ZOOLOGICAL RESEARCH

A new species of *Triplophysa* (Cypriniformes, Nemacheilidae) from Weihe River in Gansu Province, China

DEAR EDITOR,

A new species of Tibetan loach, *Triplophysa weiheensis* **sp. nov.,** is described from the Weihe River in Gansu Province, China, based on morphological and molecular analyses. The new species can be distinguished from all known congeners by a unique combination of the following characters: scaleless; snout abruptly sloping downward, anterior to anterior nostril; lower jaw crescentic, not sharp; body without obvious mottling; lateral line interrupted on posterior trunk at pelvic-fin distal extremity; caudal-peduncle length 2.0–2.7 times its depth; branched rays of pectoral fin 10–11; branched rays of pelvic fin 5–6; inner gill rakers on 1st gill arch 14–16; vertebrae 4+34–36; intestine with 6–7 loops, length *ca.* 1.8 times SL (*n*=3); bony capsule of air bladder small and thin; posterior chamber of air bladder absent.

Species of the genus *Triplophysa* Rendahl 1933 are the most common fish among the nemacheilids found in the Qinghai-Tibet Plateau (QTP) (Wu & Wu, 1992; Zhu, 1989). These fish are found in almost all water bodies in the region, with new species of *Triplophysa* still being reported (Huang et al., 2019; Liu et al., 2017; Wu et al., 2018; Yang et al., 2016). As a result, a total of 147 valid species of *Triplophysa* have been recorded to date (Froese & Pauly, 2019).

Weihe River is a tributary of the Yellow River and originates from the southern part of Gansu Province (Figure 1A). Previous studies have reported that southern Gansu is a hotspot area for *Triplophysa*, with an extensive distribution of species across the river systems (Feng et al., 2017a, 2017b, 2019b). Until now, 17 species of *Triplophysa* have been reported from the Yellow River system (Chen et al., 1987; Ding, 1994; Feng et al., 2017a; Wu & Wu, 1992; Zhu, 1989), five of which have been recorded from Weihe River (Supplementary Table S1). Following an investigation of *Triplophysa* species from Weihe River (Figure 1A), 15 specimens superficially resembling *Triplophysa stoliczkae* Steindachner 1866 (Supplementary Figure S1) were collected and are described herein as a new species based on morphological and molecular analyses.

After euthanization (see Supplementary Methods), the left ventral fin of some specimens was removed and preserved in 95% ethanol for DNA extraction. Voucher specimens were labeled and stored in 70% ethanol. Specimens were deposited in the collection of the Northwest Institute of Plateau Biology (NWIPB), Chinese Academy of Sciences, Xining, Qinghai, China. Morphological measurements and counts followed Kottelat (1990) and Prokofiev (2007). Additional measurements are described in the Supplementary Methods. Measurements were taken with digital calipers to the nearest 0.1 mm. Previous research has reported that *T. stoliczkae* is a striking case of morphological convergence and consists of distinct lineages that are not close relatives (Feng et al., 2019a). As there is no formal taxonomic revision for *T. stoliczkae*, we treated it as a morphological species in this study but considered its different genetic lineages in phylogeny. These lineages, which were initially mistaken as *T. stoliczkae*, exhibit very similar morphology. Thus, they represent a known morphological unit in the genus *Triplophysa*. We specifically measured 61 *T. stoliczkae* specimens collected from various water systems and used principal component analysis (PCA) (Supplementary Table S2) to visualize morphological differences between *T. stoliczkae* and the new species. Furthermore, we employed a Micro CT (Quantum GX2, PerkinElmer Corporation, USA) to build a skeletal model of the new species.

DNA extraction and complete cyt *b* gene (1 140 bp) amplification were carried out, as detailed in the

Received: 09 January 2020; Accepted: 28 May 2020; Online: 12 June 2020

Foundation items: This work was supported by grants from the National Natural Science Foundation of China (31870365 and 31572258) and Sino BON-Inland Water Fish Diversity Observation Network

DOI: 10.24272/j.issn.2095-8137.2020.050

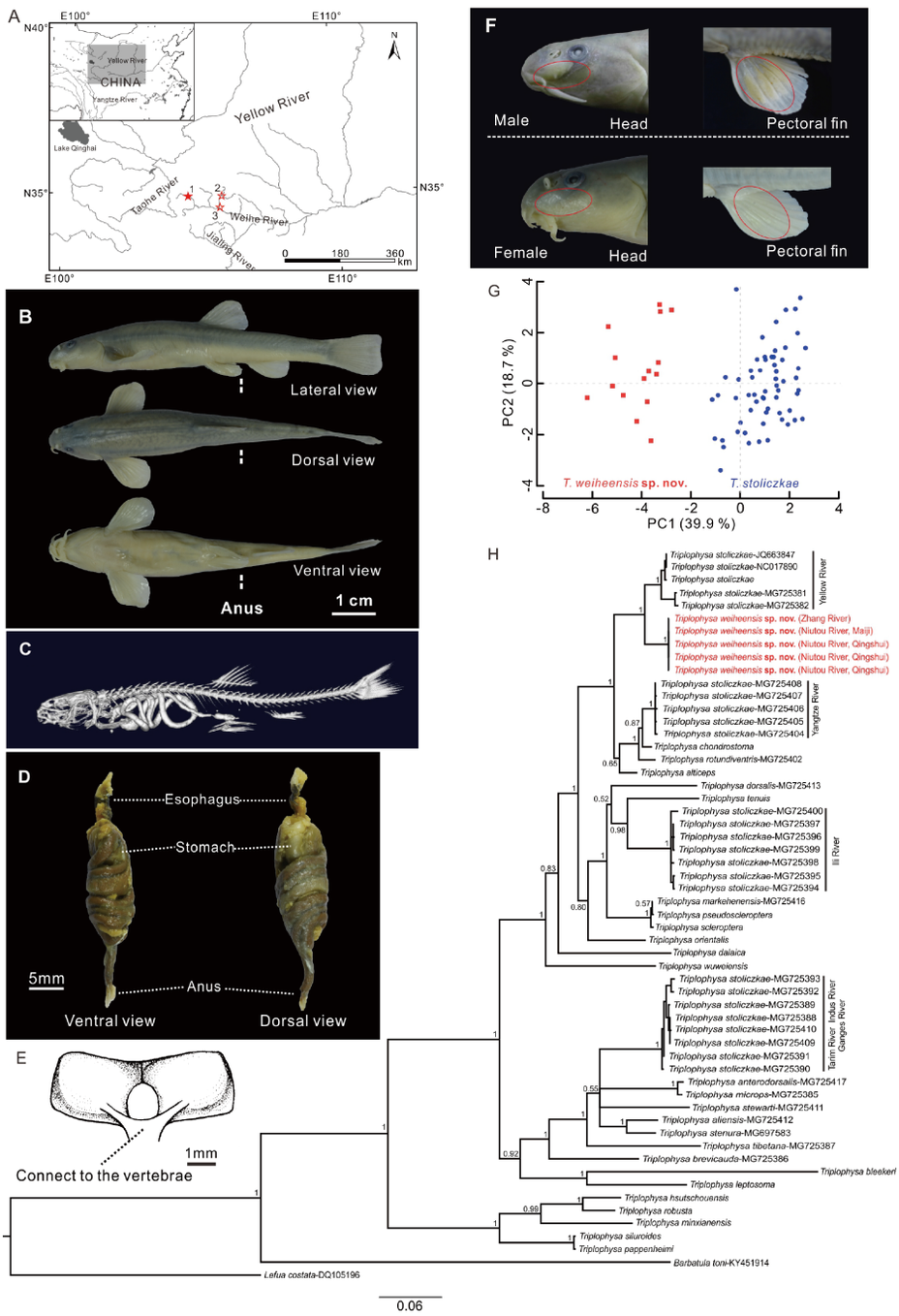

Figure 1 Collection site, morphological characters and phylogenetic position of *Triplophysa weiheensis* sp. nov.

A: Map showing sampling sites of *Triplophysa weiheensis* **sp. nov.** (star symbol, solid star for type locality). 1: Zhang River, at Chenjiamo Village, Zhangxian County; 2: Niutou River, at Hongbao Town, Qingshui County; 3: Niutou River, at Maiji District, Tianshui City. B: Lateral view, dorsal view, and ventral view of *Triplophysa weiheensis* **sp. nov.**, NWIPB 1505189, holotype, 65.8 mm SL. C: Micro CT graph of skeletal system of *Triplophysa weiheensis* **sp. nov.** NWIPB 1505183, paratype, 64.1 mm SL. D: Structure of alimentary canal, *Triplophysa weiheensis* **sp. nov.**, NWIPB 1505185, paratype, 65.5 mm SL. E: Dorsal view of bony capsule of air bladder, *Triplophysa weiheensis* **sp. nov.**, NWIPB 1505185, paratype, 65.5 mm SL. F: Characters of sexual dimorphism of *Triplophysa weiheensis* **sp. nov.** Characters are highlighted in red circles. G: Morphological comparison between *Triplophysa weiheensis* **sp. nov.** and *T. stoliczkae* by PCA with 13 morphometric characters (Supplementary Table S2). H: Phylogenetic relationships of *Triplophysa* species based on cyt *b* gene sequences. Posterior probabilities (PP) obtained from Bayesian analyses are indicated on branches.

Supplementary Methods. Polymerase chain reaction (PCR) products were sequenced from both directions using an ABI PRISM 3700 sequencing system. Finally, 20 new cyt *b* sequences of *Triplophysa* were obtained, including five sequences from the new species (Supplementary Table S3). To assess the phylogenetic status of the new species, 37 additional cyt *b* sequences of *Triplophysa* and outgroups (*Barbatula toni* and *Lefua costata*) were retrieved from GenBank (Supplementary Table S3), as per previous studies (Feng et al., 2017b; He et al., 2006; Wang et al., 2016). Phylogenetic analysis was performed using Bayesian inference (BI) (Supplementary Methods). Additionally, Kimura's 2-parameter (K2P) genetic distances (Kimura, 1980) were estimated using MEGA 6.0 (Tamura et al., 2013).

Taxonomic account

Triplophysa weiheensis **sp. nov.** Feng, Zhang, Tong, Zhou et Zhao (Figure 1B–F; Table 1)

Holotype: NWIPB 1505189, 65.8 mm SL (standard length); Zhang River, a tributary of Weihe River, at Chenjiamo Village, Zhangxian County, Dingxi City, Gansu Province, China (N34°48′, E104°31′; elevation 1 768 m a.s.l.), collected by Chenguang Feng, Chao Tong, and Kai Zhao on 12 May 2015.
Paratypes: NWIPB 1505181–183, 3 ex. (examined specimens), 64.1–77.1 mm SL; 1505185–188, 4 ex., 59.7–68.0 mm SL, same collection information as holotype. NWIPB 1505922, 53.6 mm SL, Niutou River, a tributary of Weihe River, at Maiji District, Tianshui City, Gansu Province, China (N34°34′, E105°57′; elevation 1 360 m a.s.l.), on 21 May 2015. NWIPB 1505985–988, 4 ex., 56.4–65.9 mm SL; 1505990, 48.3 mm SL; 1505992, 60.5 mm SL, Niutou River, a tributary of Weihe River, at Hongbao Town, Qingshui County, Tianshui City, Gansu Province, China (N34°42′, E106°1′; elevation 1 434 m a.s.l.), on 22 May 2015.
Etymology: The specific epithet *weiheensis* is derived from Weihe River (渭河 in Chinese, type locality) with the Latin suffix -ensis.

Table 1 Morphometric data of *Triplophysa weiheensis* sp. nov.

Morphometric character	Holotype	Paratype (Range *n*=14)	Mean	*SD*
Standard length (mm)	65.8	48.3–77.1		
Percentage of standard length (%)				
Body depth	18.1	16.2–19.2	18.2	0.9
Body width	16.5	14.1–17.6	16.0	1.0
Head length	17.9	16.3–21.5	19.6	1.7
Dorsal-fin length	16.9	14.1–20.6	17.7	1.9
Pelvic-fin length	14.2	12.2–14.7	13.6	0.7
Pectoral-fin length	18.2	15.1–20.5	17.3	1.8
Anal-fin length	15.2	13.1–17.1	15.0	1.2
Caudal-fin length	21.1	16.4–22.4	19.3	1.6
Predorsal length	49.5	47.9–55.1	50.9	2.2
Preanus length	68.5	61.9–71.0	67.3	2.6
Preanal length	72.2	63.5–73.4	69.8	2.5
Prepelvic length	56.7	51.1–58.4	54.6	2.4
Prepectoral length	19.7	16.9–21.9	19.8	1.3
Caudal-peduncle length	23.1	19.4–24.3	22.0	1.3
Caudal-peduncle depth	10.3	8.7–10.7	9.7	0.6
Pectoral-pelvic distance	32.5	31.6–38.8	35.2	2.4
Pectoral-anal distance	48.4	46.1–54.9	50.3	2.9
Pelvic-anal distance	15.5	12.7–18.1	15.3	1.7
Head length (mm)	11.8	10.0–14.0		
Percentage of head length (%)				
Head depth	67.4	59.2–72.2	63.5	4.4
Head width	81.9	68.1–91.3	79.7	8.0
Snout length	35.6	31.2–38.8	35.0	2.3
Eye length	19.4	16.6–22.9	18.7	1.7
Interorbital width	34.7	28.6–43.0	36.2	4.0
Postorbital length	50.6	41.4–53.0	48.4	3.1
Inner rostral barbel length	28.9	15.1–28.1	21.6	3.6
Outer rostral barbel length	27.0	16.7–27.5	22.9	2.9
Maxillary barbel length	31.4	18.7–28.9	23.4	3.2

Diagnosis: *Triplophysa weiheensis* **sp. nov.** can be distinguished from all known congeners by a combination of the following characters: (1) scaleless; (2) snout abruptly bending down before anterior nostril; (3) snout length shorter than postorbital length; (4) lower jaw crescentic, not sharp; (5) body without obvious mottling; (6) lateral line interrupted on posterior trunk behind vertical line of pelvic-fin distal extremity; (7) caudal-peduncle length 2.0–2.7 times its depth; (8) caudal fin slightly emarginate; (9) branched rays of pectoral fin 10–11; (10) pelvic-fin insertion behind vertical line through dorsal-fin origin, distal fin tip attaining anal-fin origin when adpressed, branched rays 5–6; (11) inner gill rakers on 1st gill arch 14–16; (12) vertebrae 4+34–36; (13) intestine with 6–7 loops, length *ca*. 1.8 times SL; (14) posterior chamber of air bladder absent, bony capsule of air bladder small and thin.

Description: Morphometric and meristic data are given in Table 1 and Supplementary Table S4, respectively.

Body thick, cylindrical. Dorsal profile of body arch-like (Figure 1B). Maximum depth of body slightly greater than maximum width, occurring between pectoral and dorsal fins. Caudal peduncle laterally compressed, depth nearly uniform toward caudal-fin base, length longer than head length (HL, 101.0–133.7% of HL). Head width greater than depth. Cheeks slightly inflated, V-shaped outline in ventral view. Snout obtuse, sloping downward anterior to anterior nostril (Figure 1B, C). Snout length shorter than postorbital length. Anterior and posterior nostrils close together. Valves around anterior nostrils, but not around posterior. Eyes small, dorsolaterally in head. Interorbital space wide (28.6–43.0% of HL, 155.6–239.0% of eye length). Mouth inferior (Supplementary Figure S2). Lips thick and well-developed with furrows; lower lip continuous with shallow median incision. Lower jaw crescentic, uncovered by lower lip. Three pairs of barbels thick, moderately short; inner rostral barbels almost reaching corner of mouth; outer rostral barbels horizontally reaching posterior nostril; maxillary barbels horizontally reaching mid-point of eyes.

Fins short (Figure 1B). Dorsal fin rounded distally, originating anterior to pelvic-fin origin; dorsal-fin origin near midway between tip of snout and caudal-fin base or slightly nearer to caudal-fin base. Pectoral fin short, not reaching halfway point to pelvic-fin base. Pelvic fin reaching past anus, distal fin tip attaining anal-fin origin when adpressed. Anal fin rounded distally, just posterior to anus. Caudal fin slightly emarginate.

Skin scaleless. Lateral line tapering, interrupted on posterior trunk at pelvic-fin distal extremity; few lateral line pores close to caudal-fin base. Stomach U-shaped, expanded. Intestine long, with 6–7 loops (Figure 1D and Supplementary Figure S3; one hidden loop surrounded by 5–6 loops). Bony capsule of air bladder small and thin, closed laterally; posterior chamber of air bladder absent (Figure 1E).

Sexual dimorphism: In mature males, unbranched and four outer branched pectoral-fin rays thickened, covered with breeding tubercles on dorsal surface. Small breeding tubercles also present on both sides of head in liber apophyses extending from anterior lower margin of orbit to base of outer rostral barbel. These characters do not occur in females (Figure 1F).

Color pattern: In life: silver-gray base in individuals. In 70% EtOH: ground color of body brown dorsally and laterally, becoming yellowish ventrally. Fin membranes hyaline and slightly gray, with dark-brown faint spots on both sides of dorsal and caudal fins and upper side of pectoral fin. Body without obvious mottling. Peritoneum silvery with scattered dark melanophores becoming dense at vertebral column.

Ecology: Specimens were collected from flowing streams with gravel or sandy substrates at 1 360–1 768 m a.s.l. (Supplementary Figure S4). River water became muddy at about 0.5 m deep. Periphytic algae, sand, and insect larvae (e.g., chironomids) were found in the stomach of specimens. Other species collected with *Triplophysa weiheensis* **sp. nov.** included *T. dalaica* Kessler, *T. minxianensis* Wang and Zhu, *Pseudorasbora parva* Temminck and Schlegel, and *Gobio huanghensis* Lo, Yao et Chen.

Distribution: *Triplophysa weiheensis* **sp. nov.** is known only from the upper reaches of the Weihe River (Figure 1A).

Comparisons: Intestine and posterior chamber of air bladder are two important characters in the morphological taxonomy of the genus *Triplophysa* (He et al., 2006; Wu and Wu, 1992; Zhu, 1989). *Triplophysa weiheensis* **sp. nov.** differs from most *Triplophysa* species based on the combination of intestine screw shaped and posterior chamber of air bladder absent (Figure 1D, E), and can be classified into the same category as *T. stoliczkae* Steindachner, *T. tanggulaensis* Zhu, *T. crassilabris* Ding, *T. alticeps* Herzenstein, *T. cakaensis* Cao and Zhu, *T. chondrostoma* Herzenstein, *T. stenura* Herzenstein, *T. rotundiventris* Wu and Chen, *T. nujiangensa* Chen et al., and *T. daochengensis* Wu et al. (Chen et al., 2004; Ding, 1994; Wu & Wu, 1992; Wu et al., 2016; Zhu, 1989). Among these, the new species is most similar to *T. stoliczkae*.

Triplophysa weiheensis **sp. nov.** can be distinguished from *T. stoliczkae* by a combination of the following characters: lower jaw crescentic, not sharp (vs. spade-like, sharp); lateral line incomplete (vs. complete); vertebrae 4+34–36 (vs. 4+38–41); intestine *ca.* 1.8 times as long as SL (vs. 1.0–1.3 times); body without obvious mottling (vs. with mottling). The PCA results also indicated that the new species differs from *T. stoliczkae* in its whole morphology (Figure 1G) and the two species can be clearly separated from each other by PC1. Five morphometric measurements, including head depth/head length, interorbital width/head length, body depth/standard length, caudal peduncle depth/standard length, and postorbital length/head length, were highly correlated with and substantially contributed to PC1 (Supplementary Figure S5A, B). The five morphometric measurements were greater in the new species than in *T. stoliczkae* (Supplementary Figure S5C), suggesting a comparatively stubby body for the new species relative to *T. stoliczkae*.

Additionally, *Triplophysa weiheensis* **sp. nov.** can be distinguished from *T. tanggulaensis* by the following

characters: pelvic fin i, 5–6 (vs. i, 7–9); inner gill rakers on 1st gill arch 14–16 (vs. 10–13); vertebrae: 4+34–36 (vs. 4+37–38); intestine with 6–7 loops (vs. 3–4 loops). The new species can be distinguished from *T. crassilabris* by the following characters: pectoral fin i, 10–11 (vs. i, 8–9); pelvic fin i, 5–6 (vs. i, 7–8); inner gill rakers on 1st gill arch 14–16 (vs. 8–9); intestine with 6–7 loops (vs. 4–5 loops). The new species can be distinguished from *T. alticeps* by the following characters: intestine with 6–7 loops (vs. 3–4 loops); bony capsule of air bladder small and thin (vs. large and inflated). The new species can be distinguished from *T. cakaensis* by the following characters: snout abruptly sloping downward anterior to nostril (vs. snout gently sloping downward); lateral line interrupted on posterior trunk at pelvic fin distal extremity (vs. ending above pectoral fin); body without obvious mottling (vs. with mottling). The new species can be distinguished from *T. chondrostoma* by the following characters: lower jaw crescentic, not sharp (vs. spade-like, sharp); bony capsule of air bladder small and thin (vs. large and inflated); caudal-peduncle length 2.0–2.7 times its depth (vs. 3.2–3.8 times); body without obvious mottling (vs. with distinct mottling). The new species can be distinguished from *T. stenura* by the following characters: caudal peduncle depth nearly uniform towards caudal-fin base (vs. tapered); lateral line interrupted (vs. complete); intestine *ca.* 1.8 times as long as SL (vs. 1.0–1.3 times). The new species can be distinguished from *T. rotundiventris* by the following characters: caudal-peduncle length 2.0–2.7 times its depth (vs. 3.0–3.6 times); intestine *ca.* 1.8 times as long as SL (vs. 2.0–2.5 times); body without obvious mottling (vs. with mottling). The new species can be distinguished from *T. nujiangensa* by the following characters: vertebrae: 4+34–36 (vs. 4+38–39); intestine with 6–7 loops (vs. 3 loops); pelvic fin reaching past anus (vs. not reaching). The new species can be distinguished from *T. daochengensis* by the following characters: intestine with 6–7 loops (vs. 3 loops); pelvic fin reaching past anus (vs. not reaching); caudal fin slightly emarginate (vs. deeply emarginate).

Molecular analysis: Our results were in accordance with previous study, which suggested that *T. stoliczkae* consists of distinct lineages that are not close relatives (Feng et al., 2019a; Figure 1H). Phylogenetic analysis recovered the monophyly of *Triplophysa weiheensis* **sp. nov.** with strong support and showed that it was close to the *T. stoliczkae* population from the Yellow River system. The K2P genetic distance between *Triplophysa weiheensis* **sp. nov.** and *T. stoliczkae* was 3.7%, which is larger than that between several pairs of recognized species (Supplementary Table S5). These analyses suggest that *Triplophysa weiheensis* **sp. nov.** is a separately evolving lineage and genetic differences from its sister lineage in the genus *Triplophysa* may have reached species-level differentiation. Morphologically, this distinct phylogenetic lineage differed from all described *Triplophysa* species. Thus, based on an integrative taxonomic approach (Wu et al., 2019), the specimens collected from Weihe River are designated as a distinct species.

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 prefixhttp://zoobank.org/.

Publication LSID:

urn:lsid:zoobank.org:pub:4AF9CC56-E859-46E5-B13C-D739D76F01F1.

Triplophysa weiheensis LSID:

urn:lsid:zoobank.org:act:C89F2F7D-7FBA-4C01-90DB-48E3826C75D7.

SCIENTIFIC FIELD SURVEY PERMISSION INFORMATION

The field surveys in the Weihe River area in Gansu Province were approved by the Department of Fisheries of Gansu Province, China.

SUPPLEMENTARY DATA

Supplementary data to this article can be found online.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

K.Z. and C.G.F. conceived and designed the study. K.Z., C.G.F., and C.T. collected specimens in the field. C.G.F., Y.Z., B.Z.Z., X.H.L., Y.T.T., and W.Z.S. performed the experiments and analyzed the data. C.G.F., Y.Z., C.T., and K.Z. prepared the manuscript. All authors read and approved the final version of the manuscript.

ACKNOWLEDGMENTS

We extend our sincere gratitude to the editors and anonymous reviewers for their constructive comments, which have helped to improve the manuscript. We express our gratitude to Wen-Jing Li and Xiao-Cheng Chen for their assistance in the specimen room of NWIPB. We thank Drs. Tao Qin and Yun-He Wu from the Kunming Institute of Zoology for their kind help. We also give our thanks to the staff from the Department of Fisheries of Gansu Province.

Chen-Guang Feng[1,2,#], Yu Zhang[1,3,#], Chao Tong[1,4], Bing-Zheng Zhou[1,3], Xiao-Hui Li[1,3], Yong-Tao Tang[1,5], Wen-Zhu Song[1], Kai Zhao[1,*]

[1] *Key Laboratory of Adaptation and Evolution of Plateau Biota*, *Laboratory of Plateau Fish Evolutionary and Functional Genomics*, *and Qinghai Key Laboratory of Animal Ecological Genomics*, *Northwest Institute of Plateau Biology*, *Chinese Academy of Sciences*, *Xining* 810008, *China*
[2] *School of Ecology and Environment*, *Northwestern Polytechnical University*, *Xi'an* 710072, *China*
[3] *University of Chinese Academy of Sciences*, *Beijing* 100049, *China*
[4] *Biology Department*, *University of Pennsylvania*, *Philadelphia*, *Pennsylvania* 19104, *USA*
[5] *College of Fisheries*, *Henan Normal University*, *Xinxiang* 453007, *China*
[#]Authors contributed equally to this work
*Corresponding author, E-mail: zhaokai@nwipb.cas.cn

REFERENCES

Chen JX, Xu TQ, Fang SM, Song SL, Wang XT. 1987. Fishes in Qinling Mountain Area. Beijing: Science Press, 19–29. (in Chinese)

Chen XY, Cui GH, Yang JX. 2004. A new fish species of genus *Triplophysa* (Balitoridae) from Nu Jiang, Yunnan, China. *Zoological Research*, **25**(6): 504−509. (in Chinese)

Ding RH. 1994. The Fishes of Sichuan, China. Chengdu: Sichuan Publishing House of Science and Technology, 63–94. (in Chinese)

Feng CG, Tang YT, Liu SJ, Tian F, Zhang CF, Zhao K. 2019a. Multiple convergent events created a nominal widespread species: *Triplophysa stoliczkae* (Steindachner, 1866) (Cobitoidea: Nemacheilidae). *BMC Evolutionary Biology*, **19**(1): 177.

Feng CG, Tong C, Zhang RY, Li GG, Wanghe KY, Tang YT, Zhang CF, Zhao K. 2017a. Biodiversity and distribution patterns of *Triplophysa* species in the northeastern margin of the Tibetan Plateau. *Biodiversity Science*, **25**(1): 53−61. (in Chinese)

Feng CG, Wu YJ, Tian F, Tong C, Tang YT, Zhang RY, Li GG, Zhao K. 2017b. Elevational diversity gradients of Tibetan loaches: the relative roles of ecological and evolutionary processes. *Ecology and Evolution*, **7**(23): 9970−9977.

Feng CG, Zhou WW, Tang YT, Gao Y, Chen JM, Tong C, Liu SJ, Wanghe KY, Zhao K. 2019b. Molecular systematics of the *Triplophysa robusta* (Cobitoidea) complex: extensive gene flow in a depauperate lineage. *Molecular Phylogenetics and Evolution*, **132**: 275−283.

Froese R, Pauly D. 2019. FishBase. World Wide Web electronic publication. version (08/2019). www.fishbase.org.

He DK, Chen YX, Chen YF. 2006. Molecular phylogeny and biogeography of the genus *Triplophysa* (Osteichthyes: Nemacheilinae) in the Tibetan Plateau inferred from cytochrome b DNA sequences. *Progress in Natural Science*, **16**(11): 1395−1404. (in Chinese)

Huang TF, Zhang PL, Huang XL, Wu T, Gong XY, Zhang YX, Peng QZ, Liu ZX. 2019. A new cave-dwelling blind loach, *Triplophysa erythraea* sp. nov. (Cypriniformes: Nemacheilidae), from Hunan Province, China. *Zoological Research*, **40**(4): 331−336.

Kimura M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, **16**(2): 111−120.

Kottelat M. 1990. Indochinese Nemacheilines: A Revision of Nemacheiline Loaches (Pisces: Cypriniformes) of Thailand, Burma, Laos, Cambodia, and Southern Viet Nam. Münich: Verlag Dr. Friedrich Pfeil, 262.

Liu SW, Pan XF, Yang JX, Chen XY. 2017. A new cave-dwelling loach, *Triplophysa xichouensis* sp. nov. (Teleostei Nemacheilidae) from Yunnan, China. *Journal of Fish Biology*, **90**(3): 834−846.

Prokofiev AM. 2007. Materials towards the revision of the genus *Triplophysa* Rendahl, 1933 (Cobitoidea: Balitoridae: Nemacheilinae): a revision of nominal taxa of Herzenstein (1888) described within the species "*Nemachilus*" *stoliczkae* and "*N.*" *dorsonotatus*, with the description of the new species *T. scapanognatha* sp. nova. *Journal of Ichthyology*, **47**(1): 1−20.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, **30**(12): 2725−2729.

Wang Y, Shen YJ, Feng CG, Zhao K, Song ZB, Zhang YP, Yang LD, He SP. 2016. Mitogenomic perspectives on the origin of Tibetan loaches and their adaptation to high altitude. *Scientific Reports*, **6**(1): 29690.

Wu TJ, Wei ML, Lan JH, Du LN. 2018. *Triplophysa anshuiensis*, a new species of blind loach from the Xijiang River, China (Teleostei, Nemacheilidae). *ZooKeys*, **744**: 67−77.

Wu YF, Wu CZ. 1992. The Fishes of the Qinghai-Xizang Plateau. Chengdu: Sichuan Publishing House of Science & Technology. (in Chinese)

Wu YH, Suwannapoom C, Poyarkov Jr NA, Paawangkhanant P, Xu K, Jin JQ, Murphy RW, Che J. 2019. A new species of the genus Xenophrys (Anura: Megophryidae) from northern Thailand. *Zoological Research*, **40**(6): 564−574.

Wu YY, Sun ZY, Guo YS. 2016. A new species of the genus *Triplophysa* (Cypriniformes: Nemacheilidae), *Triplophysa daochengensis*, from Sichuan Province, China. *Zoological Research*, **37**(5): 290−295.

Yang HF, Li WX, Chen ZM. 2016. A new cave species of the Genus *Triplophysa* from Yunnan, China. *Zoological Research*, **37**(5): 296−300.

Zhu SQ. 1989. The Loaches of the Subfamily Nemacheilinae in China (Cypriniformes: Cobitidae). Nanjing: Jiangsu Science and Technology Publishing House. (in Chinese)