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 1^{st} 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 (1140 bp) amplification were carried out, as detailed in the

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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

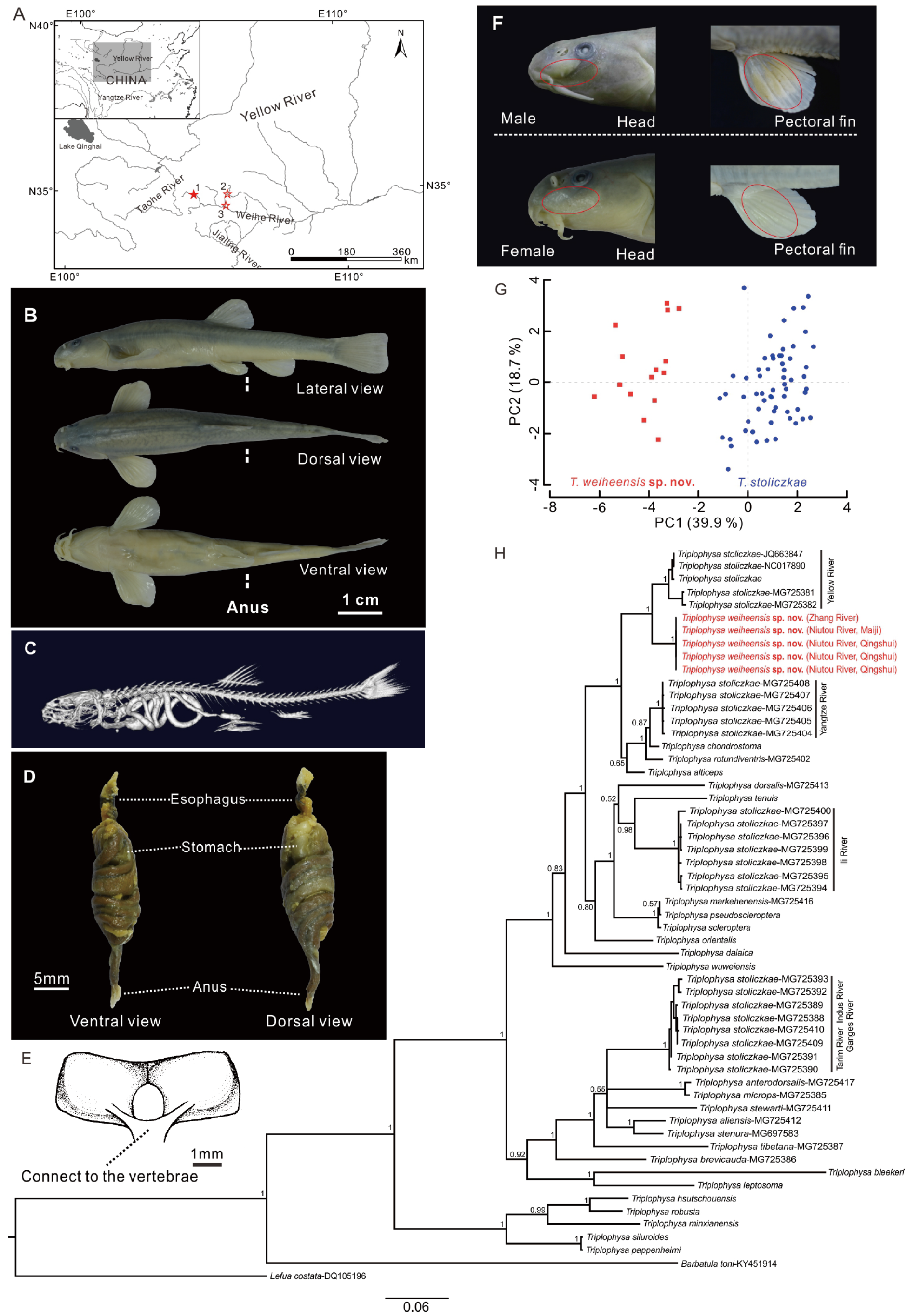

Figure 1 Collection site, morphological characters and phylogenetic position of *Triplophysa weiheensis* sp. nov.

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.

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Supplementary Materials
Supplementary Methods

Euthanization and ethics statement

After preliminary identification, collected specimens were euthanized as per Feng et al. (2019). They were placed in a dry ice box for rapid hypothermic anesthesia within about 20 s. Then, they were preserved in 70% ethanol for laboratory works. All animal experiments for this project were approved by the Ethics Committee of the Northwest Institute of Plateau Biology, Chinese Academy of Sciences [NWIPB201503018].

Additional measurements

Measurements and counts follow that of Kottelat (1990) and Prokofiev (2007). Additional measurements are the following: postorbital length is measured from the posterior margin of the orbit to the posterior end of the operculum; pectoral-pelvic distance is measured from the pectoral-fin origin to the pelvic-fin origin; the number of gill rakers was counted on the inner side of the first arch.

Amplification, sequencing and phylogenetic analysis

Total genomic DNA was extracted from fins using the standard 3-step phenol-chloroform method (Sambrook et al., 1989). The complete cyt *b* gene (1 140 bp) was amplified with the universal primer pairs L14724 (5'-GACTTGAAAAACCACCGTTG-3') and H15915 (5'-CTCCGATCTCCGGATTACAAGAC-3') (Xiao et al., 2001) in total reaction volumes of 35μL, containing approximately 100 ng of template DNA, 0.7μL of each primer (10 μmol/L), 3.5μL 10× reaction buffer, 3μL dNTPs (2.5 mmol/L each), and 1.0 U TaKaRa rTaq (TaKaRa Corp., Dalian, China). The PCR cycling profile was at 94°C for 5 min, 35 cycles of at 94°C for 30 sec, at 52°C for 30 sec and at 72°C for 1 min 30 sec, followed by 72°C for 10 min. The PCR products were sequenced from both directions using an ABI PRISM 3700 sequencing system.

The presence of cyt *b* pseudogene was checked by scanning for stop codons or indels in MEGA v6.0 (Tamura et al., 2013). Phylogenetic analysis was performed using the Bayesian inference (BI) implemented in MRBAYES 3.2.0 (Ronquist et al., 2012). The best model of DNA substitution (GTR+I+G) was identified by jModelTest 0.1.1 (Guindon and Gascuel, 2003; Posada, 2008) based on Bayesian Information Criterion (BIC) (Schwarz, 1978; Luo et al., 2010). Markov Chain Monte Carlo (MCMC) was set with one cold chain and three heated chains. Samples of trees and the parameters were drawn every 100 steps from a total of 1 000 000 MCMC generations, with the first 25% samples discarded as burn-in.

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Comparative materials

Triplophysa stoliczkae.

(The Indus river system): NWIPB 1407013–018, 6 ex., 63.7–85.2 mm SL, middle reach of Changchuan River, Rutog County, Ngari Prefecture, Tibet Autonomous Region, China; NWIPB 74–(280, 282, 284, 285, 289, 292, 294, 295), 1106009, 9 ex., 50.7–78.1 mm SL, a tributary of Lake Bangong, Rutog Town, Rutog County, Ngari Prefecture, Tibet Autonomous Region, China; NWIPB 74–(297, 312, 318), 3 ex., 67.0–80.6 mm SL, glang-chen gtsang-po, Zanda Zong, Ngari Prefecture, Tibet Autonomous Region, China.

(The Yellow river system): NWIPB 1505471–480, 10 ex., 44.51–72.69 mm SL, a tributary of Taohe River, Sigou Town, Minxian County, Gansu province, China; NWIPB 1205321, 1205322, 2 ex., 88.7–95.0 mm SL, Datong River, Haomen Town, Menyuan County, Haixi Mongolian and Tibetan Autonomous Prefecture, Qinghai Province, China; NWIPB 1707001, 1 ex., 85.1 mm SL, Huangshui River, Xihai Town, Haiyan County, Haixi Mongolian and Tibetan Autonomous Prefecture, Qinghai Province, China.

(The Ganges river system): NWIPB 1106001, 1106002, 2 ex., 104.9–112.2 mm SL, a tributary of Manasarovar Lake, Burang County, Ngari Prefecture, Tibet Autonomous Region, China; NWIPB 74–(257, 269, 267), 3 ex., 58.2–82.6 mm SL, Kongque River, Burang County, Ngari Prefecture, Tibet Autonomous Region, China.

(The Heihe river system): NWIPB 1205128, 1205134, 2 ex., 85.9–94.0 mm SL, Babao River, Zhamashi Town, Qilian County, Haixi Mongolian and Tibetan Autonomous Prefecture, Qinghai Province, China; NWIPB 1205095-097, 1205099-102, 7 ex., 65.6–101.2 mm SL, Heihe River, Longshou Town, Zhangye City, Gansu Province, China.

(The Tarim river system): NWIPB 1107083, 1 ex., 97.5 mm SL, Yarkand River, Yecheng County, Kashgar Prefecture, Xinjiang Uygur Autonomous Region, China; NWIPB 1107084, 1 ex., 73.9 mm SL, Qaraqash River, Pishan County, Kashgar Prefecture, Xinjiang Uygur Autonomous Region, China.

(The Ili river system): NWIPB 1305044, 1 ex., 98.8 mm SL, Kashi River, Nilka County, Ili Prefecture, Xinjiang Uygur Autonomous Region, China; NWIPB 1305111, 1305113-115, 4 ex., 68.8–91.0 mm SL, Kunes River, Xinyuan County, Ili Prefecture, Xinjiang Uygur Autonomous Region, China; NWIPB 1305046-048, 1305052, 1305056, 1305060, 6 ex., 56.0–102.5 mm SL, Jinghe Forest Farm, Jinghe County, Bortala Mongol Autonomous Prefecture, Xinjiang Uygur Autonomous Region, China; NWIPB 1305131, 1305141, 1305142, 3 ex., 64.0–75.4 mm SL, Tekes River, Tekes County, Ili Prefecture, Xinjiang Uygur Autonomous Region, China.

Triplophysa chondrostoma. Topotype. NWIPB 1006052–055, 4 ex., 74.4–89.2 mm SL, Caidam River, Nuomuhong Town, Dulan County, Haixi Mongolian and Tibetan Autonomous Prefecture, Qinghai Province, China.

Triplophysa tanggulaensis. NWIPB 75-061, holotype, 1 ex., 62.2 mm SL, 75-(051–054, 058, 060, 064), paratypes, 7ex., 61.3–73.2 mm SL, a hot spring between Yanshiping and T'ang-ku-la Pass, T'ang-ku-la Town, Geermu city, Haixi Mongolian and Tibetan Autonomous Prefecture, Qinghai Province, China.

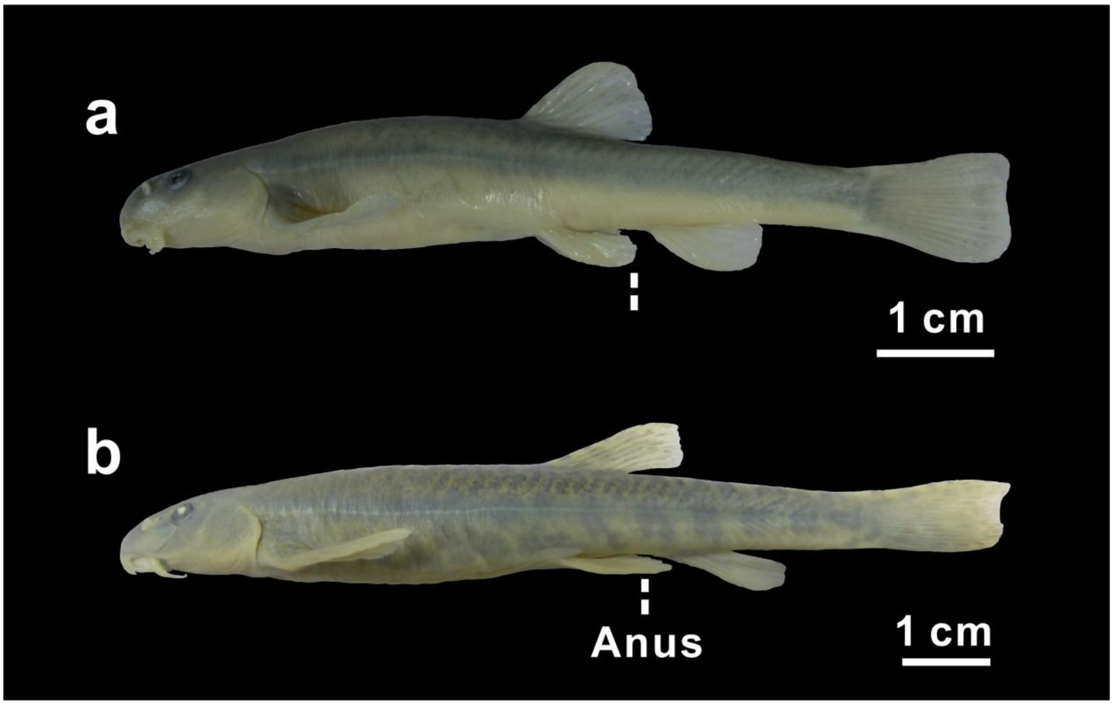
Triplophysa alticeps. Topotype. NWIPB 1206001–004, 4 ex., 45.6–60.1 mm SL, Lake Qinghai, in the Haiyan County, Tibetan Autonomous Prefecture of Haibei, Qinghai province, China; NWIPB 1310001–002, 2 ex., 74.35–86.54 mm SL, Huangshui River, at Haiyan County, Tibetan Autonomous Prefecture of Haibei, Qinghai province, China.

Triplophysa stenura. Topotype. NWIPB 1108062–1108065, 4 ex., 63.1–76.8 mm SL, Zhaqu River, Nangqian County, Yushu Tibetan Autonomous Prefecture, Qinghai province, China; NWIPB 1106110–1106112, 1106114–1106115, 1106117–1106119, 8 ex., 51.7–101.3 mm SL, headstream of Brahmaputra River, Zhongba County, Xigazê City, Tibet Autonomous Region, China.

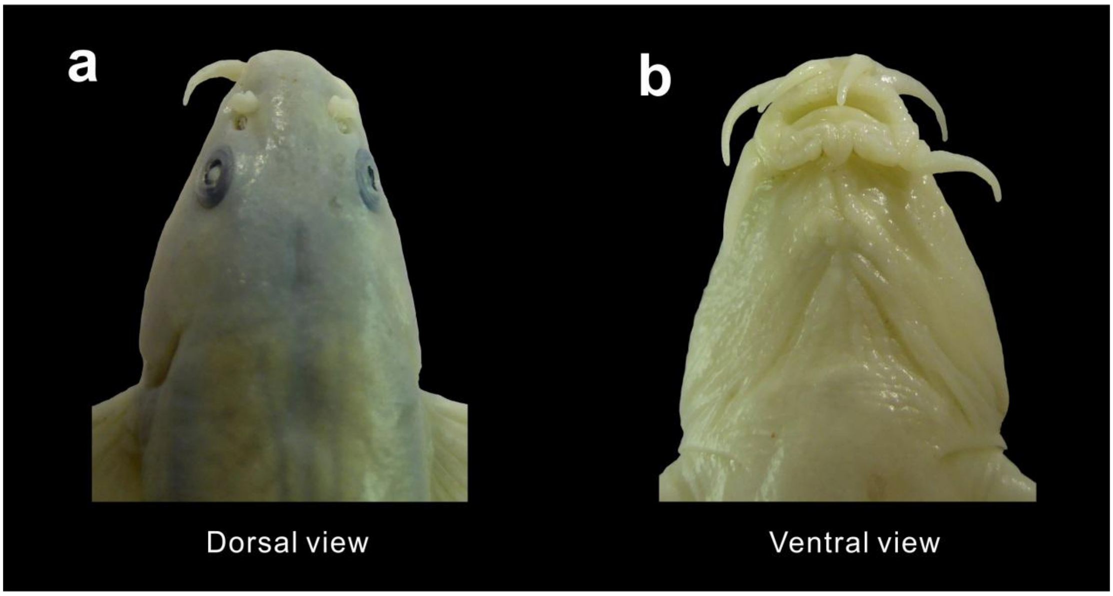
Triplophysa nujiangensa. Topotype. IHB 00915579, 00915605, 00915826, 3 ex., 65.7–77.3 mm SL, Pi River, Liuku Town, Lushui County, Nujiang of the Lisu Autonomous Prefecture, Yunnan Province, China.

Triplophysa rotundiventris. NWIPB 1107006, 1 ex., 70.1 mm SL, Naqu River, Naqu County, Naqu City, Tibet Autonomous Region, China.

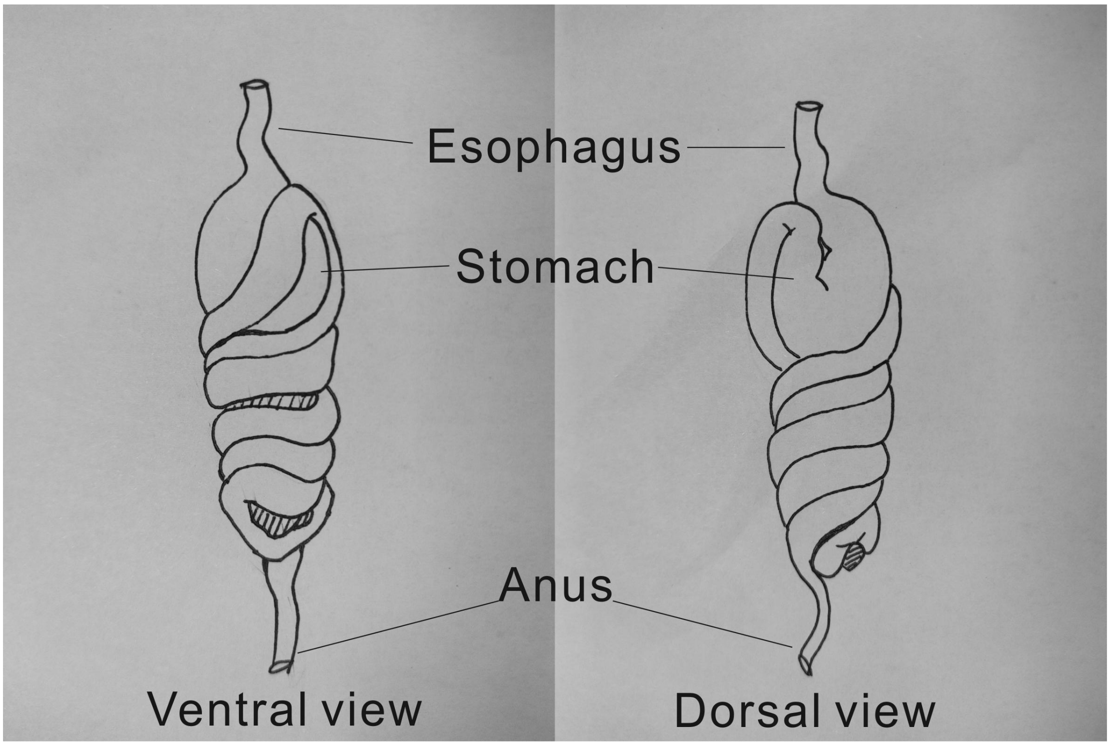
Supplementary Figures



Supplementary Figure S1 Lateral view of *Triplophysa weiheensis* **sp. nov.**, NWIPB 1505189, holotype, 65.8 mm SL**(a)** and *T. stoliczkae* NWIPB 1707001, 85.1 mm SL, from the Yellow River system**(b)**.

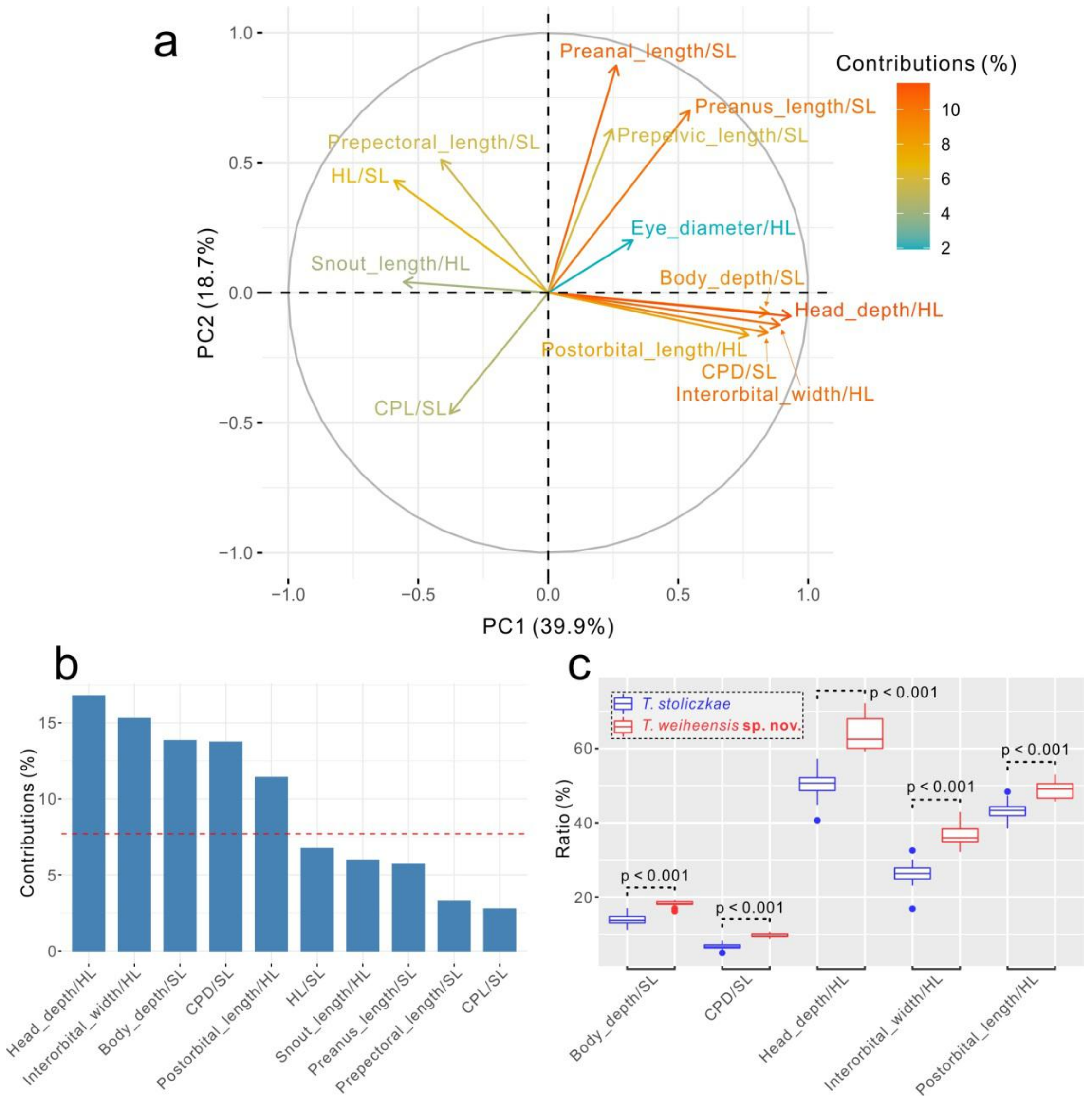


Supplementary Figure S2 *Triplophysa weiheensis* **sp. nov.**, NWIPB 1505189, holotype, 65.8 mm SL. **(a)** dorsal and **(b)** ventral view of head.



Supplementary Figure S3 Hand drawing of alimentary canal, corresponding to the photograph of Figure 1d. The shaded area indicates the fat attached to the intestine. (Drawing by Yu Zhang).

Supplementary Figure S4 Habitat of *Triplophysa weiheensis* **sp. nov.**. **(a)** Type locality: Zhang River, at Chenjiamo Village, Zhangxian County; **(b)** Niutou River, at Hongbao Town, Qingshui County. (photo by Kai Zhao).



Supplementary Figure S5 Visualize principal component analysis (PCA) results for 13 variables of morphometric characters (Table S2). **(a)** Variable correlation plot. Variables on the correlation plot are colored according to their contribution values. **(b)** The contribution of variables to PC1. The red dashed line on the graph indicates the expected average contribution. Only the top 10 contributing variables are shown. **(c)** Boxplot of morphometric measurements of five high contributing variables to PC1. The p values indicate significance from the *t*-test analysis. Abbreviation: CPD, caudal peduncle depth; CPL, caudal peduncle length; HL, head length; SL, standard length; *T.*, *Triplophysa*.

Supplementary Tables

Supplementary Table S1 List of *Triplophysa* species that have been reported in the Yellow River system

Species name	Authors and year	Whether in the Weihe river
Triplophysa minxianensis	Wang and Zhu 1979	Yes
Triplophysa sellaefer	Nichols 1925	Yes
Triplophysa shaanxiensis	Chen in Chen et al 1987	Yes
Triplophysa dalaica	Kessler 1876	Yes
Triplophysa stoliczkae	Steindachner 1866	Yes
Triplophysa robusta	Kessler 1876	
Triplophysa orientalis	Herzenstein 1888	
Triplophysa obscura	Wang in Chen et al 1987	
Triplophysa scleroptera	Herzenstein 1888	
Triplophysa pseudoscleroptera	Zhu and Wu 1981	
Triplophysa pappenheimi	Fang 1935	
Triplophysa siluroides	Herzenstein 1888	
Triplophysa leptosoma	Herzenstein 1888	
Triplophysa brevicauda	Herzenstein 1888	
Triplophysa longianguis	Wu and Wu 1984	
Triplophysa crassilabris	Ding 1994	
Triplophysa alticeps	Herzenstein 1888	

Supplementary Table S2 13 morphometric characters used in the PCA analysis

Including body_depth/standard_length (SL), head_length (HL)/SL, preanus_length/SL, preanal_length/SL, prepelvic_length/SL, prepectoral_length/SL, caudal peduncle length (CPL)/SL, caudal peduncle depth (CPD)/SL, head_depth/HL, snout_length/HL, eye_diameter/HL, interorbital_width/HL, and postorbital_length/HL.

Supplementary Table S2 was listed as a separate csv file, because it's too big.

Supplementary Table S3 Mitochondrial cytochrome *b* (cyt *b*) sequence samples of *Triplophysa* species and outgroups used in the present study

Species	Voucher ID	Accession number	Sample site	Origin
Triplophysa hsutschouensis	NWIPB 0710002	KX373852	Beidahe river, Sunan, Gansu	This study
Triplophysa stoliczkae	NWIPB 1550000	KX373851	Daxiahe river, Linxia, Gansu	This study
Triplophysa wuweiensis	NWIPB 1205606	KX373838	Shiyanghe river, Wuwei, Gansu	This study
Triplophysa tenuis	NWIPB 1250174	KX373841	Heihe (Hexi drainage), Zhangye, Gansu	This study
Triplophysa leptosoma	NWIPB 1250353	KX373839	Datonghe river, Menyuan, Qinghai	This study
Triplophysa pseudoscleroptera	NWIPB 1505061	KX373844	Taohe river, Lintao, Gansu	This study
Triplophysa minxianensis	NWIPB 1505925	KX373849	Weihe river, Qingshui, Gansu	This study
Triplophysa dalaica	NWIPB 1205219	KX373845	Weihe river, Wushan, Gansu	This study
Triplophysa robusta	NWIPB 1505995	KX373850	Daxiahe river, Linxia, Gansu	This study
Triplophysa pappenheimi	NWIPB 1410049	KX373843	Taohe river, Luqu, Gansu	This study
Triplophysa siluroides	NWIPB 1410007	KX373842	Yellow river, Maduo, Qinghai	This study
Triplophysa scleroptera	NWIPB 1250405	KX373840	Yellow river, Guide, Qinghai	This study
Triplophysa orientalis	NWIPB 1505576	KX373846	Heihe (Yellow river), ruoergai, Sichuan	This study
Triplophysa alticeps	NWIPB 1108012	KX373837	Lake Qinghai, Qinghai	This study
Triplophysa bleekeri	NWIPB 1505622	KX373847	Baishui river, Wenxian, Gansu	This study
Triplophysa weiheensis **sp. nov.**	NWIPB 1505992	KX373834	Niutou river, Qingshui, Gansu	This study
Triplophysa weiheensis **sp. nov.**	NWIPB 1505181	KY781400	Zhang River, Zhangxian, Gansu	This study
Triplophysa weiheensis **sp. nov.**	NWIPB 1505922	KY781401	Niutou River, Maiji, Gansu	This study
Triplophysa weiheensis **sp. nov.**	NWIPB 1505985	KY781402	Niutou river, Qingshui, Gansu	This study
Triplophysa weiheensis **sp. nov.**	NWIPB 1505986	KY781403	Niutou river, Qingshui, Gansu	This study
Triplophysa chondrostoma	NA	KT213589	Caidam	Wang et al., 2016
Triplophysa stoliczkae	NA	JQ663847	Yellow river	Li et al., 2013
Triplophysa stoliczkae	NA	NC017890	Yellow river	Li et al., 2013

Triplophysa stoliczkae	NWPU 1208003	MG725381	Huangshui river, Huangyuan, Qinghai	Feng et al., 2019
Triplophysa stoliczkae	NWPU 1208004	MG725382	Huangshui river, Huangyuan, Qinghai	Feng et al., 2019
Triplophysa stoliczkae	NWPU 1108034	MG725408	Zhaqu river, Chindu, Qinghai	Feng et al., 2019
Triplophysa stoliczkae	NWPU 1307052	MG725407	Zhaqu river, Chindu, Qinghai	Feng et al., 2019
Triplophysa stoliczkae	NWPU 1307016	MG725406	Zhaqu river, Chindu, Qinghai	Feng et al., 2019
Triplophysa stoliczkae	NWPU 1307007	MG725405	Zhaqu river, Chindu, Qinghai	Feng et al., 2019
Triplophysa stoliczkae	NWPU 1307005	MG725404	Zhaqu river, Chindu, Qinghai	Feng et al., 2019
Triplophysa stoliczkae	NWPU 1305148	MG725400	Kunes river, Xinyuan, Xinjiang	Feng et al., 2019
Triplophysa stoliczkae	NWPU 1305127	MG725397	Tekes river, Tekes, Xinjiang	Feng et al., 2019
Triplophysa stoliczkae	NWPU 1305125	MG725396	Tekes river, Tekes, Xinjiang	Feng et al., 2019
Triplophysa stoliczkae	NWPU 1305111	MG725399	Kunes river, Xinyuan, Xinjiang	Feng et al., 2019
Triplophysa stoliczkae	NWPU 1305065	MG725398	Kashi river, Nilka, Xinjiang	Feng et al., 2019
Triplophysa stoliczkae	NWPU 1305045	MG725395	Kashi river, Nilka, Xinjiang	Feng et al., 2019
Triplophysa stoliczkae	NWPU 1305043	MG725394	Kashi river, Nilka, Xinjiang	Feng et al., 2019
Triplophysa stoliczkae	NWPU 1106008	MG725393	Kongque river, Purang, Tibet	Feng et al., 2019
Triplophysa stoliczkae	NWPU 1106007	MG725392	Kongque river, Purang, Tibet	Feng et al., 2019
Triplophysa stoliczkae	NWPU 1007084	MG725389	Qaraqash river, Pishan, Xinjiang	Feng et al., 2019
Triplophysa stoliczkae	NWPU 1007083	MG725388	Yarkand river, Yecheng, Xinjiang	Feng et al., 2019
Triplophysa stoliczkae	NWPU 1407014	MG725410	Changchuan river, Rutog, Tibet	Feng et al., 2019
Triplophysa stoliczkae	NWPU 1407013	MG725409	Changchuan river, Rutog, Tibet	Feng et al., 2019
Triplophysa stoliczkae	NWPU 1106002	MG725391	Lake Manasarovar, Purang, Tibet	Feng et al., 2019
Triplophysa stoliczkae	NWPU 1106001	MG725390	Lake Manasarovar, Purang, Tibet	Feng et al., 2019
Triplophysa rotundiventris	NWPU 1107006	MG725402	Naqu river, Amdo Zong, Tibet	Feng et al., 2019
Triplophysa dorsalis	NWPU 1305232	MG725413	Tekes river, Tekes, Xinjiang	Feng et al., 2019
Triplophysa markehenensis	NWPU 0907001	MG725416	Dadu river, Danba, Sichuan	Feng et al., 2019
Triplophysa anterodorsalis	NWPU 1506001	MG725417	Jinsha river, Gonjo, Tibet	Feng et al., 2019

Triplophysa microps	NWPU 1307038	MG725385	Lancang river, Zadoi, Qinghai	Feng et al., 2019
Triplophysa stewarti	NWPU 1107007	MG725411	Lake Duoqing, Kangmar, Tibet	Feng et al., 2019
Triplophysa aliensis	NWPU 1106031	MG725412	Lake Manasarovar, Purang, Tibet	Feng et al., 2019
Triplophysa stenura	NWPU 1108064	MG725583	Lancang, Nangqian, Qinghai	Feng et al., 2019
Triplophysa tibetana	NWPU 1160067	MG725387	Lake Manasarovar, Purang, Tibet	Feng et al., 2019
Triplophysa brevicauda	NWPU 1106029	MG725386	Pengqu river, Nyalam, Tibet	Feng et al., 2019
Barbatula toni	NA	KY451914	NA	GenBank, unpublished
Lefua costata	NA	DQ105196	NA	Tang et al., 2006

Supplementary Table S4 Meristic counts of *Triplophysa weiheensis* sp. nov. and *T. stoliczkae*

Characters	*T. weiheensis* **sp. nov.**		*T. stoliczkae*[a] (*n*=278)	*T. stoliczkae*[b] (*n*=261)	*T. stoliczkae*[c] (*n*=13)
	Holotype	Paratypes (*n*=14)			
Dorsal fin	iii, 7	iii, 6–7	iv, 6–9	iii~iv, 6–9	iv, 7–8
Pelvic fin	i, 6	i, 5–6	i, 6–8	i, 6–8	i, 6–8
Pectoral fin	i, 10	i, 10–11	i, 10–12	i, 10–12	i, 10–11
Anal fin	iii, 5	iii , 5	iii, 5	iii, 5	iii, 5
Caudal fin	16	15–16	16	13~17	16
Gill rakers	15	14–16	9–24	13–23	15–18
Vertebrae	4+34	4+34–36	4+38–41	4+36–41 (n = 95)	4+38–39 (n = 8)

[a]Data from Wu and Wu (1992);

[b]Data from Zhu (1989);

[c]Measurement data of *T. stoliczkae* from the Yellow River system (See the Comparative material for specimen information).

Supplementary Table S5. K2P genetic distances based on mitochondrial Cyt *b* sequences between species of genus *Triplophysa*. Genetic distance between *Triplophysa weiheensis* **sp. nov.** and *T. stoliczkae* (from the Yellow River system) was colored red, others less than this value were colored orange.

Supplementary Table S5 was listed as a separate excel file, because it's too big.

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