

PREPRINT

Author-formatted, not peer-reviewed document posted on 15/09/2021

DOI: <https://doi.org/10.3897/arphapreprints.e74357>

A new species of *Homatula* (Teleostei: Cobitoidea: Nemacheilidae) from the Pearl River, China

 Rui Min, Jun-Xing Yang

Disclaimer on biological nomenclature and use of preprints

The preprints are preliminary versions of works accessible electronically in advance of publication of the final version. They are not issued for purposes of botanical, mycological or zoological nomenclature and **are not effectively/validly published in the meaning of the Codes**. Therefore, nomenclatural novelties (new names) or other nomenclatural acts (designations of type, choices of priority between names, choices between orthographic variants, or choices of gender of names) **should NOT be posted in preprints**. The following provisions in the Codes of Nomenclature define their status:

International Code of Nomenclature for algae, fungi, and plants (ICNafp)

Article 30.2: "An electronic publication is not effectively published if there is evidence within or associated with the publication that its content is merely preliminary and was, or is to be, replaced by content that the publisher considers final, in which case only the version with that final content is effectively published." In order to be validly published, a nomenclatural novelty must be effectively published (Art. 32.1(a)); in order to take effect, other nomenclatural acts must be effectively published (Art. 7.10, 11.5, 53.5, 61.3, and 62.3).

International Code of Zoological Nomenclature (ICZN)

Article: 21.8.3: "Some works are accessible online in preliminary versions before the publication date of the final version. Such advance electronic access does not advance the date of publication of a work, as preliminary versions are not published (Article 9.9)".

Running title: A new species of nemacheilids from China
A new species of *Homatula* (Teleostei, Cobitoidea, Nemacheilidae) from the Pearl
River, China

Rui Min¹, Junxing Yang^{2,3*}

¹ Kunming Natural History Museum of Zoology, Kunming Institute of Zoology,
Chinese Academy of Sciences, Kunming, Yunnan 650223, China

² State Key Laboratory of Genetic Resources and Evolution, Kunming Institute of
Zoology, The Innovative Academy of Seed Design, Chinese Academy of Sciences,
Kunming, Yunnan 650223, China

³ Yunnan Key Laboratory of Plateau Fish Breeding, Kunming Institute of Zoology,
Chinese Academy of Sciences, Kunming, Yunnan 650223, China

* Corresponding author: yangjx@mail.kiz.ac.cn

CRedit author statement

Min R and Yang JX designed the study and wrote the draft. Min R performed
morphological comparisons and conducted molecular analysis.

Competing interests

The authors have declared that no competing interests exist.

Abstract

Based on morphological and molecular analyses of *Homatula* specimens from the
Nanpanjiang River in Yunnan, China, we reveal a previously undescribed species.
Furthermore, *H. acuticephala* is reported as a synonym of *H. anguillioides*. The new
species differs from its congeners by a combination of the following characters: naked
and robust body with well-developed adipose crest (caudal peduncle depth as
percentage of length: 70.53%–78.48%), median notch on lower jaw, median gap on
lower lip, three pairs of short barbels, with maxillary barbels extending posteriorly to
anterior edge of eyes, branched dorsal fin rays $8^{1/2}$, vertebrae 37–38, and lateral line
complete. Phylogenetic analysis of the mitochondrial cytochrome c oxidase subunit I
(COI) gene indicated that the new species represents an independent lineage, closely
related to *H. longidorsalis* and *H. nanpanjiangensis*. The genetic distances (Kimura-2-
parameter) between the new species and *H. longidorsalis* and *H. nanpanjiangensis*,
were 5.0% and 5.9%, respectively. The new species is named *Homatula robusta* **sp. nov.**
and is described herein. To date, the new species is known only from the type locality.

Keywords

Molecular phylogeny, morphology, Nemacheilids loaches, taxonomy, Nanpanjiang
River, Yunnan Province

Introduction

The genus *Homatula* Nichols, 1925 of Nemacheilidae can be recognized by having
dorsal and ventral crests supported by rudimentary procurrent caudal-fin rays.
Homatula is restricted to China but is widely distributed in rivers, streams, and lakes.
However, *Homatula* species rarely cross river distributions and are narrowly dispersed

to limited geographical areas and drainages (Min et al. 2010, 2012, 2013; Yang et al. 2017; Endruweit et al. 2018; Li et al. 2019). Currently, 20 valid species are recognized (Fricke et al. 2021), including five species in the Yangtze River, i.e., *H. variegata* (Dabry de Thiersant, 1874), *H. wujiangensis* (Ding and Deng, 1990), *H. berezowskii* (Günther, 1896), *H. guanheensis* Zhou, Ma, Wang, Tang, Meng and Nie, 2021 and type species *H. potanini* (Günther, 1896); one species in the Yellow River, i.e., *H. laxiclathra* Gu and Zhang, 2012; four species in the Lancangjiang River, i.e., *H. anguillioides* (Zhu and Wang, 1985), *H. acuticephala* (Zhou and He, 1993), *H. pycnolepis* Hu and Zhang, 2010, and *H. wuliangensis* Min, Yang and Chen, 2012; three species in the Nujiang River, i.e., *H. anteriodorsalis* Li, Che and Zhou, 2019, *H. cryptoclathrata* Li, Che and Zhou, 2019, *H. nigra* Li, Che and Zhou, 2019; four species in the Red River, i.e., *H. disparizona* Min, Yang and Chen, 2013, *H. change* Endruweit, 2015, *H. wenshanensis* Li, Yang, Li and Liu, 2017 and *H. coccinocola* Endruweit, Min and Yang, 2018; and three species in the Nanpanjiang River, i.e., *H. oligolepis* (Cao and Zhu, 1989), *H. longidorsalis* (Yang, Chen and Kottelat, 1994) and *H. nanpanjiangensis* (Min, Chen and Yang, 2010). Several *Homatula* species are also found in lake systems, including *H. acuticephala* in Haixihai Lake, *H. anguillioide* in Erhai Lake, and *H. oligolepi* in Yangzonghai Lake. When we examined the collections from the Nanpanjiang River in Luoping county, Yunnan, we recognized the collection may be a different species.

Here, we describe the new species and construct a molecular phylogeny of 16 valid *Homatula* species, including genetic distance. We aimed to confirm the validation of the suspected species and describe it herein.

Methods

Specimens were collected in China and were fixed into 95 % ethanol or 10 % formalin and transferred to 75% ethanol for preservation after fixation. Tissue samples for DNA analysis were taken from one or more specimens by excising the left pelvic fin and placing it in 95% ethanol. All measurements and counts followed Kottelat (1990) and pore counts followed Armbruster (2012). Measurements were made with digital calipers to the nearest 0.1 mm from the left side. X-ray images were used to count vertebrae and simple fin rays. Lateral line pores and rays of paired fins were counted under a binocular microscope. Weberian apparatus was counted as four vertebrae. Caudal vertebrae encompassed all centra bearing a hemal spine, including the urostyle, which was counted as one vertebra. Eye diameter was measured horizontally. Body depth was measured at the dorsal-fin origin. Lateral head length was measured to the posterior margin of the operculum, excluding the opercular membrane. Examined specimens were deposited in the collection of the Kunming Natural History Museum of Zoology, Kunming Institute of Zoology (KIZ), Chinese Academy of Sciences (CAS).

DNA was extracted from fin tissues using standard phenol-chloroform extraction (Sambrook et al. 1989). Mitochondrial cytochrome c oxidase subunit 1 (COI) was amplified by polymerase chain reaction (PCR). The PCR protocols were conducted in 50-µl reactions as follows: initial denaturation step at 95 °C for 5 min, 35 cycles at 94 °C for 30 s, 56 °C for 45 s, and 72 °C for 1 min, and final extension at 72 °C for 10 min. The primers used for COI were LCOIa (CCT ACC TgT ggC AAT CAC RCg C), HCOI (gTg AAT Agg ggg AAT CAg Tg) (Liu et al. 2012). Fragments were sequenced by the Shanghai DNA Biotechnologies Company (China). DNA sequences were

aligned using default settings in MAFFT v7 (<http://mafft.cbrc.jp/alignment/server/>) (Kato and Standley 2013), and, if necessary, adjusted by eye. MEGA7 (Kumar et al. 2016) was used to calculate the Kimura's 2-parameter genetic distance (K2P). The phylogeny was analyzed using MrBayes 3.2 (Ronquist et al. 2012) with the generalized time reversible model (nst = 6) and the gamma-distributed rate variation and proportion of invariable positions (GTR+ I) for the COI datasets. We ran four simultaneous Monte Carlo Markov chains for 2000000 generations, with sampling every 1,000 generations, and the first 25% of samples were discarded as burn-in.

Results

The COI molecular dataset included 43 terminal taxa representing 25 species, 16 of which belonged to *Homatula* (Table 1). The COI was 1116 bp in length. Our results confirmed the monophyly of *H. robusta* **sp. nov.** and most species. The relationships among species from the Nanpanjiang River (*H. robusta* **sp. nov.**, *H. nanpanjiangensis*, and *H. longidorsalis*) were not resolved. Phylogenetic analysis is shown in Fig. 1. The K2P genetic distances among species from the Nanpanjiang River were 5.4% between *H. robusta* **sp. nov.** and *H. nanpanjiangensis* and, 5.3% between *H. robusta* **sp. nov.** and *H. longidorsalis*. *Homatula potanini* and *H. wujiangensis* were clustered together, with a K2P distance of 1.5%, and *H. acuticephala* and *H. anguillioides* were clustered together, with a K2P distance of 0.2%.

Homatula robusta **sp. nov.**

Figs. 2, 3, Table 2

Holotype. KIZ 2009000125, 83.12 mm SL; collected by Wansheng Jiang, Weiying Wang on 14 March 2009 in Changdi village, Luoping County, Qujing City, Yunnan Province, China; Nanpanjiang River, upper Pearl River (25°02'N, 104°30.35'E; ca. 1210 m).

Paratypes. KIZ 2009000122, 144, 146, 3 ex. 61.16–81.32 mm SL, same data as for holotype.

Diagnosis

Homatula robusta **sp. nov.** differs from all other *Homatula* by the following combination of characters: well-developed caudal peduncle crest, reaching beyond origin of anal-fin base; caudal peduncle depth (CPD) 70.53%–78.48% (74.07%) of caudal peduncle length (CPL); short barbels, with maxillary barbels extending posteriorly to anterior edge of eyes; median notch on lower jaw, median gap on lower lip, small gap on upper lip; body naked, occasional scales along lateral line of caudal peduncle; lateral line complete, branched dorsal fins $8\frac{1}{2}$, and vertebrae 37–38.

The K2P genetic distances indicated that the new species is closest to species from the same drainage, with distances of 5.4% from *H. nanpanjiangensis* and 5.3% from *H. longidorsalis*. Morphologically, *H. robusta* **sp. nov.** differs from *H. longidorsalis* by naked body (vs. scales on posterior body), anterior nostril in flap (vs. anterior nostril in short tube), lower jaw median notch present (vs. absent), branched dorsal fins $8\frac{1}{2}$ (vs. $9\frac{1}{2}$), and raised crest reaching or beyond origin of anal-fin base (vs. straight crest not reaching origin of anal-fin base) (Fig. 4A). Although they are distributed in relatively close villages in Luoping County (Fig. 5), *H. robusta* **sp. nov.** can be distinguished from *H. nanpanjiangensis* by developed crest with raised margin, reaching beyond origin of anal-fin base (vs. straight and flat crest), CPD 70.5%–78.5% of CPL (vs. CPD 54.7%–63.8% of CPL), body depth (BD) 15.9%–17.3% of SL (vs. BD 12%–15.3% of SL),

maxillary barbel reaching front margin of eye (vs. between middle and posterior margin of eye), ~13 bars (vs. ~16), and swollen cheek (vs. flat) (Fig. 4B).

Homatula robusta **sp. nov.**, *H. potanini*, and *H. wujiangensis* are characterized by a relatively stubby body and well-developed dorsal adipose crest with raised edge, and thus differ from all other *Homatula* species (very elongated body and almost straight edge) (Fig. 4C). However, *H. robusta* **sp. nov.** can be distinguished from *H. potanini* and *H. wujiangensis* by complete lateral line (vs. incomplete lateral line) and naked body (vs. posterior body (at least) covered by scales). *Homatula robusta* **sp. nov.** further differs from *H. potanini* based on vertebrae 37–38 (vs. 32–34). *Homatula robusta* **sp. nov.** also shows a large molecular distance (10.5%–10.8%) from *H. potanini* and *H. wujiangensis*.

Homatula robusta **sp. nov.** differs from all scaled species (i.e., all *Homatula* except for *H. disparizona*, *H. wenshanensis*, *H. nanpanjiangensis*, and *H. oligolepis*) based on naked body (vs. scaled) and genetic distances of 5.3%–10.8%. *Homatula robusta* **sp. nov.**, which inhabits the Nanpanjiang River, and *H. disparizona* and *H. wenshanensis*, which inhabit the Red River, show genetic distances of between 10.2% to 10.5%. *Homatula robusta* **sp. nov.** also differs from these species based on short body (vs. very elongated), vertebrae 37–38 (vs. more than 39), long and high crest (vs. short and flat), and median notch on lower jaw present (vs. absent). *Homatula oligolepis* is distributed in the same drainage (Nanpanjiang River) as *H. robusta* **sp. nov.**, and inhabits the Yangzonghai Lake and upper Nanpanjiang River. But, *H. oligolepis* differs from the new species with large elongated body, dorsal head covered with worm-shaped stripes, branched dorsal fins $9\frac{1}{2}$ and vertebrae 39–41.

Description

Morphometric data in Table 2.

Homatula robusta **sp. nov.**: body robust, caudal peduncle compressed with well-developed adipose crest, containing muscular keel and tiny internal fin rays, extending beyond origin of anal-fin base. Body naked, occasional scales scattered along lateral line of caudal peduncle. Snout blunt, cheeks swollen; mouth slightly arched; lips moderately thick, upper lip smooth with shallow tiny notch; lower lip with shallow furrows and median gap. Anterior nostril in flap, next to posterior nostril. Processus dentiformis present with circular arc edge, median notch on lower jaw. Three pairs of barbels, maxillary barbel reaching front margin of eye, outer rostral barbel reaching inner corner of mouth, but inner rostral barbel not. Eye elliptical horizontally, dorsolaterally positioned. Pectoral fin reaching about halfway from origin of pectoral fin to origin of pelvic fin; pelvic fin origin at 1st dorsal ray, reaching close to anus. Anus about one and a half eye length in front of origin of anal-fin base. Distal margin of dorsal fin concave, caudal fin slightly concaved with upper and lower lobes almost equal in length. Moderate axillary pelvic lobe with free tip. Dorsal fin rays iv- $8\frac{1}{2}$, pectoral fin rays I-11, pelvic fin rays i-6–8, anal fin rays iii- $5\frac{1}{2}$, upper caudal lobe 9 rays, lower caudal lobe 8 rays. Lateral line complete. Supraorbital pores 7, postorbital pores 3, sub- and preorbital pores 12, preoperculo-mandibular pores 10, supratemporal pores 3. Vertebrae (two specimens), 4+37–38. Intestine almost straight, with small bend next to stomach posterior.

Coloration of preserved specimens

Yellow on back, darker on upper side, four or five dark bars on side of posterior body,

bars on side of anterior body faint; 10–12 bars on side of body, except for bar on caudal-fin base, four bars at end slightly wider than those at front, bars on front closer to each other and wider than interspaces; front bars terminate around lateral line, posterior bars almost reach ventral margin. Black bar on posterior end of caudal peduncle, joining at both ends. All fin rays covered by melanopores, series of stripes halfway up each dorsal-fin ray.

Sexual dimorphism

All specimens were collected in March; no sexual dimorphism was observed.

Etymology

Robusta is Latin meaning “strong”, in reference to the strong and thick body and caudal peduncle (粗壮荷马条鳅).

Distribution

Only known from the type locality (Fig. 5).

Discussion

The new species was collected from the Nanpanjiang River. Three other species are also distributed in the Nanpanjiang River (i.e., *H. oligolepis*, *H. longidorsalis*, and *H. nanpanjiangensis*). In addition, two uncertain species were collected from the Nanpanjiang River: i.e., *H. sp1* (sympatric distribution with new species) and *H. sp2* (neighbor distribution with new species). They can be easily distinguished from *H. robusta* **sp. nov.** by elongated body (vs. short and robust), less developed crest (CPD/CPL 53.8%–66% vs. 70.5%–78.5%), oblique tail with upper lobe longer than lower lobe (vs. same length), and branched dorsal fins $9\frac{1}{2}$ (vs. $8\frac{1}{2}$). Both *H. sp1* and *H. sp2* exhibit similar morphology and show close genetic distance (1.1%) but differ in number of body stripes (~13 in *H. sp1* vs. ~8 in *H. sp2*). Furthermore, although *H. sp1* and *H. sp2* are most similar to *H. nanpanjiangensis*, they differ in number of stripes (~13 or ~8 in *H. sp1* and *H. sp2*, respectively, vs. ~16 in *H. nanpanjiangensis*), tail shape (upper lobe longer than lower lobe in *H. sp1* and *H. sp2* vs. same length in *H. nanpanjiangensis*), branched dorsal fins ($9\frac{1}{2}$ vs. mainly $8\frac{1}{2}$). Based on the above differences, and the lack of molecular data for *H. nanpanjiangensis*, there is currently insufficient evidence to distinguish *H. sp1*, *H. sp2*, and *H. nanpanjiangensis*. Therefore, we temporarily identified *H. sp1* and *H. sp2* as populations of *H. nanpanjiangensis* in the phylogenetic analysis and classified the specimens as *H. nanpanjiangensis*.

In this study, *H. acuticephala* and *H. anguillioides* were collected from the type locality (Haixihai Lake and a stream of Longtan Spring, both located in Yousuo Town, Eryuan County, Yunnan Province, China). In the original description of these species, *H. acuticephala* was said to differ from *H. anguillioides* by body depth decreasing from dorsal base to caudal peduncle (vs. uniform depth) (Zhou and He 1993). However, this difference may be due to diversity between lake and river populations of the same species, as reported for *H. erhaiensis* (synonymous with *H. anguillioides*) and *H. anguillioides* (Endruweit et al. 2018). Here, the K2P genetic distance between *H. anguillioides* and *H. acuticephala* was only 0.2%. Thus, we confirm the conclusion of Min et al. (2012), with *H. acuticephala* considered a synonym of *H. anguillioides*.

Homatula potanini and *H. wujiangensis* were clustered in our phylogeny.

Morphologically, *H. potanini* and *H. wujiangensis* both have incomplete lateral lines

and high crests. However, *H. potanini* can be distinguished from *H. wujiangensis* by longer dorsal crest extending forward to origin of anal-fin base (vs. not) and scales covering post-dorsal body (vs. scaleless or with rudimentary scales) (Ding and Deng 1990). However, as the sequences of *H. wujiangensis* were downloaded from GenBank, identification of the species could not be verified. Thus, validation of these two species retained for now.

Specimens examined

Homatula longidorsalis (n=24): **Holotype:** CHINA; Yunnan, Yiliang, Jiuxiang; KIZ 1987003989. **Paratypes:** CHINA; Yunnan, Yiliang, Jiuxiang; KIZ 1987003990, 3991–3993, 5090, 5091, 5736–5752.

Homatula nanpanjiangensis (n=21): **Holotype:** CHINA; Yunnan, Qujing, Luoping; KIZ 1994000023. **Paratypes:** CHINA: Yunnan: Qujing: Luoping; KIZ 1994000022, 024–026, 028, 029, 031, 034–036. *H. sp1:* CHINA; Yunnan, Qujing, Luoping; KIZ 2009000121, 123, 124, 145, 147. *H. sp2:* CHINA; Yunnan, Qujing, Luoping; KIZ 2008006704, 48–52.

Homatula oligolepis (n=2): CHINA; Yunnan, Zhanyi; KIZ 1985000829, KIZ 652099.

Homatula potanini (n=5): CHINA; Sichuan, Meishan; KIZ 2010000266; CHINA; Sichuan, Luoshan; KIZ 2010000279–82.

Homatula variegata (n=9): CHINA; Sichuan, Panzhihua; KIZ 2009002724–2727; CHINA; Yunnan, Zhaotong, Yanjin; KIZ 2004008050, 52–53, 57, 61.

Homatula laxiclathra (n=2): CHINA; Shanxi: Ankang: Ningshan: Weihe River: KIZ 2012002359–60.

Homatula berezowskii (n=6): CHINA: Shanxi, Ankang, Ningshan, Yangtze River; KIZ 2005014508–13.

Homatula wuliangensis (n=34): **Holotype:** CHINA; Yunnan, Jingdong; KIZ 2008008158. **Paratypes:** CHINA; Yunnan, Jingdong; KIZ 2008008156–157, 159–172, 175–176, 179, 184, 197, 199–201, 203, 205, 207, 211, 214–215, 316–318.

Homatula disparizona (n=21): **Holotype:** CHINA; Yunnan, Wenshan, Xichou; KIZ 2012000623. **Paratypes:** CHINA; Yunnan, Wenshan, Xichou; KIZ 2012000622, 624–634. CHINA; Yunnan, Wenshan, Xichou; KIZ 2014005623–30.

Homatula acuticephala (n=26): CHINA; Yunnan, Dali, Haixihai; KIZ 2008005990–6015.

Homatula anguillioides (n=12): CHINA; Yunnan, Dali, Eryuan, KIZ 2008006532–6543.

Homatula pycnolepis (n=6): CHINA; Yunnan, Dali, Yangbi; KIZ 1998004817, 19, 22, 25, KIZ 2009005288, KIZ 2009005388.

Homatula change (n=12): **Holotype:** CHINA; Yunnan, Puer, Jiangcheng; KIZ

2012004205. **Paratypes:** CHINA; Yunnan, Puer, Jiangcheng; KIZ 2012004208, 4209, 4211, 4215–18, 4221–24.

Homatula coccinocola (n=5): **Holotype:** CHINA, Yunnan, Honghe; KIZ 2011002847. **Paratypes:** CHINA, Yunnan, Honghe; KIZ 2012001866–1869.

Homatula wenshanensis (n=3): CHINA; Wenshan; KIZ 2014005685–87.

References

- Armbruster JW (2012) Standardized Measurements, Landmarks, and Meristics for the Cypriniformes. *Zootaxa* 3586: 8–16.
- Ding R, Deng Q (1990) The Noemacheilinae fishes from Sichuan, with description of a new species I. *Paracobitis*, *Nemacheilus* AND *Oreias* (CYPRINIFORMES: COBITIDAE). *Zoological Research* 11: 285–290. [In Chinese]
- Endruweit M, Min R, Yang J (2018) A new species of *Homatula* from the Red River drainage in Yunnan based on morphological and genetic data (Teleostei: Nemacheilidae). *Zootaxa* 4375: 555–566. <https://doi.org/10.11646/zootaxa.4375.4.5>
- Fricke R, Eschmeyer WN, Van der, Laan R (eds) (2020). Eschmeyer's Catalog of Fishes: Genera, Species, References. (<http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp>).
- Katoh K, Standley DS (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular biology and evolution* 30: 772–780.
- Kottelat M (1990) Indochinese Nemacheilines: A Revision of Nemacheiline Loaches (Pisces, Cypriniformes) of Thailand, Burma, Laos, Cambodia and Southern Vietnam. München, 262 pp.
- Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Molecular Biology and Evolution* 33: 1870–1874. <https://doi.org/10.1093/molbev/msw054>
- Li X, Che XJ, Zhou W (2019) Loaches of *Homatula* (Teleostei: Nemacheilidae) from the upper Salween River in Yunnan, China with description of three new species. *Zootaxa* 4711: 330–348–330–348. <https://doi.org/10.11646/zootaxa.4711.2.6>
- Liu S, Mayden RL, Zhang J, Yu D, Tang Q, Deng X, Liu H (2012) Phylogenetic relationships of the Cobitoidea (Teleostei: Cypriniformes) inferred from mitochondrial and nuclear genes with analyses of gene evolution. *Gene* 508: 60–72. <https://doi.org/10.1016/j.gene.2012.07.040>
- Min R, Chen X, Yang J (2010) *Paracobitis nanpanjiangensis*, a new loach (Balitoridae: Nemacheilinae) from Yunnan, China. *Environmental Biology of Fishes* 87: 199–204. <https://doi.org/10.1007/s10641-010-9587-z>
- Min R, Yang JX, Chen XY (2013) *Homatula disparizona*, a new species of loach from

the Red River drainage in China (Teleostei: Nemacheilidae). *Ichthyological Exploration of Freshwaters* 23: 289–384.

Min R, Chen XY, Yang JX, Winterbottom R, Mayden R (2012) Phylogenetic Relationships of the Genus *Homatula* (Cypriniformes: Nemacheilidae), with Special Reference to the Biogeographic History around the Yunnan-Guizhou Plateau. *Zootaxa* 3586: 78–94.

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice Across a Large Model Space. *Systematic Biology* 61: 539–542. <https://doi.org/10.1093/sysbio/sys029>.

Sambrook J, Fritsch EF, Maniatis T (1989) *Molecular cloning: a laboratory manual*. Cold Spring Harbor Laboratory Press, 626 pp.

Yang H, Li C, Liu T, Li W (2017) A Report on a New Species of *Homatula* from Yunnan (Cypriniformes: Noemacheilidae). *Journal of Yunnan Agricultural University* 32: 1140–1144. [In Chinese]

Zhou W, He J (1993) Paracobitis distributed in Erhai area, Yunnan, China (Pisces: Cobitidae). *Zoological Research* 14: 5–9. [In Chinese]

Acknowledgements

We gratefully appreciate Dr. WS Jiang (Jishou University) and Dr. WY Wang for collecting the specimens. This project was supported by the National Natural Science Foundation of China (31401956), Biological Resources Programme, Chinese Academy of Sciences (KFJ-BRP-017-16) and Strategic Priority Research Program of the Chinese Academy of Sciences (XDA24030505).

Table 1. Information of study samples, the sequences downloaded from GenBank are not shown the Voucher number.

Taxon	Voucher number	GenBank number
<i>Triplophysa brevicauda</i>	KIZ 050422024	MZ677092
<i>Triplophysa scleroptera</i>	KIZ 20100076	MZ677093
<i>Triplophysa obscura</i>		MG238209
<i>Claea dabryi</i>	KIZ 2009003600	MZ677094
<i>Schistura fasciolata</i>	KIZ 2012003668	MZ677096
<i>Schistura macrocephalus</i>	KIZ 2010003135	MZ677098
<i>Schistura latifasciata</i>	KIZ CXY2008062	MZ677099
<i>Schistura callichroma</i>	KIZ 200401055	MZ677095
<i>Schistura caudofurca</i>	KIZ 20150307022	MZ677097
<i>Homatula wujiangensis</i>		IHB0301075
<i>Homatula potanini</i>	KIZ 2010000235	MZ677100
<i>Homatula potanini</i>	KIZ 2010000281	MZ677101
<i>Homatula berezowskii</i>	KIZ 2005014512	MZ677105
<i>Homatula berezowskii</i>	KIZ 2005014513	MZ677104
<i>Homatula nanpanjiangensis</i>	KIZ 2009000145	MZ677112

<i>Homatula nanpanjiangensis</i>	KIZ 2009000147	MZ677113
<i>Homatula nanpanjiangensis</i>	KIZ 2008006749	MZ677119
<i>Homatula nanpanjiangensis</i>	KIZ 2008006752	MZ677120
<i>Homatula longidorsalis</i>	KIZ 2008005909	MZ677121
<i>Homatula longidorsalis</i>	KIZ 2008005910	MZ677118
<i>Homatula variegata</i>	KIZ 2009002770	MZ677110
<i>Homatula variegata</i>	KIZ 2009002724	MZ677115
<i>Homtula robusta</i>	KIZ 2009000146	MZ677106
<i>Homtula robusta</i>	KIZ 2009000144	MZ677107
<i>Homatula coccinocola</i>	KIZ 2012001867	MF953210
<i>Homatula coccinocola</i>	KIZ 2012001868	MF953211
<i>Homatula change</i>	KIZ 2015005116	MZ677109
<i>Homatula change</i>	KIZ 2015005117	MZ677108
<i>Homatula cryptoclathrata</i>	KIZ 2005012639	MZ677116
<i>Homatula cryptoclathrata</i>	KIZ 2005012637	MZ677117
<i>Homatula pycnolepis</i>	KIZ 2009003860	MZ677111
<i>Homatula pycnolepis</i>	KIZ 20050423002	MZ677114
<i>Homatula anguillioides</i>	KIZ 2008006539	MZ677124
<i>Homatula acuticephala</i>	KIZ 2008005994	MZ677122
<i>Homatula anguillioides</i>	KIZ 2008006536	MZ677125
<i>Homatula acuticephala</i>	KIZ 2008005990	MZ677123
<i>Homatula wuliangensis</i>	KIZ 2008008160	MF953221
<i>Homatula wuliangensis</i>	KIZ 2008008159	MF953220
<i>Homatula wenshanensis</i>	KIZ 2014005686	MZ677102
<i>Homatula wenshanensis</i>	KIZ 2014005687	MZ677103
<i>Homatula disparizona</i>	KIZ 2012000626	MF953194
<i>Homatula disparizona</i>	KIZ 2012000622	MF953190
<i>Botia dario</i>		KT781503

Table 2. Morphometric data for *Homatula robusta* sp. nov. (all types), *H. longidorsalis* (all types), *H. nanpanjiangensis* (all types), *H. sp1*, and *H. sp2*.

Measurements	<i>H. robusta</i> sp. nov. n=4			<i>H. nanpanjiangensis</i> n=5			<i>H. sp1</i> and <i>H. sp2</i> n=11			<i>H. longidorsalis</i> n=4		
	Rangs	Mean	SD	Ranges	Mean	SD	Ranges	Mean	SD	Ranges	Mean	SD
Standard length SL (mm)	61.1–83.12			63.82–88.74			51.6–116.5			68.46–86.88		
In percent of SL												
Head length	13.28–18.76	16.73	2.15	15.46–20.36	18.11	1.78	13.12–23.9	17.71	3.30	14.84–17.7	15.94	1.15
Predorsal length	45.92–47.71	46.95	0.72	45.97–48.42	47.08	0.89	44.31–50.27	47.15	1.58	46.85–48.41	47.50	0.57
Preventral length	49.02–50.34	49.98	0.56	49.58–51.21	50.15	0.62	47.33–55.12	51.28	2.36	48.36–50.42	49.32	0.75
Preanal length	72.69–74.56	73.89	0.73	71.84–75.68	74.44	1.37	70.93–76.78	73.96	1.78	71.85–72.98	72.50	0.42
Preanus length	67.28–68.86	68.20	0.61	68.06–70.45	69.20	0.83	66.25–71.51	68.91	1.42	67.5–68.27	67.94	0.28
Body depth	15.90–17.32	16.76	0.53	12.83–15.3	14.03	0.83	13.29–15.78	14.52	0.93	12.59–13.88	13.00	0.51
Caudal peduncle length (CPL)	16.29–19.92	18.11	1.29	17.14–19.16	18.57	0.74	16.98–20.32	18.52	0.95	18.97–20.48	19.66	0.54
Caudal peduncle depth (CPD)	11.51–14.26	13.42	1.11	10.34–12.06	10.85	0.64	10.11–12.24	11.22	0.56	11.26–11.71	11.51	0.19
Body width	11.26–11.75	11.52	0.17	9.95–11.54	10.75	0.58	10.1–13.47	11.44	0.88	10.43–10.95	10.73	0.21
Dorsal-fin length	11.64–14.12	13.31	0.98	12–15.83	14.05	1.34	9.8–16.21	13.26	2.02	10.07–11.98	10.90	0.78
Anal-fin length	14.68–15.71	15.04	0.40	14.47–16.99	15.53	0.90	12.88–15.63	14.86	0.71	12.79–14.78	13.70	0.88
Pelvic-fin length	14.26–15.22	14.79	0.39	13.47–15.67	14.42	0.75	10.35–15.47	13.16	1.33	11.2–13.29	11.83	0.85
Pectoral-fin length	17.83–18.32	18.12	0.18	15.35–19.3	17.40	1.25	11.59–19.15	15.90	2.21	12.39–14.69	13.79	0.89
In percent of head length												
Head depth at neck	63.11–64.54	64.06	0.57	50.81–60.71	53.16	3.80	46.36–55.9	52.34	2.89	46.46–48.65	48.03	0.91
Snout length	45.11–46.39	45.87	0.47	40.92–45.78	42.29	1.79	40.77–46.22	43.33	1.85	39.39–43.62	41.71	1.59
Head width at eye	69.94–74.79	72.23	1.83	50.05–72.79	57.74	7.87	51.58–65.94	58.57	4.37	46.43–48.87	47.87	0.93
Max head width	75.05–81.17	77.49	2.33	67.53–72.25	69.42	1.59	62.3–69.21	65.70	2.34	59.16–62.71	60.42	1.36
Interorbital width	22.92–28.16	25.24	1.93	23.74–26.67	25.01	1.03	16.79–26.99	23.50	2.63	25.99–27.49	26.62	0.60
eye diameter	12.80–15.81	14.08	1.09	16.54–17.94	17.29	0.52	13.25–20.27	16.61	2.56	14.69–15.9	15.10	0.48
CPD/CPL (%)	70.53–78.48	74.07	3.53	54.65–63.8	58.50	4.01	53.77–65.98	60.74	4.26	57.2–61.53	58.59	1.74
D	8 1/2			8 1/2 or 9 1/2			9 1/2			9 1/2		
A	5 1/2			5 1/2			5 1/2 or 6 1/2			5 1/2		
P	11			10 or 11			10 or 11			11		
V	8			7 or 8			6 or 8			7 or 8		
C	17			16 or 17			17			17		

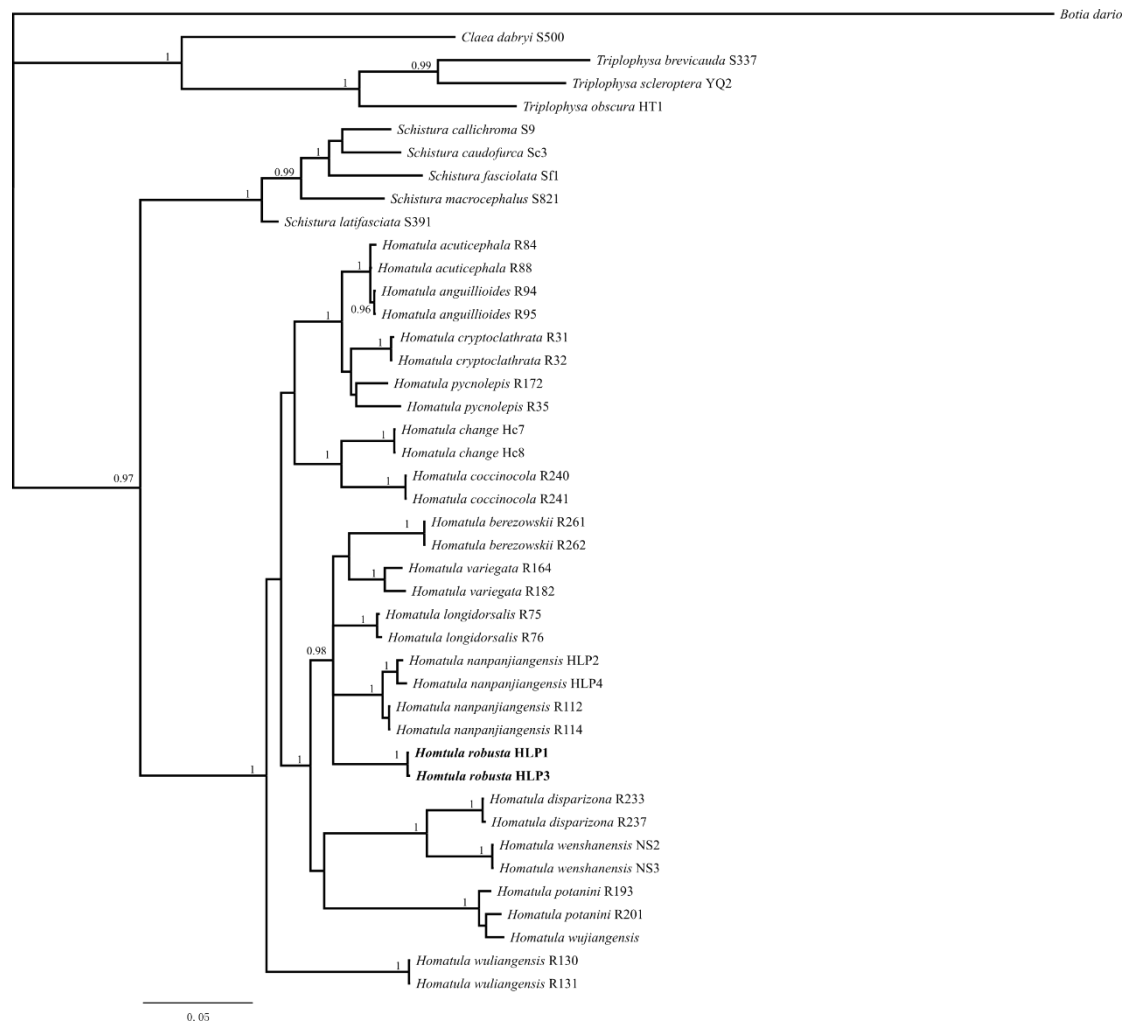


Figure 1. COI MrBayes phylogenetic reconstruction of *Homatula*. BI posterior probabilities of more than 0.95 are plotted on the branches.

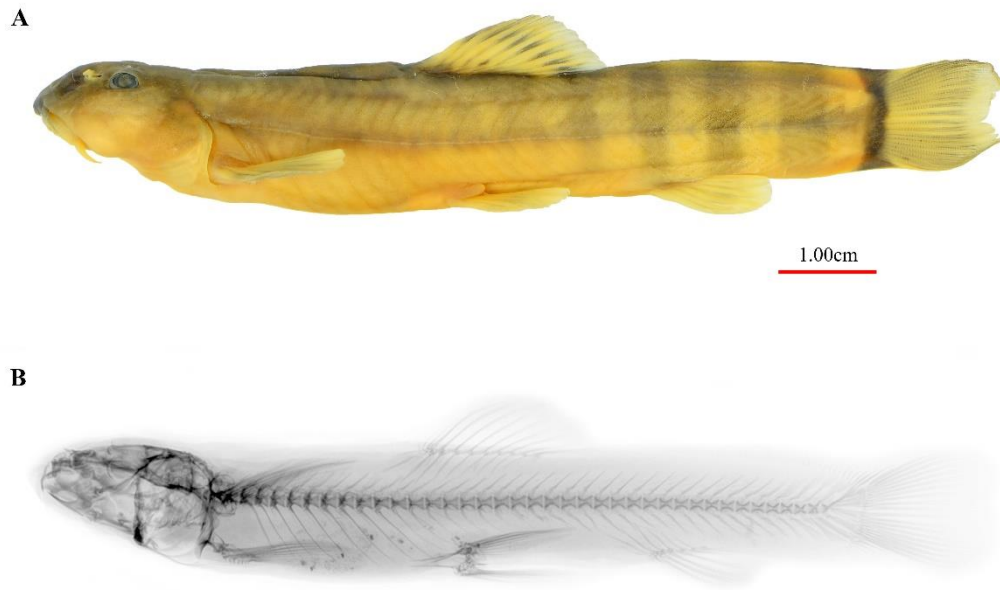


Figure 2. Lateral view (A) and X-ray (B) of *H. robusta* sp. nov., holotype, KIZ 2009000125, 83.12 mm SL.

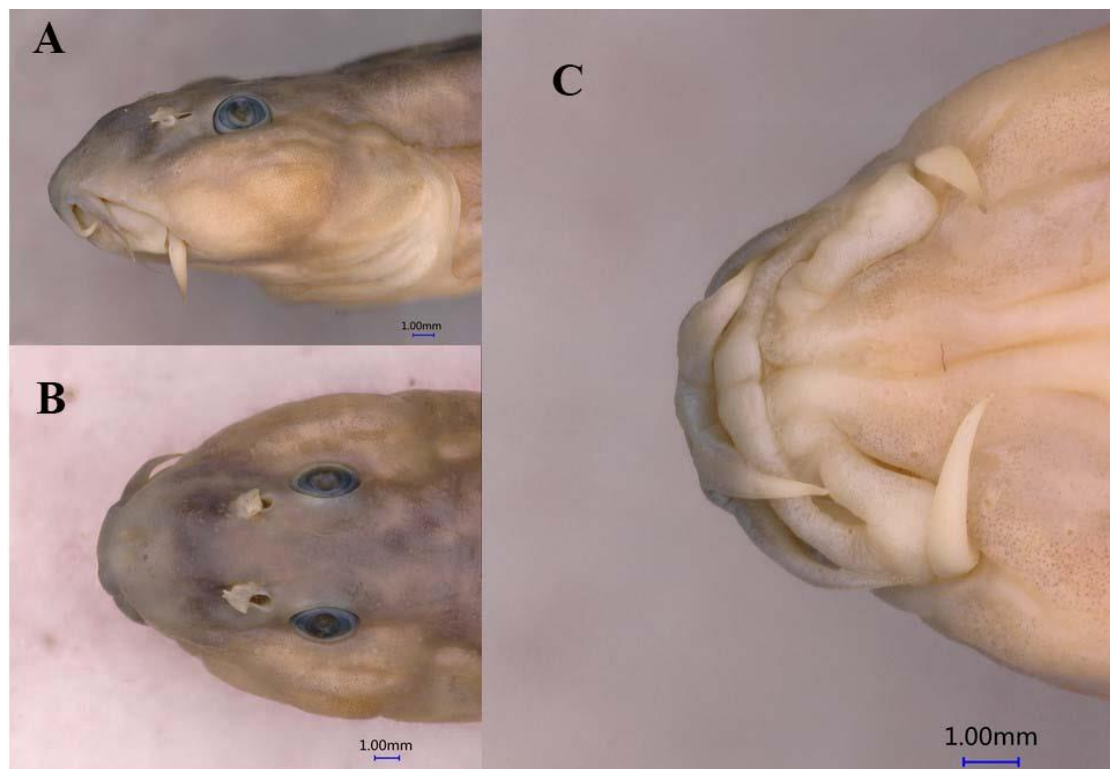


Figure 3. *Homatula robusta*, paratype, KIZ 2009000122, 81.32 mm SL, head in lateral (A), dorsal (B) and ventral (C) view



Figure 4. *H. longidorsalis* 1987003989 (a), *H. nanpanjiangensis* 1994000023 (b), *H. potanini* 2010000266 (c).

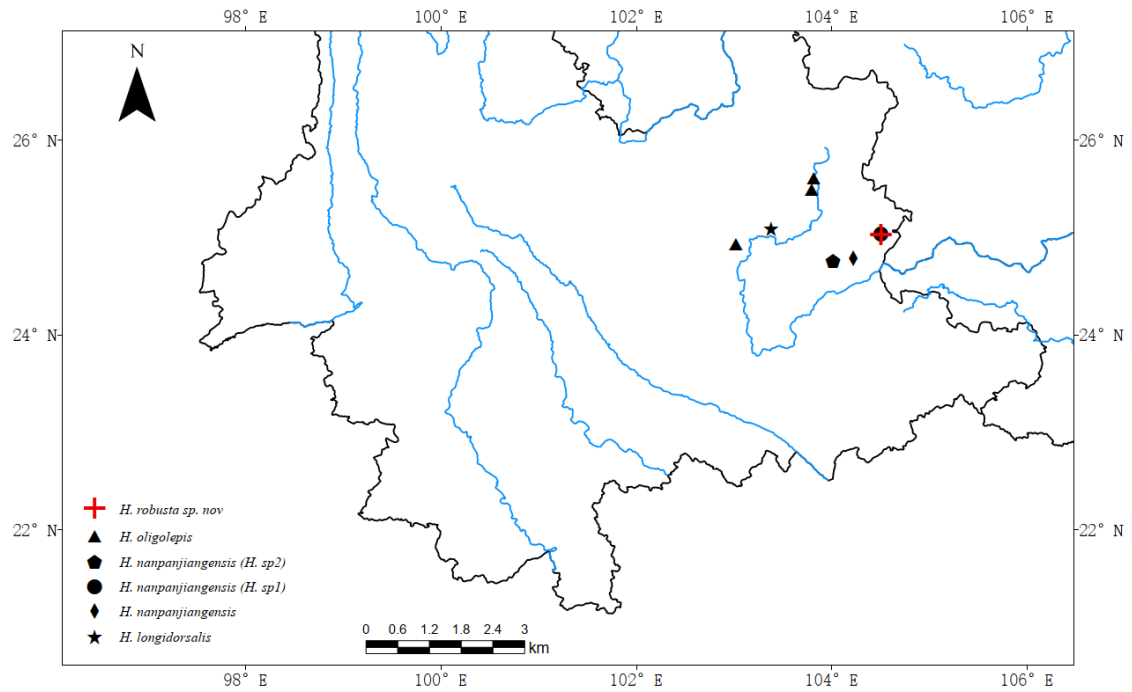


Figure 5. Distribution of *Homatula* from the Nanpanjiang River.