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* Steindacner 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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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             | 50.9 | 2.2 |
| 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 |
| Preanal length          | 68.5     | 61.9–71.0             | 67.3 | 2.6 |
| Prepelvic length        | 72.2     | 63.5–73.4             | 69.8 | 2.5 |
| Prepectoral length      | 56.7     | 51.1–58.4             | 54.6 | 2.4 |
| Caudal-pelvic length    | 19.7     | 16.9–21.9             | 19.8 | 1.3 |
| Caudal-peduncle depth   | 23.1     | 19.4–24.3             | 22.0 | 1.3 |
| Pectoral-pelvic distance| 10.3     | 8.7–10.7              | 9.7  | 0.6 |
| Pectoral-anal distance  | 32.5     | 31.6–38.8             | 35.2 | 2.4 |
| Pelvic-anal distance    | 48.4     | 46.1–54.9             | 50.3 | 2.9 |
| Head length (mm)        | 11.8     | 10.0–14.0             | 13.2 | 1.7 |
| 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) vertebræ 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 anterior nostrils, but not around posterior. 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 head length, caudal peduncle depth/standard length, and postorbital 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 S5C), suggesting a comparatively stubby body for the new species relative to *T. stolitzkazae*. 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. cacaensis 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. nuijiangensa 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 prefix http://zoobank.org/.

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**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.

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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'-GACCTGAAAAACCACCGTTG-3') and H15915 (5'-CTCCGATCTCCGGATTACAAGAC-3') (Xiao et al., 2001) in total reaction volumes of 35 µL, containing approximately 100 ng of template DNA, 0.7 µL of each primer (10 µmol/L), 3.5 µL 10× reaction buffer, 3 µL 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, Nilk 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, 7 ex., 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 nuijiangensa.** 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.
| 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, 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         | Daxiahe river, Linxia, Gansu | This study      |
| *Triplophysa robusta*       | NWIPB 1505995| KX373850         | Taohe river, Luqu, Gansu     | This study      |
| *Triplophysa pappenhaimi*   | NWIPB 1410049| KX373843         | Yellow river, Maduo, Qinghai | This study      |
| *Triplophysa siluroides*    | NWIPB 1410007| KX373842         | Yellow river, Guide, 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 weihemensis sp. nov.* | NWIPB 1505992| KX373834         | Niutou river, Qingshui, Gansu | This study      |
| *Triplophysa weihemensis sp. nov.* | NWIPB 1505181| KY781400         | Zhang River, Zhangxian, Gansu | This study      |
| *Triplophysa weihemensis sp. nov.* | NWIPB 1505922| KY781401         | Niutou River, Maiji, Gansu   | This study      |
| *Triplophysa weihemensis sp. nov.* | NWIPB 1505985| KY781402         | Niutou river, Qingshui, Gansu | This study      |
| *Triplophysa weihemensis 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 |
| Taxon                     | Specimen Code | Accession Code | Location                          | Authors                |
|--------------------------|---------------|----------------|-----------------------------------|------------------------|
| *Triplophysa stoliczkae* | NWPU 108003   | MG725381       | Huangshui river, Huangyuan, Qinghai | Feng et al., 2019     |
| *Triplophysa stoliczkae* | NWPU 108004   | MG725382       | Huangshui river, Huangyuan, Qinghai | Feng et al., 2019     |
| *Triplophysa stoliczkae* | NWPU 108034   | 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, Amo 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    |
| Species               | Accession | GenBank ID | Location                        | Authors          |
|----------------------|-----------|------------|---------------------------------|------------------|
| *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*<sup>a</sup> (n=278) | *T. stoliczkae*<sup>b</sup> (n=261) | *T. stoliczkae*<sup>c</sup> (n=13) |
|------------|--------------------------|----------------------------------|----------------------------------|----------------------------------|
|            | Holotype | Paratypes (n=14) | T. stoliczkae<sup>a</sup> (n=278) | T. stoliczkae<sup>b</sup> (n=261) | T. stoliczkae<sup>c</sup> (n=13) |
| 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) |

<sup>a</sup>Data from Wu and Wu (1992);
<sup>b</sup>Data from Zhu (1989);
<sup>c</sup>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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