rays, origin closer to the anal fin origin than the pectoral fin origin; the tip of the pelvic fin does not reach the anus. Pectoral fin with 1 unbranched and 8 (n = 3) or 9 (n = 2) branched fin rays, extending beyond the halfway to pelvic-fin origin. Anal fin with 3 unbranched and 5 branched fin rays, origin closer to pelvic-fin insertion than to caudal-fin base. The caudal fin forked, with 14 branched fin rays, the upper lobe slightly longer than the lower.

Intestine short, with a zigzag-shaped bend behind the stomach. The bony capsule of the air bladder is dumbbell-shaped (Fig. 5C), posterior chamber of the air bladder degenerated. Inner gill rakers on first gill arch 6. Vertebrae 4 + 36 (holotype).

Colouration: In life specimens, the body is generally pale, slightly pinkish, with sparse and irregular pigments laterally and dorsally, semi-transparent ventrally, and interradial membranes of all fins are transparent (Fig. 2B). In 10% formalin-preserved specimens, body pale, with slightly yellowish, pigments faded in some specimens (Figs. 5A, 5B).

Sexual dimorphism: No sexual dimorphism is observed based on the present type specimens of *T. qingzhenensis*.

Distribution and habitat: This species is presently only known from a subterranean tributary of the River Wujiang in the River Yangtze drainage in Qingzhen County, Guiyang City, Guizhou Province, China (Fig. 4). The species inhabited in complete darkness at the bottom of the slowly-flowing underground river (Fig. 6), where the water temperature was 14.5 °C and pH value was 7.8 during the survey period in November 2019.

Etymology: The specific epithet is derived from its type locality, Qingzhen County.

Molecular phylogenetic analysis: After alignment, 1,061 bps of cyt*b* gene sequences belonging to 31 individuals of 26 species of *Triplophysa* and two species of *Homatula* were involved in molecular phylogenetic analysis, including 592 conservative sites, 469 variable sites, 430 parsimony informative sites and 39 singleton sites.

Phylogenetic tree reconstructed by the ML method is shown in Fig. 7. Topology of the phylogenetic tree indicated that the sampled individuals of *T. wudangensis* and *T. qingzhenensis* grouped into a monophyletic clade with the bootstrap values of 99 and 86, respectively. These two species clustered closely with *T. rosa*. In addition, *T. xiangxiensis* was sister to the branch composed of *T. qini*, *T. sanduensis* and *T. longliensis*. *Triplophysa erythraea* formed an independent lineage.

Based on the K2P model, pairwise genetic distances between these two new species and their congeners are given in Table 4. The genetic distances between *T. wudangensis* and its cave-dwelling congeners ranged from 1.4% (*vs. T. rosa*) to 18.4% (*vs. T. zhenfengensis*); between *T. qingzhenensis* and its congeners ranged from 1.5% (*vs. T. rosa*) to 18.3% (*vs. T. baotianensis* and *T. zhenfengensis*). The genetic distance between both new species was 1.6%.

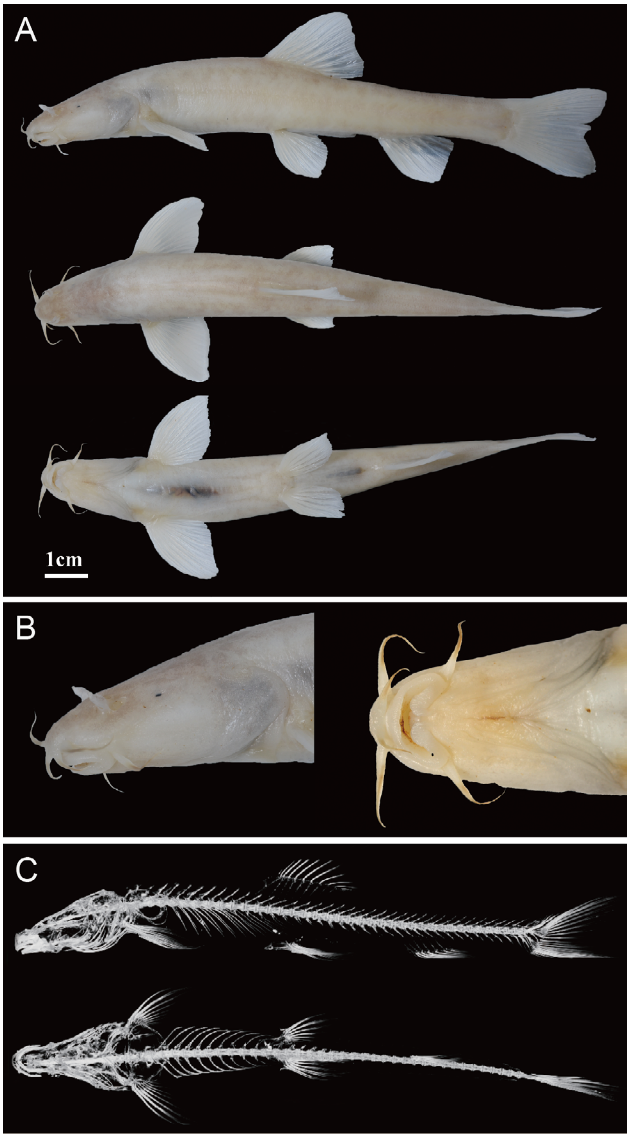

Fig. 5. *Triplophysa qingzhenensis*, holotype, IHB 2019111501 103.9 mm SL; from a subterranean tributary of the River Wujiang in the River Yangtze drainage in Qingzhen County, Guiyang City, Guizhou Province, China. A) lateral, dorsal and ventral views; B) lateral and dorsal views of the head; C) lateral and ventral views of the micro-CT graph.

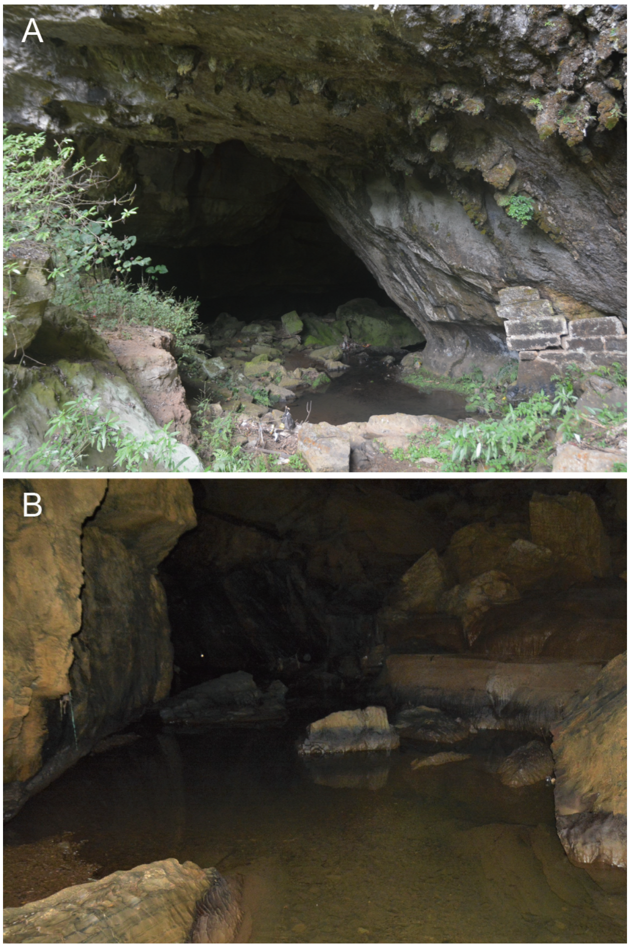

Fig. 6. Habitat of *Triplophysa qingzhenensis*. A) entrance to the subterranean tributary; B) the pool where type specimens were collected.

Discussion

There is an increasing number of cave species of *Triplophysa* described in the karst areas in the River Yangtze drainage. The descriptions of these two new species bring the total number to seven: *T. rosa* Chen & Yang, 2005, *T. wulongensis* Chen, Sheraliev, Shu & Peng, 2021, *T. qini* Deng, Wang & Zhang, 2022, *T. xiangxienesis* Yang, Yuan & Liao, 1986, *T. erythraea* Liu & Huang, 2019. from Yuanjiang system of Lake Dongting. *Triplophysa wudangensis* and *T. qingzhenensis* are significantly distinguishable by eye diameter (5.1-6.5% HL in *T. wudangensis vs.* 2.1-4.4% HL in *T. qingzhenensis*), interorbital width (33.1-35.8% HL in *T. wudangensis vs.* 25.1-30.4% HL in *T. qingzhenensis*), and vertebrae (4 + 34 in *T. wudangensis vs.* 4 + 36 in *T. qingzhenensis*). *Triplophysa rosa* (Fig. 8) was the first described cavefish from the River Wujiang system (Chen & Yang 2005), which was the closest relative to *T. wudangensis* and *T. qingzhenensis* inferred from intraspecies genetic distances. The morphometric characters of *T. rosa* are summarised in Table 2. The two new species distinctly differ from *T. rosa* by the presence of reduced eyes (*vs.* eye absent), a shorter pectoral fin (18.6-19.7% SL in *T. wudangensis* and 17.1-19.3% SL in *T. qingzhenensis vs.* 25.5% SL in *T. rosa*), distal margin of dorsal fin truncate (*vs.* concave), less branched dorsal-fin rays (7 in *T. wudangensis* and 7-8 in *T. qingzhenensis vs.* 9 in *T. rosa*), less branched anal-fin rays (5 in *T. wudangensis* and *T. qingzhenensis vs.* 6 in *T. rosa*). The two species are distinct from *T. qini*, *T. xiangxiensis* and *T. erythraea* by the presence of reduced eyes (*vs.* eye absent) and pelvic-fin tip not reaching to the anus (*vs.* reaching to); further differ from *T. qini* by the presence (*vs.* absence) of a barbel-like tip on the anterior nostril, and distal margin of pelvic fin truncate (*vs.* concave); from *T. xiangxiensis* by the posterior chamber of the air bladder degenerated (*vs.* developed), pectoral-fin tip not reaching to pelvic-fin origin (*vs.* reaching to), distal margin of dorsal fin truncate (*vs.* concave), and branched anal-fin rays 5 (*vs.* 6); from *T. erythraea* by the posterior chamber of air bladder degenerated (*vs.* developed), presence (*vs.* absence) of a barbel-like tip on the anterior nostril, branched anal-fin rays 5 (*vs.* 6), and branched caudal-fin rays 14 (*vs.* 16). *Triplophysa wulongensis* is a unique hypogean species in the River Yangtze drainage with normal-sized eyes (eye diameter 11.1-19.1% HL). The two species are further distinct from *T. wulongensis* by shorter interorbital width (33.1-35.8% HL in *T. wudangensis* and 25.1-30.4% HL in *T. qingzhenensis vs.* 38.5-43.1% HL in *T. wulongensis*), distal margin of dorsal fin truncate (*vs.* concave), branched caudal-fin rays 14 (*vs.* 18) and vertebrae (4 + 34 in *T. wudangensis* and 4 + 36 in *T. qingzhenensis vs.* 4 + 38 in *T. wulongensis*).

Molecular phylogenetic evidence corroborated the validities of *T. wudangensis* and *T. qingzhenensis* by the highly supported monophyly and sufficient genetic divergence from their congeners. Furthermore, the genetic distances between both species and their closest congener, *T. rosa*, were slightly less than the 2% threshold value, implying the differentiation of these species was at an early stage. Meanwhile, it should be noted that *T. rosa*, *T. qini*, *T. xiangxiensis* and *T. erythraea* are more analogous in morphology (eyes absent, loss of pigmentation, for instance), while the topology of the phylogenetic tree demonstrated that these hypogean species clustered neither by the river-system pattern nor by the morphological analogousness. On the one hand, this phenomenon was possibly caused by convergent evolution under similar selection pressure to other cave species (Wilkens & Strecker 2003, Xiao et al. 2005). On the other hand, it can be inferred that these hypogean species of *Triplophysa* may originate from

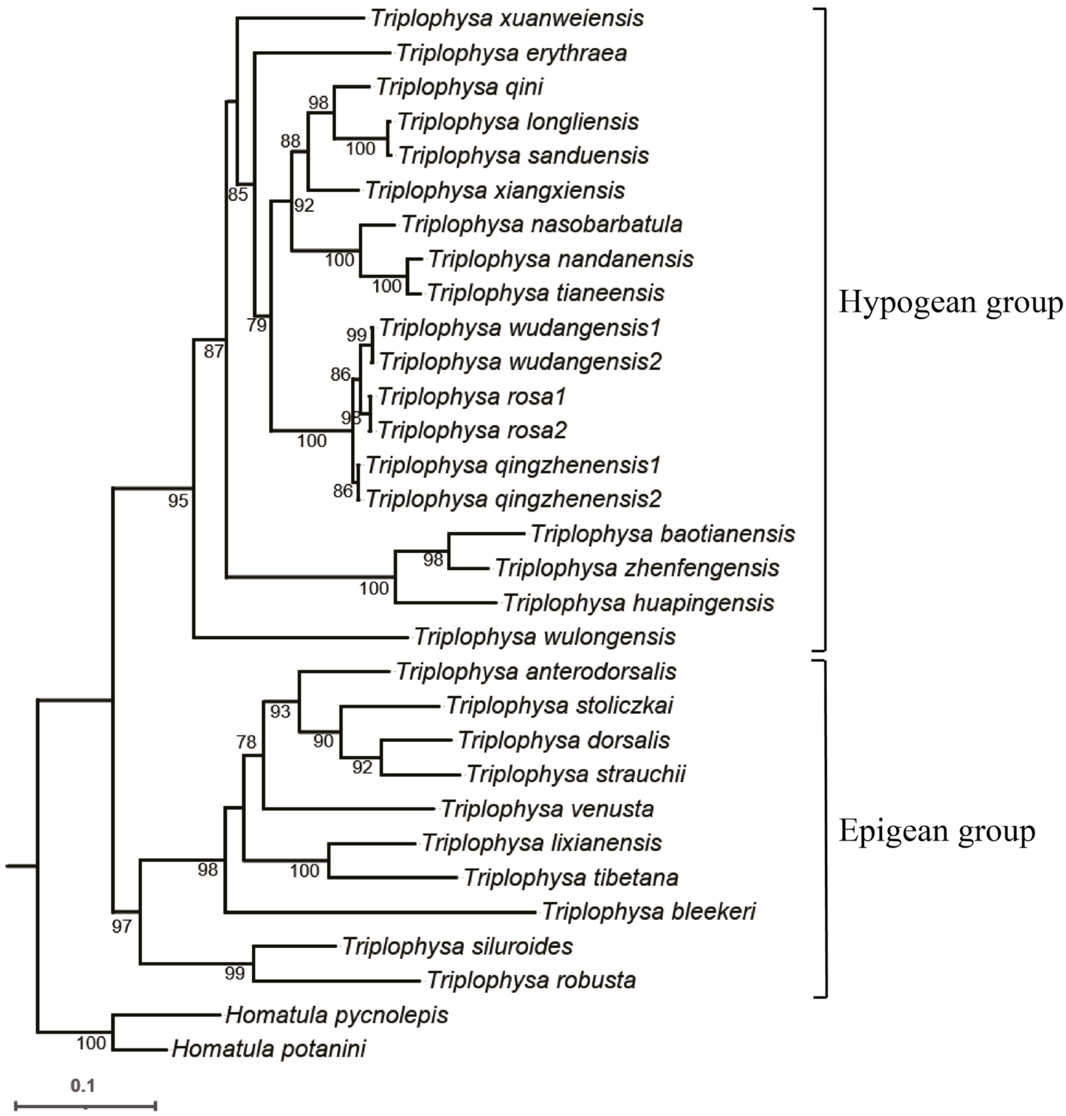

Fig. 7. Maximum likelihood tree derived from cyt*b* gene sequences of 26 species of *Triplophysa*. Numbers below the branches represent bootstrap values (> 50 shown).

heterogenetic ancestors and spread across different river systems. To be specific, the diversified hypogean species originated from the River Pearl drainage may disperse to the Wujiang system through subterranean river capture and connection and then spread to the Wuling Mountain area in Chongqing City and Hunan Province, as Wujiang is the largest river in Guizhou Province with concentrative-distributed and well-developed karst landform (Che & Yu 1985). A similar conclusion has also been drawn in the study on the dispersal process of *Sinocyclocheilus cyphotergous* species complex in the River Yangtze drainage (Wen et al. 2022). Therefore, the evolutionary history and distribution pattern of hypogean *Triplophysa* species were rather complex. A detailed field survey in Wujiang and the adjacent drainages is warranted to clarify the diversity pattern of this taxon in the River Yangtze drainage.

Due to sampling difficulties, the current distribution range and population sizes of *T. wudangensis* and *T. qingzhenensis* remain unclear, while most reported cavefishes are deemed endemic to a narrow region, even a single cave (Zhao & Zhang 2006). In the survey, we found anthropogenic activities posed threats to the two species in their type localities. For *T. wudangensis*, the subterranean river has been utilised as a water source for a mineral water factory; and for *T. qingzhenensis*, factories have discharged sewage into the groundwater. Domestic pollution, illegal fishing and alien invasion (*Gambusia affinis*, *Procambarus clarkii* and *Rana catesbiana*, for instance) also threaten these two species. Guiyang City and its surrounding area is the type locality for many cavefishes, as well as endemic fishes of the River Yangtze (Zeng et al. 2022), which plays essential roles in biodiversity conservation and biogeographical research. At the same time, it is the

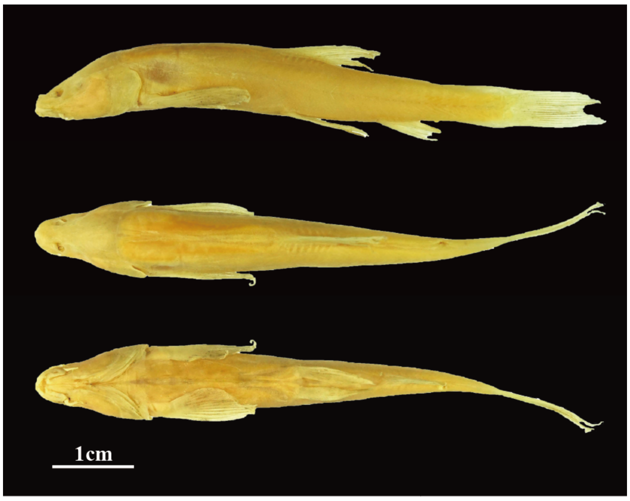

Fig. 8. Lateral, dorsal and ventral views of *Triplophysa rosa*, holotype, KIZ 200211001, holotype, 56.4 mm SL (photo Rui Min).

most urbanised area of Guizhou Province. Therefore, environmental conservation measures are urgently needed to protect these species and their habitats.

Comparative Materials

Triplophysa baotianensis: GZNU 20190524001, 69.08 mm SL; a tributary of the River Pearl at Baotian Town, Panzhou City, Guizhou Province.

Triplophysa guizhouensis: IHB 201906180001, GZNU 20200806001-20200806003, 4 specimens, 64.7-92.9 mm SL; a tributary of the Mengjiang system in the River Pearl drainage at Jialie Village, Huishui County, Guizhou Province.

Triplophysa nasobarbatula: GZNU 201731004-201731006, 3 specimens, 64.7-92.9 mm SL; a tributary of the Liujiang system in the River Pearl drainage at Yaosuo Village, Libo County, Guizhou Province.

Triplophysa qini: IHB 201809049887, holotype, 76.8 mm SL; Erwang Cave of the Wujiang system in the River Yangtze drainage at Houping Village, Wulong County, Chongqing City.

Triplophysa rosa: KIZ 200211001, holotype, 56.4 mm SL; Dongba Cave, of the Wujiang system in the River Yangtze drainage at Tianxing Town, Wulong County, Chongqing City.

Triplophysa sanduensis: SWU 2017061301-2017061304, paratypes, 4 specimens, 45.2-71.5 mm SL; a cave of the Liujiang system in the River Pearl drainage at Dengguang Village, Zhonghe Town, Sandu County, Guizhou Province.

Triplophysa xiangxiensis: IHB 2015010001, IHB 2015010004, IHB 2015010006, IHB 2015010009, topotypes, 4 specimens, 48.3-85.7 mm SL; Feihu Cave of the River Yuanjiang in the River Yangtze drainage at Huoyan Village, Longshan County, Hunan Province.

Triplophysa zhenfengensis: GZNU 20190514002, 79.0 mm SL; a tributary of the River Beipanjiang in the River Pearl drainage at Gaoyan village, Xingren City, Guizhou Province.

Comparative data were acquired from literatures for the following species: *T. aluensis* from Li & Zhu (2000); *T. anshuiensis* from Wu et al. (2018); *T. erythraea* from Huang et al. (2019); *T. fengshanensis* from Lan et al.

Table 4. Kimura-2-Parameter genetic distance (in %) among 14 cave species of *Triplophysa* based on cyt*b* gene sequences.

	1	2	3	4	5	6	7	8	9	10	11	12	13
1 *T. wudangensis*													
2 *T. qingzhenensis*	1.6												
3 *T. rosa*	1.4	1.5											
4 *T. qini*	10.5	9.9	10.3										
5 *T. xiangxiensis*	10.0	9.7	9.8	6.4									
6 *T. erythraea*	12.7	12.8	13.2	12.0	13.1								
7 *T. xuanweiensis*	12.6	12.9	13.1	12.8	12.8	13.6							
8 *T. nasobarbatula*	11.7	11.0	11.6	10.7	9.2	14.0	13.4						
9 *T. nandanensis*	11.9	11.5	12.1	11.7	10.9	14.0	13.3	5.9					
10 *T. baotianensis*	17.6	18.0	18.0	18.8	18.0	16.8	17.1	18.2	20.3				
11 *T. zhenfengensis*	18.4	18.3	18.6	17.6	17.3	17.4	16.9	17.2	19.7	7.0			
12 *T. wulongensis*	15.9	15.7	15.9	15.3	16.9	18.2	16.3	17.7	17.0	19.9	19.5		
13 *T. longliensis*	10.5	10.3	10.6	5.6	8.4	12.2	13.2	10.7	11.7	17.4	17.2	15.7	
14 *T. sanduensis*	10.6	10.3	10.6	5.8	8.7	12.5	13.2	10.6	11.8	17.6	17.4	15.4	0.5

(2013); *T. flavicorpus* from Yang et al. (2004); *T. gejiuensis* from Chu & Chen (1979); *T. huapingensis* from Zheng et al. (2012); *T. langpingensis* from Lan et al. (2013); *T. longipectoralis* from Zheng et al. (2009); *T. longliensis* from Ren et al. (2012); *T. luochengensis* from Li et al. (2017a); *T. macrocephala* from Yang et al. (2012); *T. nandanensis* from Lan et al. (1995); *T. nanpanjiangensis* from Zhu & Cao (1988); *T. posterodorsalus* from Ran et al. (2006); *T. qiubeiensis* from Li et al. (2008); *T. shilinensis* from Chen et al. (1992); *T. tianeensis* from Chen et al. (2004); *T. tianlinensis* from Li et al. (2017b); *T. tianxingensis* from Yang et al. (2016); *T. wulongensis* from Chen et al. (2021); *T. xiangshuingensis* from Li (2004); *T. xichouensis* from Liu et al. (2017); *T. xuanweiensis* from Lu et al. (2022); *T. yunnanensis* from Chu & Chen (1990).

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Author Contributions

Z. Zeng designed the study, collected the specimens, and took photos of the type specimens and habitats. F. Liu measured the morphometric characters of the specimens, examined the comparative materials and prepared the manuscript draft. Z. Gong analysed the molecular data and revised the manuscript.

Data Availability Statement

The data supporting this study's findings are available in the FigShare Digital Repository: doi.org/10.6084/m9.figshare.21657677.

Literature

Che Y.-T. & Yu J.-Z. 1985: Chinese Karst. *Science Press, Beijing, China. (in Chinese)*

Chen X.-Y., Cui G.-H. & Yang J.-X. 2004: A new cave-dwelling fish species of genus *Triplophysa* (Balitoridae) from Guangxi, China. *Zool. Res. 25: 227–231. (in Chinese)*

Chen X.-Y. & Yang J.-X. 2005: *Triplophysa rosa* sp. nov.: a new blind loach from China. *J. Fish Biol. 66: 599–608*.

Chen S.-J. & Peng Z.-G. 2019: *Triplophysa sanduensis*, a new loach species of nemacheilid (Teleostei: Cypriniformes) from South China. *Zootaxa 4560: 375–384*.

Chen S.-J., Sheraliev B., Shu L. et al. 2021: *Triplophysa wulongensis*, a new species of cave-dwelling loach (Teleostei, Nemacheilidae) from Chongqing, Southwest China. *ZooKeys 1026: 179–192*.

Chen Y.-R., Yang J.-X. & Xu G.-C. 1992: A new blind loach of *Triplophysa* from Yunnan Store Forest with comments on its phylogenetic relationship. *Zool. Res. 13: 17–23. (in Chinese)*

Chu X.-L. & Chen Y.-R. 1979: A new blind cobitid fish (Pisces, Cypriniformes) from subterranean waters in Yunnan, China. *Acta Zool. Sin. 25: 285–287. (in Chinese)*

Chu X.-L. & Chen Y.-R. 1990: Fishes of Yunnan, China, part 2. *Science Press, Beijing, China. (in Chinese)*

Deng S.-Q., Wang X.-B. & Zhang E. 2022: *Triplophysa qini*, a new stygobitic species of loach (Teleostei: Nemacheilidae) from the upper Chang-Jiang Basin in Chongqing, Southwest China. *Ichthyol. Explor. Freshw. 1178: 1–11*.

Eschmeyer W.-N., Fricke R. & Laan R. 2022: Catalog of fishes: genera, species, references. *California Academy of Sciences, San Francisco, USA. http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp*

Galtier N., Gouy M. & Gautier C. 1996: SEAVIEW and PHYLO-WIN: two graphic tools for sequence alignment and molecular phylogeny. *Comput. Appl. Biosci. 12: 543–548*.

Huang T.-F., Zhang P.-L., Huang, X.-L. et al. 2019: A new cave-dwelling blind loach, *Triplophysa erythraea* sp. nov. (Cypriniformes: Nemacheilidae), from Hunan Province, China. *Zool. Res. 40: 331–336*.

Kimura M. 1980: A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol. 16: 111–120*.

Kottelat M. 1984: Revision of the Indonesian and Malaysian loaches of the subfamily Noemacheilinae. *Jpn. J. Ichthyol. 31: 225–260*.

Kumar S., Stecher G. & Tamura K. 2016: MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Mol. Biol. Evol. 33: 1870–1874*.

Lan J.-H., Gan X., Wu T.-J. et al. 2013: Guangxi cave Fish. *Science Press, Beijing, China. (in Chinese)*

Lan J.-H., Yang J.-X. & Chen Y.-R. 1995: Two new species of the subfamily Nemacheilinae from Guangxi, China. *Acta Zool. Sin. 20: 366–372. (in Chinese)*

Larkin M.-A., Blackshields G., Brown N.-P. et al. 2007: Clustal W and Clustal X version 2.0. *Bioinformatics 23: 2947–2948*.

Li W.-X. 2004: The three new species of Cobitidae from Yunnan, China. *Journal of Jishou University (Natural Science Edition) 25: 93–96. (in Chinese)*

Li J., Lan J.-H., Chen X.-Y. et al. 2017a: Description of *Triplophysa luochengensis* sp. nov. (Teleostei: Nemacheilidae) from a karst cave in Guangxi, China. *J. Fish Biol. 91: 1009–1017*.

Li J., Li X.-H., Lan J.-H. et al. 2017b: A new troglobitic loach *Triplophysa tianlinensis* (Teleostei: Nemacheilidae) from Guangxi, China. *Ichthyol. Res. 64: 295–300*.

Li W.-X., Yang H.-F., Chen H. et al. 2008: A new blind underground species of the genus *Triplophysa* (Balitoridae) from Yunnan, China. *Zool. Res. 29: 674–678. (in Chinese)*

Li W.-X. & Zhu Z.-G. 2000: A new species of *Triplophysa* from cave Yunnan. *Journal of Yunnan University (Natural Science Edition) 22: 396–398. (in Chinese)*

Liu S.-W., Pan X.-F., Yang J.-X. et al. 2017: A new cave-dwelling loach, *Triplophysa xichouensis* sp. nov. (Teleostei Nemacheilidae) from Yunnan, China. *J. Fish Biol. 90: 834–846*.

Lu Z.-M., Li X.-J., Lv W.-J. et al. 2022: *Triplophysa xuanweiensis* sp. nov., a new blind loach species from a cave in China (Teleostei: Cypriniformes: Nemacheilidae). *Zool. Res. 43: 221–224*.

Nguyen L.T., Schmidt H.A., von Haeseler A. et al. 2015: IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol. Biol. Evol. 32: 268–274*.

Ran J.-C., Li W.-X. & Chen H.-M. 2006: A new species blind loach of *Paracobitis* from Guangxi, China (Cypriniformes: Cobitidae). *Journal of Guangxi Normal University (Natural Science Edition) 24: 81–82. (in Chinese)*

Ren Q., Yang J.-X. & Chen X.-Y. 2012: A new species of the genus *Triplophysa* (Cypriniformes: Nemacheilidae), *Triplophysa longliensis* sp. nov, from Guizhou, China. *Zootaxa 3586: 187–194*.

Tang Q.-Y., Freyhof J., Xiong B.-X. et al. 2008: Multiple invasions of Europe by East Asian cobitid loaches (Teleostei: Cobitidae). *Hydrobiologia 605: 17–28*.

Wen H.-M., Luo T., Wang Y.-L. et al. 2022: Molecular phylogeny and historical biogeography of the cave fish genus *Sinocyclocheilus* (Cypriniformes: Cyprinidae) in southwest China. *Integr. Zool. 17: 311–325*.

Wilkens H. & Strecker U. 2003: Convergent evolution of the cavefish *Astyanax* (Characidae, Teleostei): genetic evidence from reduced eye-size and pigmentation. *Biol. J. Linn. Soc. 80: 545–554*.

Wu T.-J., Wei M.-L., Lan J.-H. et al. 2018: *Triplophysa anshuiensis*, a new species of blind loach from the Xijiang River, China (Teleostei, Nemacheilidae). *Zookeys 744: 67–77*.

Xiao H., Chen S.-Y., Liu Z.-M. et al. 2005: Molecular phylogeny of *Sinocyclocheilus* (Cypriniformes: Cyprinidae) inferred from mitochondrial DNA sequences. *Mol. Phylogenet. Evol. 36: 67–77*.

Xiao W.-H., Zhang Y.-P. & Liu H.-Z. 2001: Molecular systematics of Xenocyprinae (Teleostei:Cyprinidae): taxonomy, biogeography, and coevolution of a special group restricted in East Asia. *Mol. Phylogenet. Evol. 18: 163–173*.

Yang J.-X., Chen X.-Y. & Lan J.-H. 2004: Occurrence of two new plateau-indicator Loaches of Nemacheilinae (Balitoridae) in Guangxi with reference to zoogeographical significance. *Zool. Res. 25: 109–114. (in Chinese)*

Yang H.-F., Li W.-X. & Chen Z.-M. 2016: A new cave species of the Genus *Triplophysa* from Yunnan, China. *Zool. Res. 37: 296–300*.

Yang J., Wu T.-J. & Yang J.-X. 2012: A new cave-dwelling loach, *Triplophysa macrocephala* (Teleostei: Cypriniformes: Balitoridae), from Guangxi, China. *Environ. Biol. Fishes 93: 169–175*.

Zeng Z.-X., Shao W.-H., Jin Z.-J. et al. 2022: *Hongshuia brevibarba*, a new species of labeonin fishes (Pisces: Cyprinidae) from the upper Chang-Jiang basin in Guizhou Province, South China. *Ichthyol. Explor. Freshw. 1184: 1–11*.

Zhang C.-G., Shao G.-Z., Wu H.-L. et al. 2020: Species catalogue of China, vol. 2. Animals, vertebrates (V), fishes(i). *Science Press, Beijing, China. (in Chinese)*

Zhao Y.-H. & Zhang C.-G. 2006: Cavefishes: concept, diversity and research progress. *Biodiversity Science 14: 451–460. (in Chinese)*

Zhao Y.-H. & Zhang C.-G. 2009: Endemic fishes of *Sinocyclocheilus* (Cypriniformes: Cyprinidae) in China – species diversity, cave adaptation, systematics and zoogeography. *Science Press, Beijing, China. (in Chinese)*

Zheng L.-P., Du L.-N., Chen X.-Y. et al. 2009: A new species of genus *Triplophysa* (Nemacheilinae: Balitoridae), *Triplophysa longipectoralis* sp. nov, from Guangxi, China. *Environ. Biol. Fishes 85: 221–227*.

Zheng L.-P., Yang J.-X. & Chen X.-Y. 2012: A new species of *Triplophysa* (Nemacheilidae: Cypriniformes), from Guangxi, southern China. *J. Fish Biol. 80: 831–841*.

Zhu S.-Q. 1989: The loaches of the Subfamily Nemacheilinae in China (Cypriniformes: Cobitidae). *Jiangsu Science and Technology Publishing House, Nanjing, China. (in Chinese)*

Zhu S.-Q. & Cao W.-X. 1988: Descriptions of two new species and a new subspecies of Noemacheilinae from Yunnan Province. *Acta Zootaxonomica Sinica 13: 95–100. (in Chinese)*

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