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A new cave-dwelling species of the genus *Triplophysa* (Cypriniformes, Nemacheilidae) from southwest China

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Abstract

Based on morphological and molecular data, we described a new cave-dwelling species, *Triplophysa xiuwenensis* **sp. nov.**, from the karst region of southwest China. The new species can be distinguished from its congeners by the following morphological characteristics: body scaleless without pigmentation; eye diameter of HL with 4.6–6.7%; tip of pelvic fin not reaching anus; outer rostral barbel reaching to posterior margin of eyes; 14–15 branched caudal fin rays. Phylogenetic relationships based on the mitochondrial cytochrome *b* (Cyt *b*) gene support the validity of the new species. The discovery of the new species brings the number of cave-dwelling *Triplophysa* species in Guizhou Province to 15, making it the region with the highest number of *Triplophysa* cave species in the southwestern karst area.

Key Words

Cavefish, karst, morphology, new loach, taxonomy

Introduction

Cavefish are species confined to cave environments or subterranean water bodies for at least part of their life history. They could not survive without these habitats (Zhao et al. 2011). According to whether they possess cave-adaptive characteristics (troglomorphism) (Romero and Green 2005), these cavefishes can be divided into two major categories: stygobionts and stygophiles (Zhao and Zhang 2006; Zhao et al. 2011). Currently, it is known that approximately 310 species of fish exhibit cave-dwelling habits worldwide, with two-thirds being stygobionts (Niemiller et al. 2019). China is the most diverse country regarding the richness of cavefish species. According to the author's statistics, China has more than 180 species of freshwater fish exhibiting cave-dwelling behavior, most of which are distributed in the karst areas of southwestern China, including the Guangxi Zhuang Autonomous Region, Yunnan, and Guizhou Provinces (Zhao et al. 2022).

The nemacheilid genus *Triplophysa* Rendahl, 1933 is an important component of Chinese cavefishes. Currently, more than 140 *Triplophysa* species are recorded worldwide (Fricke et al. 2024), including more than 100 species found in China (Zhang and Zhao 2016; Liu 2021). Among them, there are 40 valid cave-dwelling species of *Triplophysa* recorded in the karst region of southern China (Zhao et al. 2022; Luo et al. 2023; Lan et al. 2024). This genus has become the second largest cavefish group in China and indeed, in the world. With ongoing explorations of cavefish, an increasing number of cave-dwelling species of *Triplophysa* have been discovered in recent years (Lu et al. 2022; Liu et al. 2022; Luo et al. 2023). In previous studies, the group represented by *T. rosa* comprises four species (*T. rosa*, *T. wudangensis*, *T. qingzhenensis*, and *T. ziyunensis*) (Chen and Yang 2005; Liu et al. 2022; Lan et al. 2024). They exhibit cave-adapted features, such as reduced eyes and extended barbels. Interestingly, except for *T. ziyunensis*, the remaining three species are distributed in the Wujiang River Basin (Lan et al. 2024).

In 2023, we collected a number of specimens of *Triplophysa* during the cavefish surveys carried out in Guizhou Province, China. After careful examination, a new species was discovered and is reported herein.

Materials and methods

Specimens' collection and preservation

All samples were collected using hand nets and fish traps. Muscle tissues for genetic analysis were excised and preserved in anhydrous ethanol at -20 °C. The collected specimens were initially fixed in 10% formalin and subsequently transferred to 75% ethanol for long-term preservation. Comparative specimens for this study were stored at the School of Life Sciences, Guizhou Normal University (GZNUSLS) and Institute of Zoology, Chinese Academy of Sciences (ASIZB).

Morphological measurements and analyses

Counts and morphometric measurements followed Li (2018) and Tang et al. (2012). All morphometric measurements were taken from the left side, using digital calipers with an accuracy of 0.01 mm. Measurements were recorded as percentages of standard length (SL) or head length (HL). Vertebral counts were obtained through a micro-CT scan (Bruker Skyscan 1276) of the specimens. Morphometric and meristic data were tabulated and analyzed using Microsoft Excel.

DNA extraction, PCR and Sequencing

Total genomic DNA was extracted from muscle tissue using a modified high-salt method (Aljanabi and Martinez 1997). The integrity of the extracted DNA was checked by 1% agarose gel electrophoresis, and both the DNA concentration and purity were quantified by analysis with the Epoch 2 spectrophotometer system (Biotek Instruments, Inc., Winooski, VT, USA). The mitochondrial gene Cyt *b* was amplified using the primers L14724 (5'-GACTTGAAAAACCACCGTTG-3') and H15915 (5'-CTCCGATCTCCGGATTACAAGAC-3') (Xiao et al. 2001). The PCR was conducted in a total volume of 35 µL containing 17.5 µL of 2xTaq Plus MasterMix (CoWin Biosciences, Beijing, China), 14.5 µL dd H_2O, 1 µL of template DNA (100 ng/µL), and 1 µL of each primer (10 µM). The PCR conditions were as follows: initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 1 min, annealing at 54 °C for 30 s, and elongation at 72 °C for 1.5 min, with a final extension at 72 °C for 10 min. The amplified PCR products were visualized on 1% agarose gels to confirm amplification. The sizes of the amplified PCR products were estimated by comparison to a DL2000 DNA size marker (TaKaRa, Beijing, China). Finally, purification and sequencing of the PCR products were performed by Sangon Biotech (Shanghai) Co., Ltd.

Molecular data analysis

A total of 57 Cyt *b* sequences were used to construct the phylogenetic tree, including five sequences we newly sequenced and 52 published Cyt *b* sequences from species available on NCBI (https://www.ncbi.nlm.nih.gov/) (Table 1). Phylogenetic tree was constructed using the Bayesian inference (BI) method based on the Cyt *b* gene. The BI analysis was performed with MrBayes v. 3.2.6 (Ronquist et al. 2012) integrated into PhyloSuite v1.2.3 (Zhang et al. 2020). Four Markov chain Monte Carlo (MCMC) chains were run simultaneously for 2 million generations sampling every 1000 generations. Bayesian posterior probability (BPP) was calculated in a majority-rule consensus tree after discarding the first 25% of samples as burn-in. The phylogenetic trees were visualized and edited using the online tool Interactive Tree Of Life (iTOL) (https://itol.embl.de/). Genetic distances between pairs of sequences were computed using the Kimura-2-parameter (K2P) model within the MEGA v7 software (Kumar et al. 2016).

Results

***Triplophysa xiuwenensis* Zhu, Zhang & Yuan sp. nov.**
https://zoobank.org/5A37DA8F-6165-485C-9965-6FFFE5B29814
Figs 1–4, Table 2

Holotype. GZNUSLS202306086 (Fig. 1), 69.1 mm SL, collected by Ren-Yi Zhang, Lei Deng, Huan Cheng, Ren-Rong Huang on June 23, 2023, in Shilin Village, Dashi Buyi Township, Xiuwen County, Guizhou Province, China (27.055334°N, 106.476045°E; ca. 1114 m; Fig. 4).

Paratypes. Four specimens from the same locality as the holotype. GZNUSLS202306088–090, 40.5–58.2 mm SL, ASIZB 248338, 56.3 mm SL, the collected information is the same as that of the holotype specimen.

Diagnosis. The new species can be distinguished from all other species in the genus *Triplophysa* by the following combination of characteristics: body naked, without skin pigmentation, lateral line complete; eye reduced, with diameter of HL 4.6–6.7%; anterior nostril with elongated barbel-like tip; long outer rostral barbel reaching to posterior margin of eyes; distal margin of dorsal fin truncate; tip of pectoral fin not reaching to pelvic-fin origin, tip of pelvic fin near anus; dorsal fin 8, anal fin 6, and caudal fin 14–15 branched fin rays; vertebrae 41.

Description. D iii, 8; A iii, 6; P i, 9; V i, 6; C 14–15; vertebrae 4+37 (n = 1).

Morphological data of the specimens of *Triplophysa xiuwenensis* sp. nov. are given in Table 2.

Table 1. GenBank accession numbers for molecular phylogenetic analysis.

	Species	Voucher	GenBank accession number
1	*Barbatula barbatula*	/	KP715096
2	*Claea dabryi*	KIZ 2009002750	MG238215
3	*C.wulongensis*	T20	OQ754129
4	*C.minibarba*	IHB 2017097698	OP750015
5	*Homatula berezowskii*	FS-2014-Y03	NC_040302
6	*H. pycnolepis*	No. 20080819953	NC_056344
7	*T. alticeps*	H14	OP616079
8	*T. angeli*	/	NC_065113
9	*T. anlongensis*	GZNU 20230112003	OQ754140
10	*T. anterodorsalis*	F3894	MG725417
11	*T. baotianensis*	GZNU 20180421006	OQ241181
12	*T. bleekeri*	/	NC_018774
13	*T. brevicauda*	KIZ 050422005	MG238301
14	*T. cehengensis*	GZNU 20230109003	OQ754134
15	*T. dorsalis*	F740	MG725413
16	*T. erythraea*	/	NC_088519
17	*T. guizhouensis*	GZNUSLS202502020	PV394924
18	*T. huapingensis*	T13	OQ754125
19	*T. langpingensis*	T10	OQ754122
20	*T. lixianensis*	/	NC_030521
21	*T. longliensis*	SWU2016090300	MW582825
22	*T. macrocephala*	T11	OQ754123
23	*T. markehenensis*	F3893	MG725416
24	*T. nandanensis*	/	MW582824
25	*T. nanpanjiangensis*	GZNUSLS202407050	PV394925
26	*T. nasobarbatula*	GZNU 20220313011	OQ241176
27	*T. nujiangensa*	IHB201315814	KT213598
28	*T. panzhouensis*	GZNU 20220513003	OQ754121
29	*T. pappenheimi*	/	NC_033972
30	*T. qingzhenensis*	IHB 201911150005	MT700459
31	*T. qini*	/	ON528184
32	*T. qiubeiensis*	T15	OQ754127
33	*T. robusta*	/	NC_025632
34	*T. rongduensis*	GZNU 20230110003	OQ754137
35	*T. rosa*	SWU10100503	JF268621
36	*T. rotundiventris*	F2077	MG725402
37	*T. sanduensis*	SWU20170613001	MW582822
38	*T. siluroides*	/	EF212443
39	*T. stenura*	/	OR916123
40	*T. stewarti*	/	NC_030506
41	*T. stoliczkai*	F2565	MG725410
42	*T. strauchii*	CF736	KX373854
43	*T. tenuis*	IHB0917490	KT224363
44	*T. tianeensis*	/	MW582826
45	*T. tianxingensis*	GZNUSLS202309182	PV394926
46	*T. tibetana*	NWIPB1106069	KT224364
47	*T. venusta*	/	NC_029330
48	*T. weiheensis*	CF3569	KY781403
49	*T. wudangensis*	T22	OQ754131
50	*T. wuweiensis*	IHB201307124	KT224365
51	*T. xiangxiensis*	/	JN696407
52	*T. xiuwenensis* sp. nov.	GZNUSLS202306089	PV394922
53	*T. xiuwenensis* sp. nov.	GZNUSLS202306090	PV394923
54	*T. xuanweiensis*	ASIZB223820	OL675198
55	*T. yaluwang*	GZNU20240118005	PQ117067
56	*T. zhenfengensis*	GZNUSLS202311039	PV394927
57	*T. ziyunensis*	GZNU20230529003	PQ117069

Body elongated, with anterior being nearly cylindrical and posterior portion flattened laterally from dorsal-fin base to caudal-fin base increasingly. With deepest body depth anterior to dorsal-fin origin, deepest body depth 12.8–14.8% of SL. Dorsal-fin origin near body midpoint.

Table 2. Morphometric data of *Triplophysa xiuwenensis* sp. nov.

Morphometric characters	Holotype	Paratypes (n = 4)			
	GZNUSLS202306086	min	max	Mean	SD
Total length (mm)	84.9	49.9	84.9	64.4	14.6
Standard length (mm)	69.1	40.5	69.1	53.4	11.8
Head length (mm)	16.3	10.5	16.3	13.3	2.3
In percent SL%					
Body height	14.8	12.8	14.8	13.9	0.8
Body width	13.2	10.5	13.2	11.2	1.1
Predorsal length	50.8	45.8	51.9	49.8	2.4
Dorsal fin length	22.1	21.3	24.5	22.6	1.2
Dorsal fin base length	13.3	12.6	14.0	13.2	0.5
Prepelvic length	53.2	52.2	55.9	53.4	1.4
Pelvic fin length	16.1	15.6	18.0	16.9	1.0
Pelvic fin base length	3.9	3.9	4.6	4.2	0.3
Preanal length	73.6	70.2	75.4	73.3	2.0
Anal fin length	15.4	15.4	18.1	16.6	1.2
Anal fin base length	9.1	9.1	11.5	10.5	1.0
Prepectoral length	21.8	21.8	26.8	24.0	2.0
Pectoral fin length	18.2	18.2	20.7	19.4	0.9
Pectoral fin base length	5.6	4.4	5.6	5.1	0.5
Preanus length	70.3	70.3	75.2	71.7	2.0
Caudal peduncle length	18.4	14.8	18.4	17	1.4
Caudal peduncle depth	7.4	6.9	7.8	7.3	0.4
In percent HL%					
Head height	52.9	45.7	52.9	48.9	2.9
Head width	67.8	60.7	67.8	64.2	3.0
Inner rostral barbel length	21.7	15.3	23.4	19.7	3.3
Outer rostral barbel length	52.5	31.0	53.2	43.5	9.4
Maxillary barbel length	33.7	30.0	37.8	32.8	3.2
Eye diameter	4.7	4.6	6.7	5.3	0.8
Interorbital width	37.7	31.8	40.8	35.9	3.9
Distance between posterior nostrils	27.3	22.3	27.3	24.9	1.8
Preanterior nostril length	25.1	20.4	27.3	24.0	2.8
Postocular head length	51.5	48.7	53.5	51.0	1.9

Dorsal profile slightly convex from snout to dorsal-fin insertion, then gradually compressed from dorsal fin origin to caudal fin base. Ventral side flat. Head conical, slightly flattened and head length 23.6–28.6% of SL. Width greater than height. Snout short, tip blunt. Anterior and posterior nostrils closely connected, anterior nostril in a short tube with an elongated barbel-like tip, the tip of the nostril appendage not reaching the anterior margin of the eye; posterior nostril above maxillary barbel. Eye reduced to black dot, located dorsolaterally on head, orbit indistinct, eye diameter 4.6–6.7% of HL.

Three pairs of barbels; inner rostral barbel short, 15.3–23.4% of HL, extending backward not reaching front of eye; outer rostral barbel well-developed, 31.0–53.2% of HL, extending backward reaching beyond eye; maxillary barbel 30.0–37.8% of HL, extending backward reaching beyond eye. Mouth inferior, mouth angle located below midpoint between anterior and posterior nostrils.

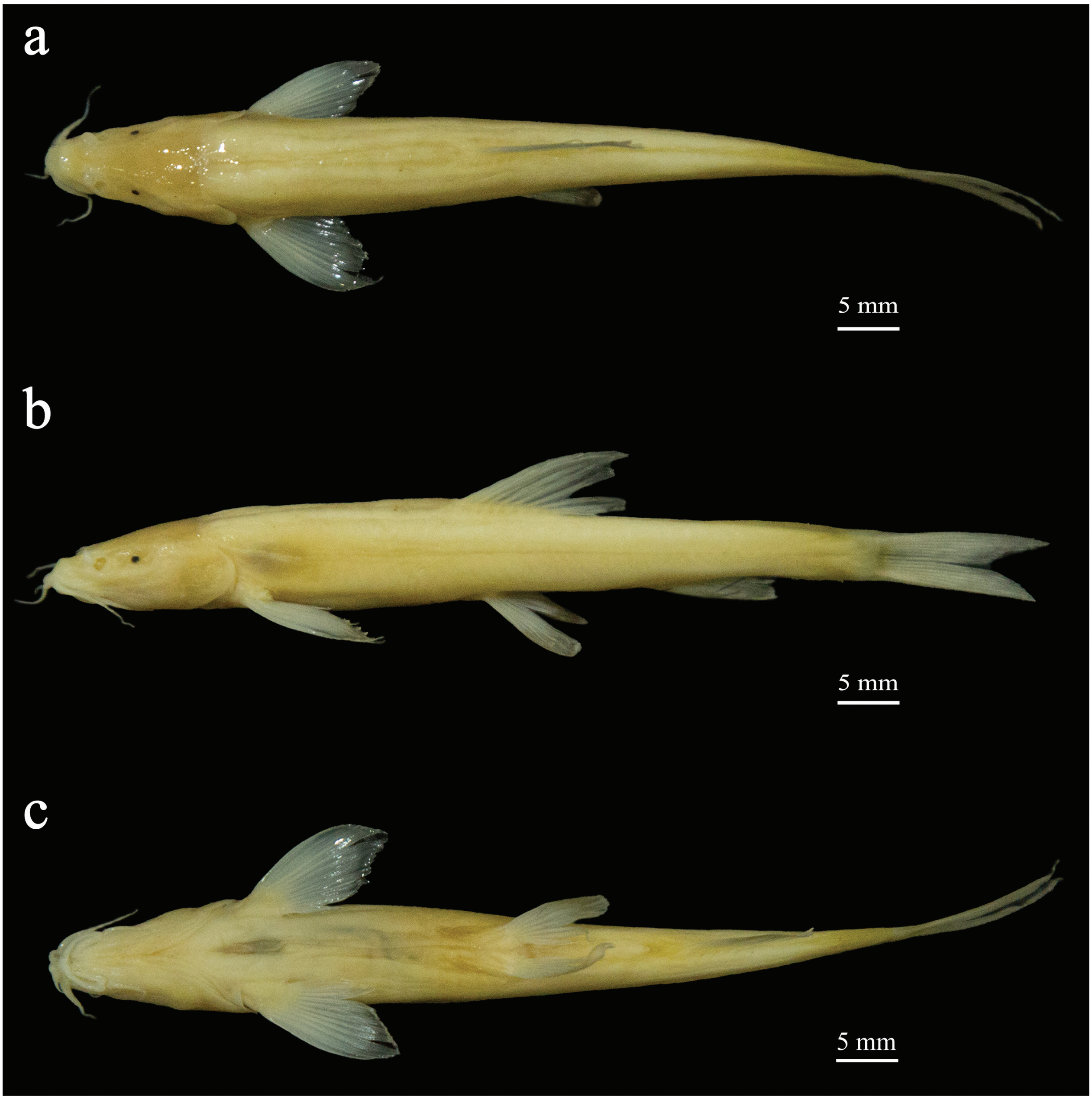

Figure 1. *Triplophysa xiuwenensis* sp. nov., holotype, GZNUSLS202306086, 69.1 mm SL. **a.** dorsal view; **b.** lateral view; **c.** ventral view.

Central lower lip with 'V'-shaped notch, interrupted centrally, forming one pair of longitudinal folds. Upper jaw arched, no median dentiform process; lower jaw arched, no central notch. Gill filaments with tufted branching, gill rakers short, with small protuberances.

Body smooth and scaleless. Lateral line pores on head indistinct, connecting posteriorly with lateral line on body; body lateral line complete, straight, extending to slightly anterior of base of caudal peduncle. The posterior chamber of air bladder degenerated.

Dorsal fin origin located near midpoint or slightly anterior between snout tip and caudal fin base; dorsal fin short, 21.3–24.5% of SL, with straight margin. First branched fin ray longest, shorter than head length, extending past vertical of anus, approaching vertical of anal fin origin. Pectoral fin spread flat, fin ray tips reaching about halfway between pectoral and pelvic fin origins. Pelvic fin spread flat, origin posterior to dorsal fin origin, fin ray tips extending near anus but not reaching anal fin origin; distance between pelvic and pectoral fin origins greater than distance to anal fin origin. Anal fin margin concave, fin ray tips not reaching caudal fin base. Anus close to anal fin origin. Caudal fin forked, upper lobe slightly longer than lower lobe, tips slightly pointed, caudal peduncle with slight adipose crest.

Coloration. Live adults of the new species exhibit a pale yellow body coloration, no pigmentation, fin bases pale yellow, membranes between fins colorless. No spots on body (Fig. 3). After fixation in 10% formalin, body turns creamy white (Fig. 1).

Sexual dimorphism. No secondary sexual characteristics were detected in the current samples.

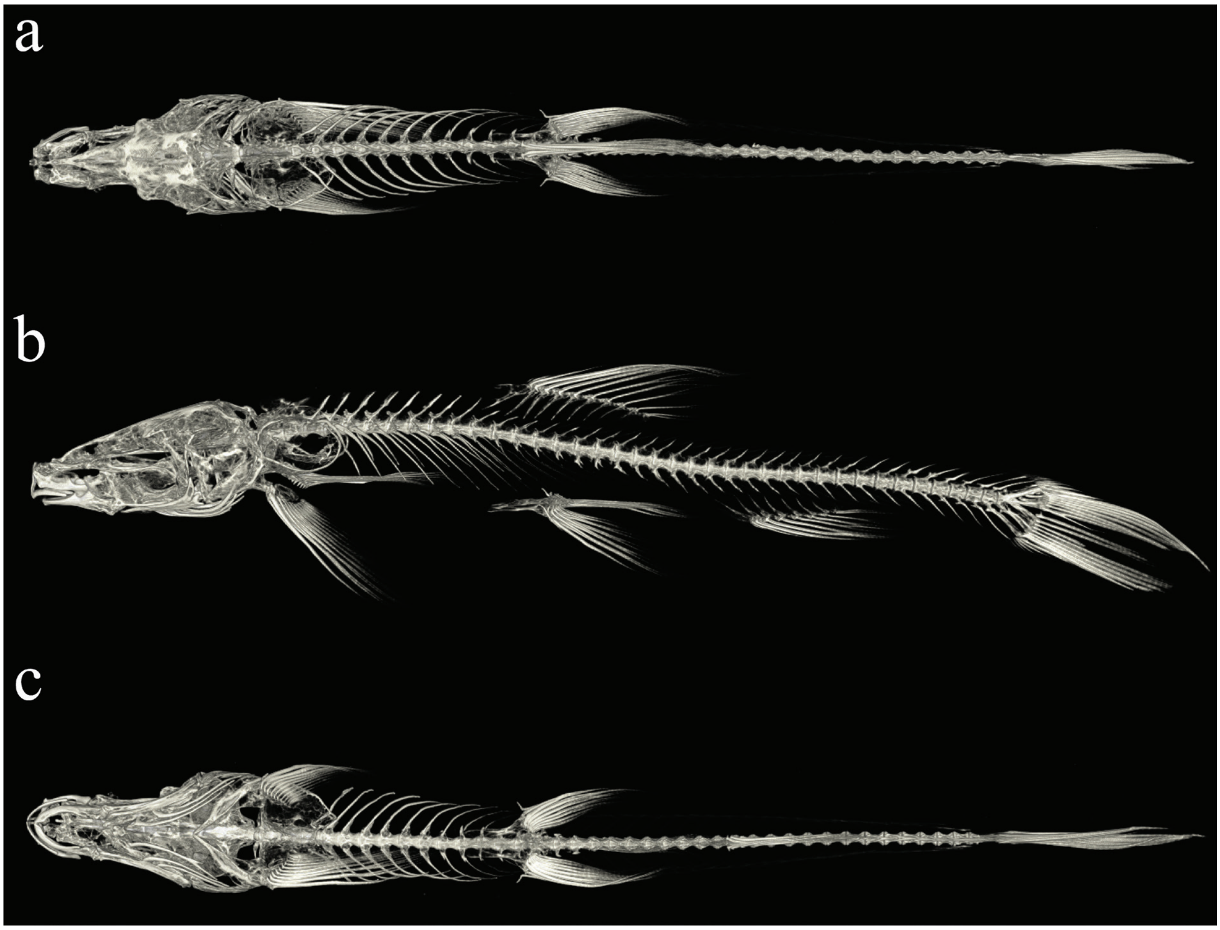

Figure 2. *Triplophysa xiuwenensis* sp. nov., holotype, micro-CT graph. **a.** dorsal view; **b.** lateral view; **c.** ventral view.

Figure 3. Live specimen of *Triplophysa xiuwenensis* sp. nov.

Distribution. The specimens were collected at the outlet of a karst cave in Shilin Village, Xiuwen County, Guizhou Province, China, where the water is connected to the upper reaches of the Wujiang River system in the Yangtze River basin (Fig. 4). So far, *Triplophysa xiuwenensis* sp. nov. has only been found in the type locality.

Etymology. The specific name refers to the type locality of the new species: Shilin Village, Xiuwen County and the Latin suffix (ensis). We propose the Chinese name '修文高原鳅'.

Molecular phylogenetic analysis

We constructed a phylogenetic tree of *Triplophysa* based on the mitochondrial Cyt *b* molecular marker. Within the hypogean group clade, two specimens from Shilin Village, Xiuwen County, Guizhou Province, formed a distinct branch that is a sister group to *T. rosa*, *T. wudangensis*, *T. qingzhenensis*, and *T. ziyunensis* (Fig. 5). Based on the K2P model, pairwise genetic distances between the new species and its congeners are given in Table 5. The new species shows a minimum genetic distance of 2.2% from other *Triplophysa* species (vs. *T. qingzhenensis*). Therefore, the species from this locality represents a new evolutionary lineage, which we describe as a new species: *Triplophysa xiuwenensis* sp. nov.

Discussion

So far, forty cave-dwelling species of *Triplophysa* have been reported in the karst region of southwest China (Table 3). Since *T. rosa* was reported in 2005, the karst region of southwest China and its underground caves have become a hotspot for scientific research, and cave fishes have become the focus of scientists' attention. As for the Guizhou region, which is the center of the southwest karst region, fourteen species of cave-dwelling of *Triplophysa* have been described.

The detailed distinguishing characteristics between the new species and 40 species of this genus are shown in Table 4. *Tripophysa xiuwenensis* sp. nov. can be distinguished from *T. anlongensis*, *T. baotianensis*, *T. flavicorpus*, *T. guizhouensis*, *T. huapingensis*, *T. longipectoralis*, *T. longliensis*, *T. luochengensis*, *T. macrocephala*, *T. nandanensis*, *T. nanpanjiangensis*, *T. nasobarbatula*, *T. panzhouensis*, *T. qingzhenensis*, *T. rongduensis*, *T. sanduensis*, *T. tianeensis*, *T. tianxingensis*, *T. wudangensis*, *T. wulongensis*, *T. xiangshuingensis*, *T. xichouensis*, *T. yaluwang*, *T. yunnanensis*, *T. zhenfengensis*, *T. ziyunensis* by skin pigmentation absence (vs. skin pigmentation presence). The new species can be distinguished from *T. anshuiensis*, *T. erythraea*, *T. fengshanensis*, *T. gejiuensis*, *T. posterodorsalus*, *T. qini*, *T. qiubeiensis*, *T. rosa*, *T. shilinensis*, *T. xiangxiensis*, *T. xuanweiensis* by eye reduced, diameter of HL with 4.6–6.7% (vs. eye absent).

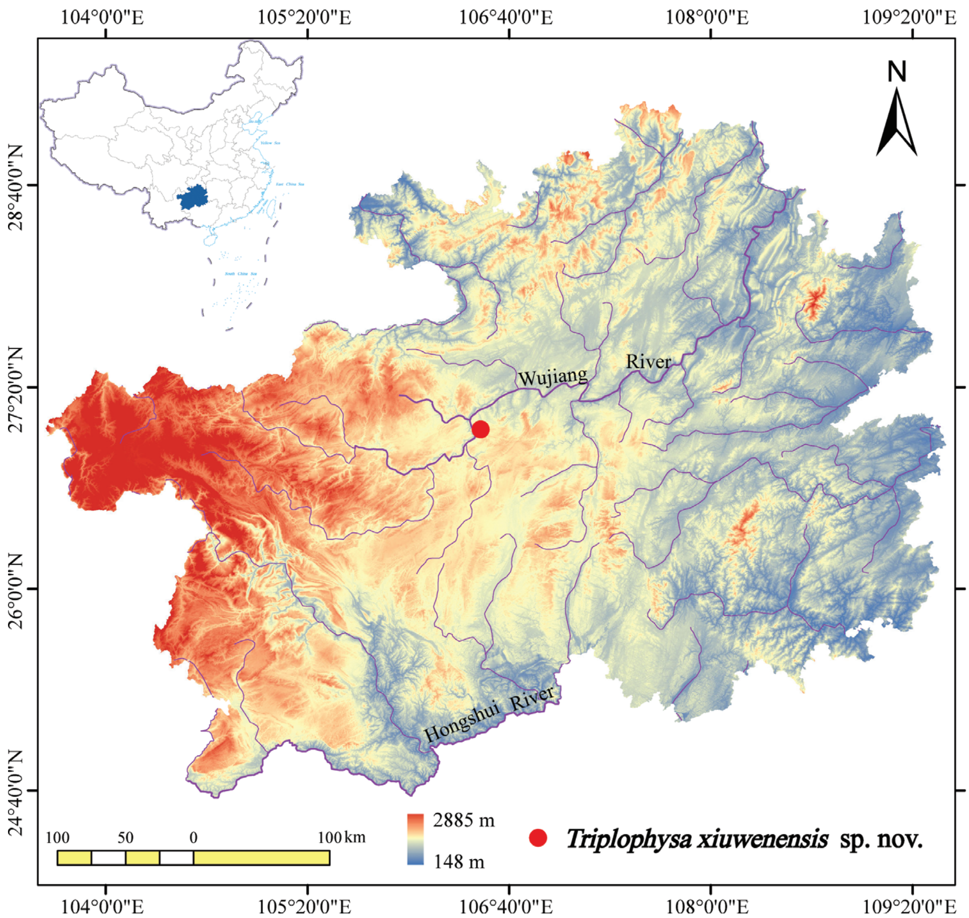

Figure 4. The distribution of *Triplophysa xiuwenensis* sp. nov.

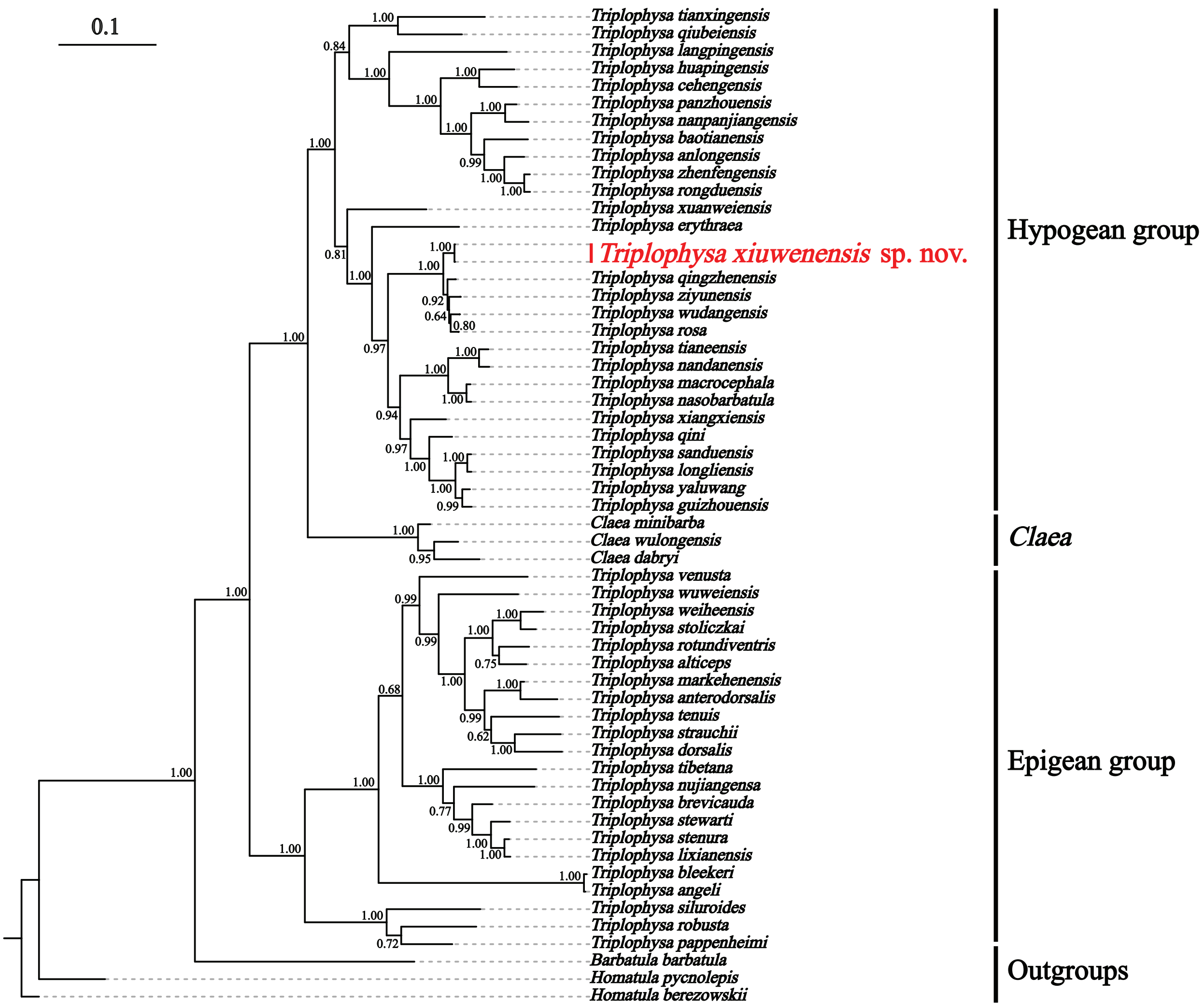

Figure 5. Phylogenetic relationships of selected species of *Triplophysa* and three outgroup species based on Bayesian inference (BI) methods using mitochondrial Cyt *b* gene sequences. The BI posterior probabilities are shown at the nodes.

Tripophysa xiuwenensis sp. nov. can be distinguished from *T. aluensis* by interorbital width of HL (31.8–40.8% vs. 22.2%), 14–15 branched caudal-fin rays (vs. 13); from *T. langpingensis* by pectoral-fin rays (i, 9 vs. i, 10–11), lateral line complete (vs. incomplete); from *T. tianlinensis* by dorsal-fin rays (iii, 8 vs. iv, 8–9), tip of maxillary barbel reaching the posterior margin of eye (vs. reaching the posterior margin of operculum); from *T. cehengensis* by dorsal-fin rays (iii, 8 vs. iv, 9), pectoral-fin rays (i, 9 vs. i, 10), eye diameter of HL (4.6–6.7% vs. 2%).

Additionally, the new species can be further distinguished from the four cave-dwelling species of *Triplophysa* in the *T. rosa* group by the following morphological characteristics. It can be distinguished from *T. qingzhenensis* by eye diameter of HL (4.6–6.7% vs. 2.1–4.4%), interorbital width of HL (31.8–40.8% vs.25.1–30.4%) and vertebrae (4 + 37 in *T. xiuwenensis* sp. nov. vs. 4 + 36 in *T. qingzhenensis*); from *T. rosa* by eye reduced, diameter of HL with 4.6–6.7% (vs. eye absent), 9 branched pectoral-fin rays (vs. 12) and tip of pelvic fin not reaching anus (vs. tip of pelvic fin reaching anus); from *T. wudangensis* by skin pigmentation absence (vs. skin pigmentation presence), 8 branched dorsal-fin rays (vs. 7), 6 branched anal-fin rays (vs. 5) and vertebrae (4 + 37 in *T. xiuwenensis* sp. nov. vs. 4 + 34 in *T. wudangensis*); from *T. ziyunensis* by skin pigmentation absence (vs. skin pigmentation presence), tip of pelvic fin not reaching anus (vs. tip of pelvic fin reaching anus) and tip of outer rostral barbel reaching beyond eye (vs. not reaching anterior margin of eye).

In this study, phylogenetic analyses show that the new species belongs to the hypogean group of *Triplophysa* and that this hypogean group is monophyletic. However, *Triplophysa* is not a monophyletic group, and the hypogean group forms a sister group to *Claea*, suggesting that more samples and molecular specimens are urgently needed to illustrate the evolutionary history of *Triplophysa*. Together with the new species, there are now five species in the *T. rosa* group clade. However, the interspecific genetic distances among these species remain relatively small. The reason for this phenomenon may be that *Triplophysa* species are influenced by the karst landscape, leading to slower species differentiation, such as traits like eye degeneration or loss. These species may be in an ongoing stage of differentiation (Liu et al. 2022).

Table 3. A list of 40 species of hypogean group of the genus *Triplophysa* distributed in the southwest China Karst.

ID	Species	Province	Main drainage	Tributary	Reference
1	*Triplophysa aluensis* Li & Zhu, 2000	Yunnan	Pearl River	Nanpanjiang River	Li and Zhu 2000
2	*Triplophysa anlongensis* Lan, Song, Luo, Zhao, Xiao & Zhou, 2023	Guizhou	Pearl River	Nanpanjiang River	Luo et al. 2023
3	*Triplophysa anshuiensis* Wu, Wei, Lan & Du, 2018	Guangxi	Pearl River	Hongshui River	Wu et al. 2018a
4	*Triplophysa baotianensis* Li, Li, Liu & Li, 2018	Guizhou	Pearl River	Nanpanjiang River	Li et al. 2018
5	*Triplophysa cehengensis* Luo, Mao, Zhao, Xiao & Zhou, 2023	Guizhou	Pearl River	Beipanjiang River	Luo et al. 2023
6	*Triplophysa erythraea* Liu & Huang, 2019	Hunan	Yangtze River	Yuanjiang River	Huang et al. 2019
7	*Triplophysa fengshanensis* Lan, 2013	Guangxi	Pearl River	Hongshui River	Lan et al. 2013
8	*Triplophysa flavicorpus* Yang, Chen & Lan, 2004	Guangxi	Pearl River	Hongshui River	Yang et al. 2004
9	*Triplophysa gejiuensis* (Chu & Chen, 1979)	Yunnan	Pearl River	Nanpanjiang River	Chu and Chen 1979
10	*Triplophysa guizhouensis* Wu, He & Yang, 2018	Guizhou	Pearl River	Hongshui River	Wu et al. 2018b
11	*Triplophysa huapingensis* Zheng, Yang & Che, 2012	Guangxi	Pearl River	Hongshui River	Zheng et al. 2012
12	*Triplophysa langpingensis* Yang, 2013	Guangxi	Pearl River	Hongshui River	Lan et al. 2013
13	*Triplophysa longipectoralis* Zheng, Du, Chen & Yang, 2009	Guangxi	Pearl River	Liujiang River	Zheng et al. 2009
14	*Triplophysa longliensis* Ren, Yang & Chen, 2012	Guizhou	Pearl River	Hongshui River	REN et al. 2012
15	*Triplophysa luochengensis* Li, Lan, Chen & Du, 2017	Guangxi	Pearl River	Hongshui River	Li et al. 2017a
16	*Triplophysa macrocephala* Yang, Wu & Yang, 2012	Guangxi	Pearl River	Liujiang River	Yang et al. 2012
17	*Triplophysa nandanensis* Lan, Yang & Chen, 1995	Guangxi	Pearl River	Hongshui River	Lan et al. 1995
18	*Triplophysa nanpanjiangensis* Zhu & Cao, 1988	Yunnan	Pearl River	Nanpanjiang River	Zhu and Cao 1988
19	*Triplophysa nasobarbatula* Wang & Li, 2001	Guizhou	Pearl River	Liujiang River	Wang and Li 2001
20	*Triplophysa panzhouensis* Yu, Luo, Lan, Xiao & Zhou, 2023	Guizhou	Pearl River	Nanpanjiang River	Luo et al. 2023
21	*Triplophysa posterodorsalus* (Li, Ran & Chen, 2006)	Guangxi	Pearl River	Liujiang River	Ran et al. 2006
22	*Triplophysa qingzhenensis* Liu, Zen, & Gong, 2022	Guizhou	Yangtze River	Wujiang River	Liu et al. 2022
23	*Triplophysa qini* Deng, Wang & Zhang, 2022	Chongqing	Yangtze River	Wujiang River	Deng et al. 2022
24	*Triplophysa qiubeiensis* Li & Yang, 2008	Yunnan	Pearl River	Nanpanjiang River	Li et al. 2008
25	*Triplophysa rongduensis* Mao, Zhao, Yu, Xiao & Zhou,2023	Guizhou	Pearl River	Beipanjiang River	Luo et al. 2023
26	*Triplophysa rosa* Chen & Yang, 2005	Chongqing	Yangtze River	Wujiang River	Chen and Yang 2005
27	*Triplophysa sanduensis* Chen & Peng, 2019	Guizhou	Pearl River	Duliujiang River	Chen and Peng 2019
28	*Triplophysa shilinensis* Chen,Yang & Xu, 1992	Yunnan	Pearl River	Nanpangjiang River	Chen et al. 1992
29	*Triplophysa tianeensis* Chen, Cui & Yang, 2004	Guangxi	Pearl River	Hongshui River	Chen et al. 2004
30	*Triplophysa tianlinensis* Li, Li, Lan & Du, 2017	Guangxi	Pearl River	Hongshui River	Li et al. 2017b
31	*Triplophysa tianxingensis* Yang, Li & Chen, 2016	Yunnan	Pearl River	Nanpangjiang River	Yang et al. 2016
32	*Triplophysa wudangensis* Liu, Zen & Gong, 2022	Guizhou	Yangtze River	Wujiang River	Liu et al. 2022
33	*Triplophysa xiangshuingensis* Li, 2004	Yunnan	Pearl River	Nanpanjiang River	Li 2004
34	*Triplophysa xiangxiensis* Yang, Yuan & Liao, 1986	Hunan	Yangtze River	Yuanjiang River	Yang et al. 1986
35	*Triplophysa xichouensis* Liu, Pan, Yang & Chen, 2017	Yunnan	Red River	Red River	Liu et al. 2017
36	*Triplophysa xuanweiensis* Lu, Li, Mao & Zhao, 2022	Yunnan	Pearl River	Beipanjiang River	Lu et al. 2022
37	*Triplophysa yaluwang* Lan, Liu, Zhou & Zhou, 2024	Guizhou	Pearl River	Hongshui River	Lan et al. 2024
38	*Triplophysa yunnanensis* Yang, 1990	Yunnan	Pearl River	Nanpanjiang River	Chu and Chen 1990
39	*Triplophysa zhenfengensis* Wang & Li, 2001	Guizhou	Pearl River	Beipanjiang River	Wang and Li 2001
40	*Triplophysa ziyunensis* Wu, Luo, Xiao & Zhou, 2024	Guizhou	Pearl River	Hongshui River	Lan et al. 2024

Additionally, we should note that the *T. rosa* group is confirmed to disperse across the watershed between the Yangtze River and Pearl River with the confirmation of *T. ziyunensis*. According to geological evidence, the Miaoling Mountains originated in the Early Pleistocene and developed into the dividing watershed between the Yangtze and Pearl River systems by the mid-to-late Pleistocene (Zhou and Chen 1993). This suggests that possible gene flow among hypogean group species between connected water systems may have occurred among species before the Miaoling Mountains formed the watershed separating the Yangtze and Pearl River systems and a similar phenomenon has been found previously in the epigean group of *Triplophysa* (Qian et al. 2023).

Together with the species in this study, 15 cave-dwelling species of *Triplophysa* have been reported from Guizhou, making it the province with the highest number of cave-dwelling species of *Triplophysa* in the southwest karst region. This phenomenon can be attributed to the highly developed karst landscape in Guizhou, which features numerous undiscovered underground caves and culverts. Most of the currently known *Triplophysa* cave species are restricted to specific areas with small populations, and only a few widespread species exist. This distribution pattern implies that additional undiscovered *Triplophysa* cave-dwelling species may still exist (Luo et al. 2023). The evolutionary history of *Triplophysa* species requires further investigation using higher-resolution molecular markers. In particular, the genetic evolution and phylogenetic relationships of cave-dwelling groups within the genus should be analyzed with additional molecular data, integrated with biogeographical studies. Furthermore, the small population sizes of some species may indicate anthropogenic damage or pollution in certain underground rivers. A similar phenomenon has also been observed in the cave-dwelling species of the genus *Sinocyclocheilus* (Fan et al. 2024; Shao et al.

Table 4. Morphology of the new species described in this paper was compared with that of 40 known species of hypogean group of *Triplophysa*.

Speices	Skin pigmentation	Eye diameter (% HL)	Interorbital width (% HL)	Vertebrae	Posterior chamber of air bladder	Dorsal fin rays	Anal fin rays	Pectoral fin rays	Pelvic fin rays	Caudal fin rays	CPL/CPD	Ventral fin tip reaching to Anal
T. aluensis	Absent	5.6	22.2	–	Degenerated	iii, 7	iii, 5	i, 9	i, 6	13	2.8	No
T. anlongensis	Whole body	5.1–9.3	32.1–35.6	4 + 37	Degenerated	iii, 8	iii, 5	i, 11	ii, 8	16	1.7	No
T. anshuiensis	Dorsal	Absent	–	–	Developed	iv, 7–8	ii, 6	i, 10	i, 6	14	1.59	Yes
T. baotianensis	Whole body	14.0–15.0	25.0–35.0	–	Degenerated	iii, 6–7	ii, 5	i, 9	ii, 6–7	11–13	1.67–2.25	No
T. cehengensis	Absent	1.5–2.2	27.2–36.5	4 + 35	Developed	iv, 9	iii, 5	i, 10	ii, 8	16	2	Yes
T. erythraea	Absent	Absent	–	–	Developed	ii, 8	i, 6	ii, 10	i, 5	17	1.98–2.35	Yes
T. fengshanensis	Absent	Absent	–	–	–	ii, 8	ii, 6	i, 8–10	i, 6–7	16	2.46–2.6	No
T. flavicorpus	Whole body	5.1–6.8	3.1–5.2	4 + 34	Degenerated	iii, 10	iii, 6–7	i, 11	i, 6–7	16	1.7	Yes
T. gejiuensis	Absent	Absent	–	–	Developed	iii, 7–8	iii, 4–6	i, 10	i, 5	14–15	1.4	Yes
T. guizhouensis	Whole body	9.4–12.1	20.3–24.3	–	Developed	iii, 8	iii, 6	i, 8–9	i, 6	14	1.7	No
T. huapingensis	Whole body	10.4–14.3	27.6–30.8	–	Degenerated	iii, 8–9	iii, 5	i, 9–10	ii, 5–6	16	1.71–1.88	No
T. langpingensis	Absent	2.7–5.9	30.6–34.5	–	–	iii, 7–8	iii, 5–6	i, 10–11	ii, 6	14	1.55	Yes
T. longipectoralis	Whole body	11.8–16.4	21.2–25.3	4 + 35	Degenerated	iii, 8	iii, 5–6	i, 9–10	i, 6	14–15	1.4	Yes
T. longliensis	Whole body	9.5–11.5	31.4–37.5	4 + 38	Developed	iii, 8	iii, 5	i, 10	i, 6	15–16	2.43	Yes
T. luochengensis	Whole body	7.5–8.6	18.4–21.3	4 + 33–34	Degenerated	iii, 8	ii, 6	i, 10	i, 6	16–17	1.6	No
T. macrocephala	Whole body	3.6–8.0	22.9–25.8	–	Degenerated	iii, 7–9	iii, 5–6	i, 9–11	i, 6	15–17	1.95	Yes
T. nandanensis	Whole body	11.1–21.3	24.4–27.8	4 + 36	Degenerated	iv, 8	iv, 5	i, 9–10	i, 6	14–16	1.69	No
T. nanpanjiangensis	Whole body	12.0–16.5	30.3–34.5	4 + 38	Degenerated	iii, 7–8	ii, 5	i, 9–10	i, 6	16	1.7–2.3	No
T. nasobarbatula	Whole body	9.1–13.3	27.0–33.3	4 + 36	Degenerated	iii, 8	iii, 5	i, 9	i, 6	15	1 .4–1.8	Yes
T. panzhouensis	Whole body	7.0–11.0	22.1–31.3	4 + 35	Degenerated	iv, 7–8	iii, 5	i, 11	ii, 7	16	2.2	No
T. posterodorsalus	Absent	Absent	–	–	–	iii, 6	ii, 4	i, 13	i, 5	15	3.33	No
T. qingzhenensis	Whole body	2.1–4.4	25.1–30.4	4 + 36	Degenerated	iii, 7–8	iii, 5	i, 8–9	i, 5	14	2.25	No
T. qini	Absent	Absent	–	4 + 34–35	–	ii, 8	ii, 5	i, 10	i, 6	14–16	2.36	Yes
T. qiubeiensis	Absent	Absent	–	4 + 35	Degenerated	iii, 7	iii, 5	i, 7–9	ii, 5	14–15	2.00–2.80	Yes
T. rongduensis	Whole body	7.2–14.7	24.1–28.6	4 + 39	Degenerated	iv, 9	iii, 5	i, 10	ii, 7	16	1.62	No
T. rosa	Absent	Absent	–	–	–	iii, 9	iii, 6	i, 12	i, 7	14	2.3	Yes
T. sanduensis	Whole body	11.9–15.4	31.2–40.2	4 + 37	Degenerated	ii, 8–9	i, 5	i, 8–9	i, 5	17–18	1.58	No
T. shilinensis	Absent	Absent	–	–	Degenerated	iii, 7	iii, 5	i, 8–10	i, 6	14	2.47	No
T. tianeensis	Whole body	3.0–5.9	21.3–25.6	4 + 35	Degenerated	iii, 6–7	iii, 6	i, 8–9	i, 5–6	15–16	1.84	No
T. tianlinensis	Absent	Absent	Absent	–	Degenerated	iv, 8–9	iii, 6	i, 10	i, 6	15–16	1.55	Yes
T. tianxingensis	Whole body	4.2–6.7	17.4–24.0	4 + 38	Developed	iii, 8	ii, 5	i, 9	i, 5	16	2	No
T. wudangensis	Whole body	5.1–6.5	33.1–35.8	4 + 34	Degenerated	iii, 7	iii, 5	i, 8	i, 5	14	2.56	No
T. xiangshuingensis	Whole body	7.5	32.3	–	Degenerated	iii, 6	iii, 5	i, 9	i, 6	14	1.9	No
T. xiangxiensis	Absent	Absent	–	–	Developed	iii, 8	iii, 6	i, 11	i, 6	16	2.37	Yes
T. xichouensis	Whole body	Absent	–	4 + 36	Developed	iii, 8	ii, 6	i, 9–10	i, 5–6	16	2.8	Yes
T. xuanweiensis	Absent	Absent	–	–	Well developed	iii, 7–8	iii, 5	i, 10–12	i, 7–8	17–18	2.02	Yes
T. yaluwang	Whole body	4.6–6.1	24.3–26.0	4 + 36	Degenerated	iii, 7	iii, 5	i, 9	i, 5–6	14	2.5	Yes
T. yunnanensis	Whole body	7.2–8.3	27.0–27.8	–	Degenerated	iii, 7	iii, 5	i, 9–10	i, 7	15–16	1.8–2.1	No
T. zhenfengensis	Whole body	7.1–16.7	22.2–34.5	4 + 36	Degenerated	iii, 7	iii, 5	i, 9	ii, 5–7	14–15	1.6–2.0	No
T. ziyunensis	Whole body	2.4–4.9	22.3–26.2	4 + 35	Degenerated	iii, 8	iii, 5	i, 10	i, 6	16	2.34	Yes
T. xiuwenensis sp. nov.	Absent	4.6–6.7	31.8–40.8	4 + 37	Degenerated	iii, 8	iii, 6	i, 9	i, 6	14–15	2.1–2.5	No

2024). The cave-dwelling species of *Triplophysa* evolved and adapted to the extreme conditions of karst caves, with its species diversity and morphological variation closely linked to environmental changes and the specific ecological conditions of their habitats. Therefore, these species hold significant ecological and scientific value.

Morphological comparisons

Comparative data for the following species were acquired by specimen examination:

Triplophysa anlongensis (n = 3): China: Guizhou: Anlong County: Xinglong Town: GZNUSLS202501337–GZNUSLS202501339.

Triplophysa baotianensis (n = 3): China: Guizhou: Panzhou City: Baotian Town: GZNUSLS202201086–GZNUSLS202201088.

Triplophysa cehengensis (n = 3): China: Guizhou: Ceheng County: Rongdu Town: GZNUSLS202407006–GZNUSLS202407008.

Triplophysa guizhouensis (n = 10): China: Guizhou: Huishui County: Baijin Town: GZNUSLS202502020–GZNUSLS202502029.

Table 5. Kimura-2-Parameter genetic distance (in %) among 28 cave species of *Triplophysa* based on Cyt *b* gene sequences.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
1	*T. xiuwenensis* sp. nov.																											
2	*T. anlongensis*	18.0																										
3	*T. baotianensis*	17.5	7.5																									
4	*T. cehengensis*	16.6	11.2	12.5																								
5	*T. erythraea*	12.7	16.9	16	17.5																							
6	*T. guizhouensis*	10.4	17.2	16.8	15.6	11.8																						
7	*T. huapingensis*	17.9	11.2	11.8	6.5	17.8	17.1																					
8	*T. langpingensis*	16.7	16.7	16.4	16.4	18.8	17.4	17.2																				
9	*T. longliensis*	10.4	17.2	16.8	16.1	12.1	2.8	17.4	17.6																			
10	*T. macrocephala*	11.7	18.0	18.5	17.8	13.3	10.5	17.4	18.4	10.4																		
11	*T. nasobarbatula*	11.3	18.1	18.4	17.6	13.4	10.4	17.2	17.5	10.2	0.7																	
12	*T. nandanensis*	11.4	19.5	19.7	18.8	13.9	11.0	18.8	19.4	11.5	5.1	5.2																
13	*T. nanpanjiangensis*	17.8	8.9	9.4	11.6	16.7	17.3	11.4	17.4	16.3	17.8	17.7	18.5															
14	*T. panzhouensis*	17.7	7.7	8.3	10.9	15.5	16.4	11.0	17.0	14.8	17.4	17.5	18.4	3.3														
15	*T. qingzhenensis*	2.2	18.2	17.7	16.5	12.7	10.0	17.2	16.6	10.3	11.0	10.6	11.3	17.3	17.7													
16	*T. qini*	10.3	17.2	18.1	16.1	11.9	5.5	17.7	18.4	5.7	10.1	10.2	11.5	17.2	16.5	9.9												
17	*T. qiubeiensis*	16.2	16.9	16.4	16.6	15.6	14.8	17.0	16.8	15.5	16.7	16.2	16.9	16.4	16.5	15.9	15.4											
18	*T. rongduensis*	17.7	4.2	7.2	10.5	16.8	15.7	10.2	16.1	16.4	17.8	17.5	18.6	9.5	8.9	17.8	16.9	16.1										
19	*T. rosa*	2.4	18.2	17.7	16.8	13.1	10.2	18.1	16.7	10.6	11.3	11.0	11.8	18.2	18.3	1.5	10.3	16.2	18.1									
20	*T. sanduensis*	10.9	17.4	17.0	16.1	12.4	2.9	17.4	17.6	0.5	10.2	10.1	11.6	16.8	15.3	10.3	5.8	15.7	16.7	10.6								
21	*T. tianeensis*	12.0	19.8	19.7	19.4	13.2	11.4	19.1	19.2	12.0	5.4	5.5	1.8	18.7	18.4	11.2	11.2	16.9	19.5	12.0	12.1							
22	*T. tianxingensis*	14.6	18.5	17.8	19.2	15.9	16.1	18.3	16.9	16.3	18.3	17.6	17.5	18.5	17.9	15.3	15.3	12.6	18.0	16.2	16.1	17.6						
23	*T. wudangensis*	2.4	17.9	17.2	16.5	12.7	10.3	17.3	16.4	10.5	11.4	11.0	11.7	17.8	18.1	1.7	10.5	16.4	17.8	1.5	10.6	11.7	15.7					
24	*T. xiangxiensis*	9.1	16.9	17.6	16.8	13.0	9.0	18.2	17.2	8.4	9.1	9.0	10.7	16.6	16.4	9.7	6.4	16.3	16.5	9.8	8.7	10.0	14.8	10.1				
25	*T. xuanweiensis*	13.4	17.4	16.7	17.3	13.5	13.1	17.3	16.2	13.3	12.8	12.7	13.1	16.3	16.1	13.0	12.8	13.6	17.2	13.1	13.3	13.4	14.9	12.5	12.8			
26	*T. yaluwang*	10.2	17.1	16.1	16.0	11.6	1.4	16.6	17.3	2.5	10.2	10.1	10.6	16.8	15.6	9.7	5.6	14.6	15.8	10.1	2.6	11.1	15.1	9.9	9.0	12.6		
27	*T. zhenfengensis*	17.9	4.2	7.7	10.6	16.8	16.3	10.4	16.3	17.1	18.3	18.0	19.2	9.5	9.1	18.1	17.3	16.1	1.0	18.3	17.3	19.7	18.5	18.1	16.8	17.2	16.4	
28	*T. ziyunensis*	2.4	17.9	17.2	16.5	12.4	10.6	17.6	15.9	10.9	11.1	10.7	11.6	18.1	18.4	1.7	10.5	15.9	17.8	1.5	10.9	11.5	15.5	1.4	10.1	12.1	10.5	18.1

Triplophysa huapingensis (n = 13): China: Guangxi: Baise City: Leye County: Chaoyang Town: GZNUSLS210702498–GZNUSLS210702510.

Triplophysa langpingensis (n = 1): China: Guangxi: Baise City: Tianlin County: Langping Town: GZNUSLS202108205.

Triplophysa longliensis (n = 3): China: Guizhou: Longli County: Basheng Township: GZNUSLS022072266–GZNUSLS022072268.

Triplophysa nasobarbatula (n = 11): China: Guizhou: Libo County: Maolan Town: GZNUSLS202001339–GZNUSLS202001349.

Triplophysa nanpanjiangensis (n = 17): China: Yunnan: Qujing City: Zhanyi District: GZNUSLS202407034–GZNUSLS202407050.

Triplophysa panzhouensis (n = 4): China: Guizhou: Panzhou City: Hongguo Town: GZNUSLS202304280–GZNUSLS202304283.

Triplophysa qingzhenensis (n = 15): China: Guizhou: Guiyang City: Qingzhen City: GZNUSLS202110074–GZNUSLS202110088.

Triplophysa qiubeiensis (n = 5): China: Yunan: Qiubei County: Shuanglongying Town: GZNUSLS202109028–GZNUSLS202109032.

Triplophysa rongduensis (n = 5): China: Guizhou: Ceheng County: Rongdu Town: GZNUSLS202407001–GZNUSLS202407005.

Triplophysa rosa (n = 5): China: Chongqing City: Wulong District: Jiangkou Town: GZNUSLS202105001–GZNUSLS202105005.

Triplophysa sanduensis (n = 4): China: Guizhou: Sandu County: Zhonghe Town: GZNUSLS202201082–GZNUSLS202201085.

Triplophysa tianxingensis (n = 1): China: Yunan: Guangnan County: Zhulin Town: GZNUSLS202307046.

Triplophysa wudangensis (n = 3): China: Guizhou: Guiyang City: Wudang District: GZNUSLS202304284, GZNUSLS202410001–GZNUSLS202410002.

Triplophysa xiangshuingensis (n = 2): China: Yunan: Kunming City: Shilin County: Lufu Town: GZNUSLS202304270–GZNUSLS202304271.

Triplophysa yunnanensis (n = 6): China: Yunnan: Yiliang County: Jiuxiang Yi and Hui Ethnic Township: GZNUSLS202304260–GZNUSLS202304265.

Triplophysa zhenfengensis (n = 5): China: Guizhou: Zhengfeng County: Longchang Town: GZNUSLS202311039–GZNUSLS202311043.

The data for the following species were obtained from literature: *T. aluensis* (Li and Zhu 2000), *T. anshuiensis* (Wu et al. 2018a), *T. erythraea* (Huang et al. 2019), *T. fengshanensis* (Lan et al. 2013), *T. flavicorpus* (Yang et al. 2004), *T. gejiuensis* (Chu and Chen 1979), *T. longipectoralis* (Zheng et al. 2009), *T. luochengensis* (Li et al. 2017a), *T. macrocephala* (Yang et al. 2012), *T. nandanensis* (Lan et al. 1995), *T. posterodorsalus* (Ran et al. 2006), *T. qini* (Deng et al. 2022), *T. shilinensis* (Chen et al. 1992), *T. tianeensis* (Chen et al. 2004), *T. tianlinensis* (Li et al. 2017b), *T. xiangxiensis* (Yang et al. 1986), *T. xichouensis* (Liu et al. 2017), *T. xuanweiensis* (Lu et al. 2022), *T. yaluwang* (Lan et al. 2024), *T. ziyunensis* (Lan et al. 2024).

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