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

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Abstract

Based on morphological and molecular analysis of Homatula species distributed in the Nanpanjiang River in Yunnan, China, we described a new species, Homatula robusta sp. nov. It differs from its congeners by a combination of the following characters: naked and robust body with well-developed crests (caudal peduncle depth as a percentage of its length: 70.5–78.5%); lateral line complete; 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 8½; and vertebrae 37–39. It can further be distinguished from H. nanpanjiangensis by several differences of the caudal skeleton such as the number of hypural elements, the presence of epurale and the shape of neural and haemal spines. Phylogenetic analysis of the mitochondrial cytochrome c oxidase subunit I (COI) gene indicated that the new species represents an independent lineage. It is separated from other Homatula species by a minimum of 5.3% Kimura-2-parameter distance in the COI gene. Furthermore, we confirmed that Homatula wenshanensis should be a member of Homatula based on both skeleton and molecular evidence.

Keywords

Molecular phylogeny, morphology, Nanpanjiang River, osteology, taxonomy
Introduction

Homatula, a group of benthic nemacheilids distributed in the eastern slope of the Qinghai-Tibetan Plateau, was established by Nichols in 1925 based on Nemachilus potanini Günther, 1896 from the Minjiang River (a tributary of the Yangtze River, Sichuan, China) (Kottelat 1990; Bănărescu and Nalbant 1995; Min et al. 2012). Species of Homatula are characterized by the crests along the dorsal and ventral margins of the caudal peduncle supported by rudimentary procurrent caudal-fin rays, the presence of a degenerated non-ossified secondary gas-bladder chamber, and a medium to large-sized body with a maximum standard length of 190 mm (Zhu 1989; Kottelat 1990; Bănărescu and Nalbant 1995; Endruweit et al. 2018).

Currently, 21 valid species are recognized, mostly distributed in China, and only one species is recently reported from Vietnam (Nguyen et al. 2021; Zhou et al. 2021). In China, six species are in the Palaearctic drainages of the Yangtze and Yellow River, four in the Lancangjiang River (upper reaches of the Mekong River), three in the Nujiang River (Salween River), four in the Red River, and three in the Nanpanjiang River (upper reaches of the Pearl River), respectively. None of them is distributed across these large river systems.

Three Homatula species have been reported from the Nanpanjiang River, i.e., H. oligolepis (Cao & Zhu, 1989), H. longidorsalis (Yang, Chen & Kottelat, 1994) and H. nanpanjiangensis (Min, Chen & Yang, 2010). In 2009, a medium-sized loach was collected from Luoping County, Yunnan Province, China, which belongs to the Nanpanjiang River drainage. By comparing it to other Homatula species, especially the species distributed in the Nanpanjiang River, we describe it as a new species here.

Materials and methods

All specimens were collected in 2009 from Yunnan Province, China and they were fixed either in 95% ethanol or 10% formalin and transferred to 75% ethanol for preservation. For DNA analysis, tissue samples from the left pelvic fin were excised from one or more specimens and placed in 95% ethanol. General methods for measurements and counts were done following Kottelat (1990), 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. The Weberian apparatus was counted as four vertebrae. Caudal vertebrae encompassed all centra bearing a haemal 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 from snout tip to the posterior margin of the operculum, excluding the opercular membrane. Examined specimens were deposited in the collection of the Kunming Natural His-
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In order to compare skeletal morphology, we applied Computed Microtomographic (μCT) scans of the holotype of *H. robusta* (KIZ 2009000125), a paratype of *H. nanpanjiangensis* (KIZ 1994000029) and a specimen of *H. wenshanensis* (KIZ 2014005686). Specimens were scanned using a GE Phoenix v|tome|x m dual tube 300/180 kV system in the Key Laboratory of Vertebrate Evolution and Human Origins, Institute of Vertebrate Paleontology and Paleoanthropology (IVPP), Chinese Academy of Sciences. The specimen was scanned with an energy beam of 80 kV and a flux of 80*μA using a 360° rotation and then reconstructed into a 4096*4096 matrix of 1536 slices. The final CT reconstructed skeleton images were exported with a minimum resolution of 6.099 μm. The skeleton images were exported from the virtual 3D model which was reconstructed using Volume Graphics Studio 3.0. Osteological terminology generally follows that of Prokofiev (2009) and Conway (2011) with modifications.

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/) (Katoh 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 2 000 000 generations, with sampling every 1000 generations, and the first 25% of samples were discarded as burn-in.

Comparative materials

*Homatula longidorsalis* (Yang, Chen & Kottelat, 1994) (*N* = 24): Holotype: CHINA; Yunnan, Yiliang, Jiuxiang; KIZ 1987003989, 82.0 mm SL. Paratypes: CHINA; Yunnan, Yiliang, Jiuxiang; KIZ 1987003990, 3991–3993, 5090, 5091, 5736–5752, 46.0–89.5 mm SL.

*Homatula nanpanjiangensis* (Min, Chen & Yang, 2010) (*N* = 20): Holotype: CHINA; Yunnan, Qujing, Luoping; KIZ 1994000023, 86.8 mm SL. Paratypes: CHINA: Yunnan: Qujing: Luoping; KIZ 1994000018–22, 024–037, 72.4–89.7 mm SL.

*Homatula oligolepis* (Cao & Zhu, 1989) (*N* = 2): CHINA; Yunnan, Zhanyi; KIZ 1985000829, KIZ 652099, 138.2–170.7 mm SL.
Homatula potanini (Günther, 1896) \((N = 5)\): China; Sichuan, Meishan; KIZ 2010000266; China; Sichuan, Luoshan; KIZ 2010000279–82, 70.6–80.1 mm SL.

Homatula variegata (Dabry de Thiersant, 1874) \((N = 9)\): China; Sichuan, Panzhihua; KIZ 2009002724–2727, 77.4–95.2 mm SL; China; Yunnan, Zhaotong, Yanjin; KIZ 2004008050, 52–53, 57, 61, 76.3–101.6 mm SL.

Homatula laxicrathra Gu & Zhang, 2012 \((N = 2)\): China; Shanxi: Ankang; Ningshan: Weihe River: KIZ 2012002359–60, 100.5–120.5 mm SL.

Homatula guanheensis Zhou, Ma, Wang, Tang & Nie, 2021 \((N = 6)\): China; Shanxi, Ankang, Ningshan, Yangtze River; KIZ 2005014508–13, 104.5–135 mm SL.

Homatula wuliangensis Min, Yang & Chen, 2012 \((N = 34)\): Holotype: China; Yunnan, Jingdong; KIZ 2008008158, 181.9 mm SL. Paratypes: China; Yunnan, Jingdong; KIZ 2008008156–157, 159–172, 175–176, 179, 184, 197, 199–201, 203, 205, 207, 211, 214–215, 316–318, 64.6–191.1 mm SL.

Homatula disparizona Min, Yang & Chen, 2013 \((N = 21)\): Holotype: China; Yunnan, Wenshan, Xichou; KIZ 2012000623. Paratypes: China; Yunnan, Wenshan, Xichou; KIZ 2012000622, 624–634. China; Yunnan, Wenshan, Xichou; KIZ 2014005623–30, 62.8–92.4 mm SL.

Homatula acuticephala (Zhou & He, 1993) \((N = 26)\): China; Yunnan, Dali, Haixihai; KIZ 2008005990–6015, 33.7–51.5 mm SL.

Homatula anguillioideis (Zhu & Wang, 1985) \((N = 12)\): China; Yunnan, Dali, Eryuan, KIZ 2008006532–6543, 68.8–143.3 mm SL.

Homatula pycnolepis Hu & Zhang, 2010 \((N = 6)\): China; Yunnan, Dali, Yangbi; KIZ 1998004817, 19, 22, 25, KIZ 2009005288, KIZ 2009005388, 120.1–177.1 mm SL.

Homatula change Endruweit, 2015 \((N = 12)\): Holotype: China; Yunnan, Puer, Jiangcheng; KIZ 2012004205, 107.6 mm SL. Paratypes: China; Yunnan, Puer, Jiangcheng; KIZ 2012004208, 4209, 4211, 4215–18, 4221–24, 37.9–76.5 mm SL.

Homatula coccinocola Endruweit, Min & Yang, 2018 \((N = 5)\): Holotype: China; Yunnan, Honghe; KIZ 2011002847, 99.6 mm SL. Paratypes: China; Yunnan, Honghe; KIZ 2012001866–1869, 51.1–79.0 mm SL.

Homatula cryptoclathrata Li, Che & Zhou, 2019 \((N = 2)\): China; Yunnan, Lincang; KIZ 2005012637, 39, 91.3–100 mm SL.

Homatula wenshanensis Li, Yang, Li & Liu, 2017 \((N = 3)\): China; Yunnan, Wenshan; KIZ 2014005685–87, 60.2–110.9 mm SL.

We obtained information on H. wujiangensis Ding & Deng, 1990 from Ding and Deng (1990), on H. anteridorsalis Li, Che & Zhou, 2019 and H. nigra Li, Che & Zhou, 2019 from Li, Che and Zhou (2019), and H. dotui Nguyen, Wu, Cao & Zhang, 2021 from Nguyen et al. (2021).

GenBank Accession numbers are listed in Table 1.
Table 1. Voucher and Genbank numbers for study samples; sequences downloaded from GenBank are without voucher numbers.

| Taxon                        | Voucher number | GenBank number |
|------------------------------|----------------|----------------|
| Triplophysa brevicauda       | KIZ 050422024  | MZ677092       |
| Triplophysa scleroptera      | KIZ 20100076   | MZ677093       |
| Triplophysa obscura          |                | MG238209       |
| Claet daphyi                 | KIZ 2009003600 | MZ677094       |
| Schistura fasciola           | KIZ 2012003668 | MZ677096       |
| Schistura macrocephalus      | KIZ 2010003135 | MZ677098       |
| Schistura latifasciata       | KIZ CXY2008062 | MZ677099       |
| Schistura callichroma        | KIZ 200401055  | MZ677095       |
| Schistura caudofurca         | KIZ 2015030722 | MZ677097       |
| Homatula wujiangensis        | KIZ 2010000235 | MZ677100       |
| Homatula potanini            | KIZ 2010000281 | MZ677101       |
| Homatula guanheensis         | KIZ 2005014512 | MZ677105       |
| Homatula guanheensis         | KIZ 2005014513 | MZ677104       |
| Homatula longidorsalis       | KIZ 2008005909 | MZ677121       |
| Homatula longidorsalis       | KIZ 2008005910 | MZ677118       |
| Homatula variegata           | KIZ 2009002770 | MZ677110       |
| Homatula variegata           | KIZ 2009002724 | MZ677115       |
| Homatula robusta             | KIZ 2009000146 | MZ677106       |
| Homatula robusta             | KIZ 2009000144 | MZ677107       |
| Homatula coccinocola         | KIZ 2012001867 | MF953210       |
| Homatula coccinocola         | KIZ 2012001868 | MF953211       |
| Homatula change              | KIZ 2015051116 | MZ677109       |
| Homatula change              | KIZ 2015051117 | MZ677108       |
| Homatula cryptoclathrata     | KIZ 2005012639 | MZ677116       |
| Homatula cryptoclathrata     | KIZ 2005012637 | MZ677117       |
| Homatula pycnolepis          | KIZ 2009003860 | MZ677111       |
| Homatula pycnolepis          | KIZ 20050423002 | MZ677114     |
| Homatula anguillinioides     | KIZ 2008006539 | MZ677124       |
| Homatula acuticephala        | KIZ 2008005994 | MZ677122       |
| Homatula anguillinioides     | KIZ 2008006536 | MZ677125       |
| Homatula acuticephala        | KIZ 2008005990 | MZ677123       |
| Homatula wulianensis         | KIZ 2008008160 | MF953221       |
| Homatula wulianensis         | KIZ 2008008159 | MF953220       |
| Homatula wenshanensis        | KIZ 2014005686 | MZ677102       |
| Homatula wenshanensis        | KIZ 2014005687 | MZ677103       |
| Homatula disparizona         | KIZ 2012000626 | MF953194       |
| Homatula disparizona         | KIZ 2012000622 | MF953190       |
| Botia dario                  |                | KT781503       |

Results

Taxonomy

Homatula robusta sp. nov.

http://zoobank.org/A27C86D0-FD58-448C-9A85-6129B1A18F66
Figs 1–5, Tables 2, 3

Material. Holotype. KIZ 2009000125, 83.12 mm SL; collected by Wansheng Jiang and Weiying Wang on 14 March 2009 at Changdi village, Luoping County, Qu-
Figure 1. Lateral A dorsal B and ventral views C of *H. robusta* sp. nov., holotype, KIZ 2009000125, 83.12 mm SL.

Figure 2. *Homatula robusta* sp. nov., paratype, KIZ 2009000122, 81.32 mm SL, head in lateral A dorsal B and ventral C views.
jing City, Yunnan Province, China; Nanpanjiang River, upper Pearl River (25°02’N, 104°30’E; ca 1210 m). **Paratypes.** KIZ 2009000122, 144, 146, 3 ex. 61.16–81.32 mm SL, same data as for holotype.

**Diagnosis.** The new species can be distinguished from all other species of *Homatula* by having the following combination of characters: naked and robust body with well-developed crests (caudal peduncle depth as a percentage of its length: 70.5–78.5%), lateral line complete, median notch on lower jaw, median gap on lower lip, three pairs of short barbels, with maxillary barbels extending posteriorly to the anterior edge of eyes, branched dorsal-fin rays 8½, emarginated caudal fin, and vertebrae 37–39.
Description. Anterior body cylindrical, posterior body laterally compressed; robust, depth 5.8–6.3 times in length. Caudal peduncle stout, depth 1.27–1.42 times in its length. Crests on dorsal and ventral midlines present and supported by rudimentary procurrent caudal-fin rays; dorsal crest starting immediately posterior of dorsal-fin base, ventral crest starting immediately posterior of anal-fin base.

Snout blunt in lateral view, cheeks inflated. Eyes elliptical horizontally, dorsolaterally positioned. Mouth inferior, slightly arched. Anterior nostril in flap, next to posterior nostril. Lips moderately thick, upper lip smooth, slightly notched medially, lower lip with shallow furrows and median gap. Processus dentiformis on upper jaw present with circular arc edge; lower jaw spoon-like with a median notch. Three pairs of barbels, maxillary barbel reaching anterior margin of eye, outer rostral barbel reaching inner corner of mouth and inner rostral barbel not.

Dorsal-fin rays iv, 8½, distal margin slightly convex. Pectoral-fin rays 11, reaching about halfway from insertion of pectoral fin to insertion of pelvic fin. Pelvic-fin rays 6–8, reaching close to anus, inserted opposite of the first branched dorsal-fin ray. Anus located 1.53–2.17 times eye diameter in front of anal-fin origin. Anal-fin rays iii, 5½. Caudal-fin rays 9+8, distal margin of caudal fin emarginated with upper and lower lobes almost equal in length. Moderate axillary pelvic lobe with free tip.

Body scaleless, or sparse scales scattered along lateral line after posterior end of anal-fin base, embedded beneath skin. Lateral line completed with 85–89 pores. Supraorbital pores 7, postorbital pores 3, sub- and preorbital pores 12, preoperculo-mandibular pores 10, supratemporal pores 3.

Table 2. Measurements of four type specimens of *Homatula robusta* sp. nov.

| Measurements (mm)               | 2009000125 | 2009000122 | 2009000144 | 2009000146 |
|--------------------------------|------------|------------|------------|------------|
| SL                             | 83.12      | 81.32      | 73.70      | 61.16      |
| Head length                    | 18.76      | 18.28      | 16.58      | 13.28      |
| Predorsal length               | 38.78      | 37.34      | 35.16      | 29.06      |
| Preventral length              | 41.80      | 40.94      | 37.06      | 29.98      |
| Preanal length                 | 60.42      | 60.46      | 54.52      | 45.60      |
| Preanus length                 | 55.92      | 56.00      | 50.16      | 41.96      |
| Body depth                     | 14.40      | 13.82      | 11.72      | 10.28      |
| Caudal peduncle length (CPL)   | 16.56      | 14.78      | 13.32      | 9.96       |
| Caudal peduncle depth (CPD)    | 11.68      | 11.60      | 10.20      | 7.04       |
| Body width                     | 9.36       | 9.38       | 8.66       | 7.06       |
| Dorsal-fin length              | 11.28      | 11.48      | 10.24      | 7.12       |
| Anal-fin length                | 12.32      | 11.94      | 11.58      | 9.14       |
| Pelvic-fin length              | 12.12      | 12.28      | 11.22      | 8.72       |
| Pectoral-fin length            | 14.82      | 14.76      | 13.50      | 11.12      |
| Head depth at neck             | 11.84      | 11.72      | 10.70      | 8.56       |
| Snout length                   | 8.64       | 8.48       | 7.48       | 6.10       |
| Head width at eye              | 13.12      | 13.34      | 12.40      | 9.46       |
| Max head width                 | 14.08      | 13.90      | 12.88      | 10.78      |
| Interorbital width             | 4.30       | 4.44       | 4.24       | 3.74       |
| eye diameter                   | 2.62       | 2.34       | 2.28       | 2.10       |
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Figure 4. Lateral CT scans of: **A** *H. robusta* sp. nov., KIZ 2009000125, lateral view and structure of caudal skeletons **B** *H. nanpanjiangensis*, KIZ 1994000029, lateral view and structure of caudal skeletons **C** *H. wenshanensis*, KIZ 2014005686, lateral view. Abbreviations: ph—partypurale, ns—neural spine, hs—haemal spine, h—hypural, epu—epurale, pst—pleurostyle.

Vertebrae (three specimens), 4+37–39; four hypural elements with h-1 & h-2 fused, epurale present, last four neural spines (ns-1 to ns-4) and last three haemal spines (hs-1 to hs-3) on the caudal vertebrae are significantly enlarged. U-shaped stomach; intestine almost straight, with small bend next to stomach posterior. Longest recorded length is 83.1 mm SL, 95.7 mm total length (KIZ 2009000125, holotype).
Table 3. Morphometrics of *Homatula robusta* sp. nov. and *Homatula nanpanjiangensis*. SD, standard deviation.

| Measurements                          | H. robusta |            |           |            | H. nanpanjiangensis |            |
|---------------------------------------|------------|------------|----------|------------|---------------------|----------|
|                                      | N | Min | Max | Mean | SD | N | Min | Max | Mean | SD |
| SL (mm)                               | 4 | 61.1 | 83.12 | 74.83 | 8.65 | 19 | 63.82 | 88.74 | 78.37 | 7.56 |
| As percent of SL                      | 4 | 13.28 | 18.76 | 16.73 | 2.15 | 19 | 15.46 | 20.36 | 18.63 | 1.53 |
| Head length                           | 4 | 45.92 | 47.71 | 46.95 | 0.72 | 19 | 45.62 | 50.47 | 47.98 | 1.37 |
| Predorsal length                      | 4 | 49.02 | 50.34 | 49.98 | 0.56 | 19 | 49.58 | 54.03 | 51.14 | 1.14 |
| Preanal length                        | 4 | 72.69 | 74.56 | 73.89 | 0.73 | 19 | 71.25 | 76.94 | 74.37 | 1.47 |
| Preanus length                        | 4 | 67.28 | 68.86 | 68.20 | 0.61 | 19 | 66.55 | 70.46 | 69.03 | 1.01 |
| Body depth                            | 4 | 15.9 | 17.32 | 16.76 | 0.53 | 19 | 12.64 | 15.30 | 13.77 | 0.73 |
| Caudal peduncle length (CPL)          | 4 | 16.29 | 19.92 | 18.11 | 1.29 | 19 | 16.49 | 20.92 | 18.64 | 1.06 |
| Caudal peduncle depth (CPD)           | 4 | 11.51 | 14.26 | 13.42 | 1.11 | 19 | 9.45  | 12.06 | 10.83 | 0.66 |
| Body width                            | 4 | 11.26 | 11.75 | 11.52 | 0.17 | 19 | 9.24  | 13.65 | 10.73 | 1.10 |
| Dorsal-fin length                     | 4 | 11.64 | 14.12 | 13.31 | 0.98 | 7  | 12.00 | 15.83 | 14.21 | 1.19 |
| Anal-fin length                       | 4 | 14.68 | 15.71 | 15.04 | 0.40 | 6  | 14.47 | 16.99 | 15.56 | 0.82 |
| Pelvic-fin length                     | 4 | 14.26 | 15.22 | 14.79 | 0.39 | 6  | 13.47 | 15.67 | 14.40 | 0.68 |
| Pectoral-fin length                   | 4 | 17.83 | 18.32 | 18.12 | 0.18 | 6  | 15.35 | 19.30 | 17.49 | 1.16 |
| As percent of head length             | 4 | 63.11 | 64.54 | 64.06 | 0.57 | 19 | 49.88 | 61.19 | 55.01 | 3.79 |
| Head depth at neck                    | 4 | 45.11 | 46.39 | 45.87 | 0.47 | 19 | 40.92 | 47.69 | 44.16 | 2.28 |
| Snout length                          | 4 | 69.94 | 74.79 | 72.23 | 1.83 | 19 | 49.63 | 72.79 | 59.56 | 6.77 |
| Head width at eye                     | 4 | 75.05 | 81.17 | 77.49 | 2.33 | 19 | 63.22 | 77.27 | 69.29 | 3.72 |
| Max head width                        | 4 | 22.92 | 28.16 | 25.24 | 1.93 | 19 | 22.21 | 26.67 | 24.29 | 1.17 |
| Interorbital width                    | 4 | 12.8 | 15.81 | 14.08 | 1.09 | 19 | 13.79 | 17.94 | 15.95 | 1.05 |
| Eye diameter                          | 4 | 70.53 | 78.48 | 74.07 | 3.53 | 19 | 49.50 | 65.66 | 58.22 | 4.22 |

Figure 5. Distribution of *Homatula* from the Nanpanjiang River.
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**Coloration of preserved specimens.** Body light brown with vertical brown bars in formalin-fixed specimens. Bars on predorsal body usually blurred and indistinct, or countable and separated by extraordinary narrow interspaces just in KIZ 2009000122. Bars and interspaces getting wider towards caudal-fin base and approximately equal width on posterior body. Usually, bars regularly shaped and jointed on dorsal midline from opposite sides, or two bars met on upper body and last bar diffused or formed by two combined bars (KIZ 2009000122). Dark black bar on caudal-fin base, reaching dorsal and ventral extremities. All fin rays pale brown and covered by melanophores, series of stripes halfway up each dorsal-fin ray. Color in alcohol-fixed specimens is paler than those in formalin-fixed specimens.

**Sexual dimorphism.** No sexual dimorphism was observed.

**Etymology.** Robusta is a Latin word meaning ‘strong’, in reference to the stout body and caudal peduncle. The Chinese common name is suggested as 粗壮荷马条鳅.

**Distribution.** Only known from the type locality, Changdi village, Luoping County, Qujing City, Yunnan Province, China (Fig. 5).

**Phylogenetic characterization and relationships**

The COI molecular dataset included 39 terminal taxa representing 25 species, 15 of which belonged to Homatula (Table 1). The COI gene was 1116 bp in length with 313 informative sites, 68 singleton sites, and 717 constant sites. The Bayesian inference (BI) phylogenetic analysis recovered the monophyly of *H. robusta*, *H. wenshanensis* and most species of *Homatula* (Fig. 6). The K2P distance between *H. robusta* and its closest species on the tree, *H. longidorsalis*, is 5.3%. *Homatula wenshanensis* was the sister group of *H. disparizona*. *Homatula potanini* and *H. wujiangensis* (Ding & Deng, 1990) 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%.

**Discussion**

*Homatula robusta* sp. nov. can be distinguished from its congeners except *H. disparizona*, *H. nanpanjiangensis*, *H. wujiangensis*, *H. oligolepis*, *H. dotui* and *H. wenshanensis* by body scaleless, or sparse scales scattered along the lateral line after anal-fin base (vs. anterior body scaleless or with rudimentary scales in *H. berezowskii*, *H. guanheensis*, *H. laxiclavata*, *H. longidorsalis*, *H. potanini*, and *H. variegata*; whole body scaled besides the head in *H. acuticephala*, *H. anguillioides*, *H. anteridorsalis*, *H. change*, *H. coccinocola*, *H. cryptoclavata*, *H. nigra*, *H. pycnolepis*, *H. wuliangensis*). The new species can be distinguished from *H. dotui* and *H. wujiangensis* by the complete lateral line (vs. incomplete), presence of brown bars on the body (vs. absence in *H. dotui*), 37–39 vertebrae (vs. 31 in *H. dotui*), normally developed eye, 12.8–15.8% of HL (vs. rudimentary, 4–6% in *H. dotui*), caudal-fin rays 9+8 (vs.
Figure 6. COI MrBayes phylogenetic reconstruction of Homatula. BI posterior probabilities of more than 0.95 are plotted on the branches.

8+7 in *H. dotui*), dorsal crest reaching forward beyond the origin of anal-fin base (vs. not reaching the posterior point of anal-fin base in *H. wuijiangensis*) and from *H. oligolepis* by the regular bars on the side of the body (vs. vermiform markings on the
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It can be distinguished from *H. disparizona* and *H. wenshanensis* by the stronger body with BD 15.9–17.3% of SL (vs. 12.1–15.4% in *H. disparizona*, 12.1–14.8% in *H. wenshanensis*), vertebrae 37–39 (vs. 39–40 in *H. disparizona*, 47–48 in *H. wenshanensis*), stout caudal peduncle with CPD 70.5–78.5% of Caudal peduncle length (CPL) (vs. 47–62% in *H. disparizona*, 27.3–35% in *H. wenshanensis*), and the median notch on the lower jaw present (vs. absent), caudal fin slightly emarginated (vs. forked in *H. wenshanensis*). *Homatula robusta* can be distinguished from its most similar species, *H. nanpanjiangensis*, on external morphology by the stouter caudal peduncle with CPD 70.5–78.5% of CPL (vs. 49.5–65.7%), deeper body depth (BD) 15.9–17.3% of SL (vs. 12.6–15.3%), shorter barbel with maxillary barbel reaching the anterior margin of the eye (vs. between middle and posterior margin of eye) (Table 3, Fig. 3C), and differed from *H. nanpanjiangensis* on the structures of the caudal skeleton by having four hypural elements with h-1 and h-2 fused (vs. five, h-1 and h-2 separated), epurale present (vs. absent), last four neural spines and last three haemal spines of the caudal centra significantly enlarged (vs. slightly enlarged) (Fig. 4).

*Homatula wenshanensis* was questioned as member of the genus *Homatula* by Nguyen et al. (2021), because of its indistinct adipose crests along the dorsal and ventral midlines of caudal peduncle, a forked caudal fin and 4+47–48 vertebrae that are not shared by species of *Homatula*. The results of our skeleton scan showed that *H. wenshanensis* has the typical generic character of *Homatula* – crests on caudal peduncle supported by rudimentary procurent rays (Fig. 4C) – and our COI-based phylogeny showed that *H. wenshanensis* formed an independent lineage sister to *H. disparizona*. Therefore, *H. wenshanensis* is confirmed as a species of the genus *Homatula*.

*Homatula* is previously believed to be restricted to China. Recently, *H. dotui*, a cave-dwelling species, was reported from central Vietnam (Nguyen et al. 2021). *Homatula dotui* is between *Schistura* and *Homatula* as an independent lineage in the phylogenetic tree built by the cytb gene (Nguyen et al. 2021), which indicates that this cavefish species could belong to an undescribed genus. As stated by Nguyen et al. (2021: 8), a further study should be addressed to confirm the placement of *H. dotui*.

Three species of *Homatula* have been previously reported from the Nanpanjiang River: *H. oligolepis* and *H. longidorsalis* are distributed in the upper Nanpanjiang River; *H. nanpanjiangensis* is distributed in the middle Nanpanjiang River. They possess an elongate body of medium to large size, scaleless (*H. oligolepis* and *H. nanpanjiangensis*) or at least scaleless on the predorsal body (*H. longidorsalis*), 9 ½ branched dorsal-fin rays (*H. oligolepis* and *H. longidorsalis*) or 8 ½ (a few 9 ½ in *H. nanpanjiangensis*), regular vertical bars on each side of body, and bars in front of dorsal-fin base conspicuously thinner than those behind (*H. longidorsalis* and *H. nanpanjiangensis*) or vermiform markings on body and dorsal head (*H. oligolepis*). Here, *H. robusta* sp. nov. is reported from the middle Nanpanjiang River with a stout body. For better identification, a key to species distributed in the Nanpanjiang River is provided.
Key to species of *Homatula* in the Nanpanjiang River

1 Body scaleless or with rudimentary scales present at caudal peduncle ............2
   - Scales clearly present, covering posterior of body at least, anterior nostril in short tube, 9 ½ branched dorsal-fin rays ...............................**H. longidorsalis**

2 Medium-sized body with regular bars on body, interspaces thinner than bars on predorsal body, SL up to 88.7 mm ........................................3
   - Large-sized body with vermiform markings on body and head, SL up to 170.7 mm .................................................................**H. oligolepis**

3 Well-developed crests with CPD 70.5–78.5% of CPL, maxillary barbel reaching anterior margin of eye, no more than 13 bars, four hypural elements, epural present, last four neural spines and last three haemal spines on caudal vertebrae significantly enlarged ...............................**H. robusta sp. nov.**
   - Medium crests with CPD 49.5%–65.7% of CPL, maxillary barbel reaching between middle and posterior margin of eye, ~16 bars on average, five hypural elements, epural absent, neural and haemal spines on caudal vertebrae slightly enlarged ...............................**H. nanpanjiangensis**

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