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Source: *Folia Zoologica*, 64(1) : 1-16

Published By: Institute of Vertebrate Biology, Czech Academy of Sciences

URL: <https://doi.org/10.25225/fozo.v64.i1.a1.2015>

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Three new species of cobitid fish genus *Cobitis* (Teleostei, Cobitidae) from the River Pearl basin of China

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Received 24 February 2014; Accepted 13 March 2015

Abstract. Morphology features and sequences of mitochondrial cytochrome *b* (cyt *b*) gene were analyzed of the species *Cobitis* Linnaeus, 1758 from the River Pearl basin of China. Three new species of *C. leptosoma* sp. nov., *C. wumingensis* sp. nov., and *C. obtusirostra* sp. nov. were described. *C. leptosoma* is distinguishable from its congeners by a long knife-shaped lamina circularis; a sharp and nearly filamentous tip of the pectoral-fin ray in males; four Gambetta pigment lines, L₂ usually being absent, L₅ consisting of 10–12 oval blotches; and a round or oblong spot smaller than or equal to the eye diameter on the upper part of the caudal peduncle. *C. wumingensis* is distinguishable by small barbels, maxillo-mandibular barbels shorter than eye diameter; a long fingerlike lamina circularis; a sharp and nearly filamentous tip of the pectoral-fin ray in males; L₅ showing sexual dimorphism; a deeper bluish band pigment in females; a surface pigment with 13–14 blotches in males; and a conspicuous jet-black roundish spot smaller than or equal to the eye diameter on the upper part of the caudal peduncle. *C. obtusirostra* is distinguished by a cystiform lamina circularis; a sharp and nearly filamentous tip of the pectoral-fin ray in males; L₅ consisting of 8–10 rounded blotches; and a conspicuous jet-black arcuate spot smaller than the eye diameter on the upper part of the caudal peduncle.

Key words: loaches, taxonomy, Guangxi, Guangdong Province, molecular phylogeny

Introduction

The cobitid fish genus *Cobitis* L., 1758, one of the largest in the subfamily Cobitinae, is widely distributed throughout Eurasia (excluding the Tibetan Plateau), its adjacent islands, and northwestern Africa (Chen 1981, Sawada 1982). Over 241 species have been described (Froese & Pauly 2014), and 15 of those are from China. Their high diversity and widespread distribution have made cobitids one of the most difficult groups of fish to understand taxonomically and systematically (Nalbant 1993, Šlechtová et al. 2008). Various researchers have attempted to classify these fish and decipher their phylogenetic history. A number of species once referred to as *Cobitis* now belong to other genera like *Iksookimia*, *Koreocobitis*, and *Microcobitis*. One of the latest efforts employing phylogenetic systematics indicated that the genera *Cobitis*, *Iksookimia*, and *Niwaëlla* are not monophyletic (Šlechtová et al. 2008). Similar results were also found in studies by Tang et al. (2005,

2006). The genera *Cobitis*, *Iksookimia*, *Kichulchoia*, *Koreocobitis*, *Misgurnus*, *Niwaëlla*, *Paramisgurnus*, *Sabanejewia*, and *Microcobitis* together form a monophyletic lineage within the family Cobitidae (Šlechtová et al. 2008). *Cobitis* is in most cases diagnosed by the characteristics: one lamina circularis is present on the second pectoral-fin ray in males, the bifid suborbital spine situated in front of eyes, and three distinct longitudinal rows of black pigmentation on the body sides; one along the dorsal line, one along the lateral line and one at about half distance between these two (Bohlen & Harant 2010).

Southeast Asia is believed to be the origin of Cobitidae due to their higher diversification in this area (Chen 1981, Chen & Zhu 1984, Bănărescu 1990). Southern China, one origin of the cobitid fish, has complex and varied land forms. The River Pearl is the biggest river in southern China, flowing throughout the Guangxi Zhuang Autonomous Region, and the Yunnan, Guizhou, and Guangdong Provinces. Only four

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species of the genus *Cobitis* are known from southern China: *C. arenae* (Lin, 1934), *C. multimaculata* Chen & Chen, 2011, *C. microcephala* Chen & Chen, 2011 and *C. australis* Chen, Chen & Chen, 2013. *C. arenae* was first described by Lin (1934) as *Misgurnus arenae* based on the specimens collected from a shallow stream near the Western Lake of Hui-yang District, Guangzhou Province.

Nichols (1943) transferred this species to the genus *Cobitis* based on the suborbital spine and the body colour characteristic. Kottelat (2001) recorded this species in the Vietnamese River Lo basin and transferred it to the genus *Acantopsis*. However, *C. arenae* did not exactly fit the proper definition of *Acantopsis*, so Chen & Chen (2005, 2011, 2013) considered it a valid species in the genus *Cobitis*. *C. arenae* is widely distributed throughout the Guangxi, Guangdong, and Hainan Provinces in China and Northern Vietnam. The other three species were recently described by Chen & Chen (2011) and Chen et al. (2013). *C. multimaculata* and *C. microcephala* are found in the River Nanliu. *C. microcephala* is also found in the River Beiliu (a tributary of the River Pearl basin). *C. australis* is found in the Yujiang and Yongjiang Rivers (tributaries of the River Pearl basin). In this paper, morphological variation and the mitochondrial cytochrome *b* gene (1140 bp) were used to re-examine cobitid specimens collected from the River Pearl basin and three new species were described.

Material and Methods

The examined materials were collected from the Lijiang and Wuming Rivers in Guangxi and the River Lianjiang in the Guangdong Province during the spring (in April 1975 and 2006) and summer (in July 1958), which all belong to the River Pearl basin south of China (Fig. 1). Materials used in morphology were preserved in 10 % formaldehyde solution, and specimens for molecular analyses were preserved in 95 % ethanol. All study specimens were deposited in the Freshwater Fish Museum (FFM) of the Institute of Hydrobiology (IHB) at the Chinese Academy of Sciences (CAS) in Wuhan (Hubei Province).

Nineteen morphometric variables were measured according to procedures by Chen & Chen (2011, 2013). All measurements are given in millimeters (mm). Fin-rays (simple and branched) were counted under transmitted light using a binocular stereomicroscope. Vertebrae (including the Weberian ossicles and the hypural complex) were counted by examining the negatives of roentgenograms. The

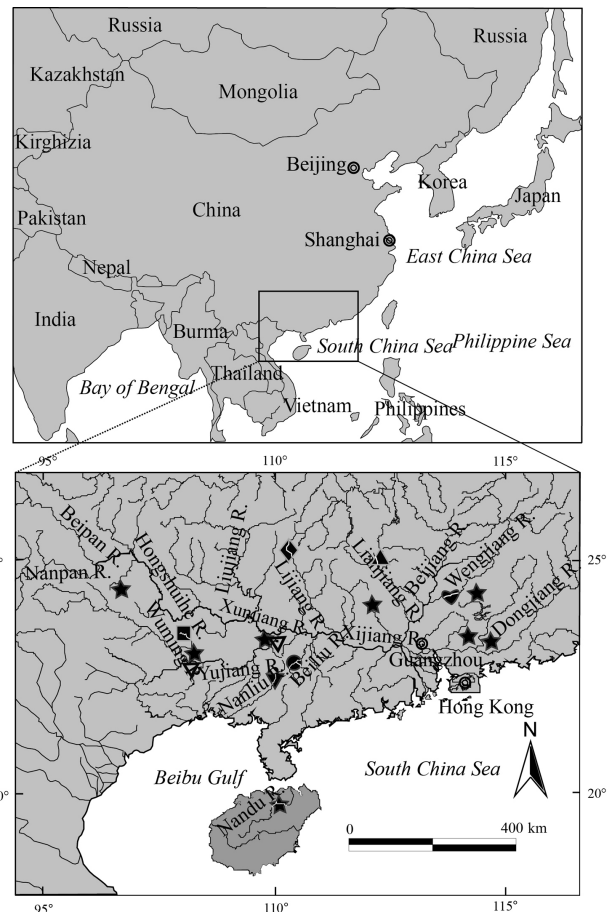


Fig. 1. Map showing the collection sites (i.e. currently known localities) of seven species of the genus *Cobitis* in southern China. (★) *C. arenae*, (▼) *C. multimaculata* and *C. microcephala*, (●) *C. microcephala*, (▽) *C. australis*, (◆) type locality of *C. leptosoma* sp. nov., (■) type locality of *C. wumingensis* sp. nov., (▲) type locality of *C. obtusirostra* sp. nov. and locality of *C. arenae*, (♥) the other locality of *C. obtusirostra*.

roentgenograms were made of the lateral aspect of the fish using a medical X-ray system. Scales were collected from the subdorsal region between the dorsal fin origin and lateral line and photographed using a Leica DC180 camera attached to a Leica GZ6 stereomicroscope. Notations (L_1 - L_5) describing lines and speckles on the dorsolateral side of the trunk followed those designated by Takeda & Fujie (1945). L_1 and L_2 altogether were identical with zone one as described by Gambetta (1934), who distinguished four zones on the dorsolateral side. L_3 , L_4 , and L_5 correspond to zones 2, 3, and 4, respectively.

Total DNA was extracted from muscle tissue or fin clips using the standard phenol-chloroform method (Sambrook et al. 1989). The complete mitochondrial cytochrome *b* gene was used to identify the status of these specimens with the following primers: L14724 (5'-GAC TTG AAA AAC CAC CGT TG-3') and H15915 (5'-CTC CGA TCT CCG GAT TAC

Table 1. Taxa analysed in this study, their sites of origin and their GenBank Accession numbers.

| Scientific name in source | Locality | Accession Nos. |
|--|--------------------------------------|----------------|
| <i>Cobitis arenae</i> | China, Hainan, R. Nanduijiang | JX888905 |
| <i>Cobitis australis</i> 1 | China, Guangxi, R. Yongjiang | KC753352 |
| <i>Cobitis australis</i> 2 | China, Guangxi, R. Yujiang | KC753353 |
| <i>Cobitis biwae</i> 1 | | AB084395* |
| <i>Cobitis biwae</i> 2 | | AB084412* |
| <i>Cobitis choii</i> 1 | Kim et al. (2008) | EU656112* |
| <i>Cobitis choii</i> 2 | | EU333980* |
| <i>Cobitis crassicauda</i> 1 | China, Jiangxi, R. Xinjiang | JX888909 |
| <i>Cobitis crassicauda</i> 2 | China, Jiangxi, R. Xinjiang | KP133114 |
| <i>Cobitis crassicauda</i> 3 | China, Jiangxi, R. Xinjiang | |
| <i>Cobitis dolichorhynchus</i> 1 | China, Fujiang, R. Jiulongjiang | JX888908 |
| <i>Cobitis dolichorhynchus</i> 2 | China, Fujiang, R. Jiulongjiang | |
| <i>Cobitis fasciola</i> 1 | China, Jiangxi, R. Xinjiang | JX888910 |
| <i>Cobitis fasciola</i> 2 | China, Jiangxi, R. Xinjiang | KP133115 |
| <i>Cobitis granoei</i> | Tang et al. (2006) | DQ105242* |
| <i>Cobitis hankugensis</i> | | EU670753* |
| <i>Cobitis leptosoma</i>1 sp. nov. | China, Guagnxi, R. Lijiang | KP133117 |
| <i>Cobitis leptosoma</i>2 sp. nov. | China, Guagnxi, R. Lijiang | |
| <i>Cobitis leptosoma</i>3 sp. nov. | China, Guagnxi, R. Lijiang | |
| <i>Cobitis lutheri</i> 1 | China, Heilongjiang, R. Heilongjiang | JX888906 |
| <i>Cobitis lutheri</i> 2 | China, Heilongjiang, R. Heilongjiang | KP133112 |
| <i>Cobitis macrostigma</i> | China, Jiangxi, L. Poyang | JX888904 |
| <i>Cobitis matsubara</i> 1 | Kitagawa et al. (2001) | AB039348* |
| <i>Cobitis matsubara</i> 2 | | AB091222* |
| <i>Cobitis melanoleuca</i> | Šlechtová et al. (2008) | EF508500* |
| <i>Cobitis microcephala</i> 1 | China, Guangxi, R. Nanliujiang | JX888907 |
| <i>Cobitis microcephala</i> 2 | China, Guangxi, R. Nanliujiang | KP133113 |
| <i>Cobitis obtusirostra</i>1 sp. nov. | China, Guangdong, R. Lianjiang | KP133118 |
| <i>Cobitis obtusirostra</i>2 sp. nov. | China, Guangdong, R. Lianjiang | |
| <i>Cobitis pacifica</i> 1 | Šlechtová et al. (2008) | EF508505* |
| <i>Cobitis pacifica</i> 2 | Šlechtová et al. (2008) | EF508506* |
| <i>Cobitis rara</i> | Šlechtová et al. (2008) | EF508507* |
| <i>Cobitis sinensis</i> 1 | China, Guizhou, R. Yuangjiang | JX888902 |
| <i>Cobitis sinensis</i> 2 | China, Guizhou, R. Yuangjiang | |
| <i>Cobitis stenocauda</i> 1 | China, Jiangxi, R. Xinjiang | JX888903 |
| <i>Cobitis stenocauda</i> 2 | China, Jiangxi, R. Xinjiang | KP133111 |
| <i>Cobitis striata</i> | Saitoh et al. (2003) | NC004695* |
| <i>Cobitis takatsuensis</i> | Kitagawa et al. (2001) | AB039337* |
| <i>Cobitis tetralineata</i> 1 | Kim et al. (2013) | KC524528* |
| <i>Cobitis tetralineata</i> 2 | | EU670756* |
| <i>Cobitis wumingensis</i>1 sp. nov. | China, Guangxi, R. Wuming | KP133116 |
| <i>Cobitis wumingensis</i>2 sp. nov. | China, Guangxi, R. Wuming | |
| <i>Iksookimia yongdokensis</i> | Šlechtová et al. (2008) | EF508516* |
| <i>Iksookimia longicorpa</i> 1 | Šlechtová et al. (2008) | EF508513* |
| <i>Iksookimia longicorpa</i> 2 | Šlechtová et al. (2008) | EF508514* |
| <i>Iksookimia koreensis</i> | Šlechtová et al. (2008) | EF508511* |
| <i>Iksookimia pumila</i> | Šlechtová et al. (2008) | EF508515* |
| <i>Niwaëlla multifasciata</i> | Šlechtová et al. (2008) | EF508574* |
| <i>Niwaëlla laterimaculata</i> | Tang et al. (2006) | DQ105236* |
| <i>Niwaëlla delicata</i> | Šlechtová et al. (2008) | EF508572* |
| <i>Sabanejewia balcanica</i> | Perdices & Doadria (2001) | AF499190* |

Sequences marked with * were retrieved from GenBank.

AAG AC-3') (Xiao et al. 2001). Amplification of the products was performed in a Biometra thermal cycler under the following conditions: four minutes initial denaturation at 95 °C, followed by 35 cycles of 40 seconds at 94 °C for denaturation, 45 seconds at 52-60 °C for annealing, one minute at 72 °C for extension, and a final extension at 72 °C for eight minutes. PCR products were purified with a BioStar glass-milk DNA purification kit following the manufacturer's instructions and sequenced by the Shanghai DNA Biotechnologies Company.

The sequence was aligned and tested for saturation at codon positions by plotting the absolute pairwise differences in transitions and transversions against the Kimura 2-parameter model using MEGA 5.05 (Tamura et al. 2011). The collection sites and corresponding GenBank sequence Accession Nos. of 42 individuals of 24 species of the genus *Cobitis*, three individuals of three species of the genus *Niwaëlla*, and five individuals of four species of the genus *Iksookimia* are listed in Table 1. *Sabanejewia balcanica* Karaman, 1922 was used as the out-group. The phylogenetic relationship among *Cobitis* species was reconstructed using Bayesian Inference (BI) with MrBayes 3.0 (Huelsenbeck & Ronquist 2001), and maximum likelihood (ML) as performed using MEGA 5.05. For the ML analyses, the substitution model was calculated by applying Tamura-Nei using Gamma distributed (G) rates among sites. Nonparametric bootstrap support for internal branches was calculated for ML with 1000 pseudoreplicates. For the BI analyses, the best-fit model of nucleotide substitution was selected using the Akaike Information Criterion (Akaike 1973) implemented in jModelTest 0.1.1 (Posada 2008).

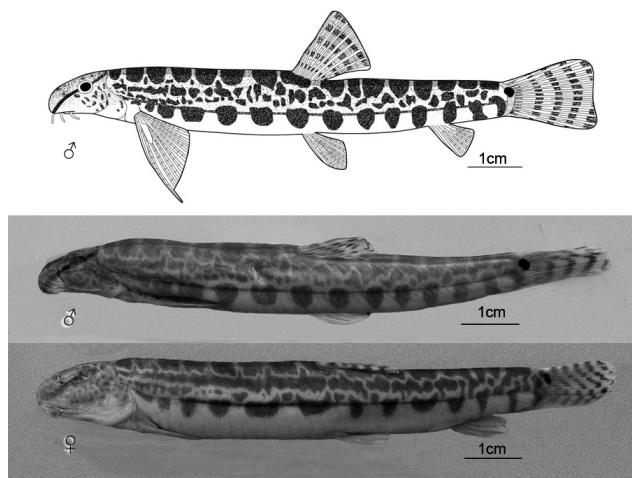


Fig. 2. *C. leptosoma* sp. nov. male, holotype, IHB 587834, 82.3 mm SL, female, IHB 75iv1682, 89.5 mm SL. The River Lijiang, Lingchuan, Guangxi, China.

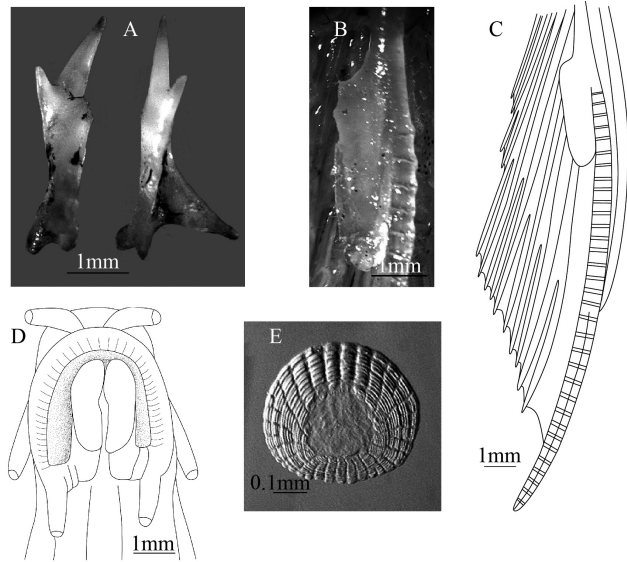


Fig. 3. *C. leptosoma* sp. nov. A, suborbital spine; B-C, lamina circularis in the pectoral fin of male; D, mouth characters; E, subdorsal scales.

Four Metropolis Coupled Markov Chains Monte Carlo (MCMCMC) were run for 2×10^6 generations, starting with random trees under the GTR + G + I and sampling frequencies of each of the 100 generations. The datasets were partitioned into codon positions and the parameter values independently estimated during the analyses for each partition. Burning values were approximated for *cyt b*. The remaining trees were used to build a 50 % majority rule consensus tree, and statistical support of clades was assessed by posterior probabilities.

The evolution of the lamina circularis and pigmentation pattern were traced onto the molecular phylogeny using MacClade 4.0 (Maddison & Maddison 2000).

Results

Cobitis leptosoma sp. nov. (Figs. 2-3)

Holotype: IHB 587834, male, 82.3 mm SL; China: Guangxi, Lingchuan County, the River Lijiang, a tributary of the River Xijiang, belonging to the River Pearl basin, 25°41' N, 110°32' E, July 1958.

Paratypes: IHB 587596, 587875, 587836, three females, 84.4-117.4 mm SL, the same data as holotype; IHB 75iv1682, 75iv1686, 75iv1688-9, four females, 85.9-103.5 mm SL, the same locality as holotype, April 1975; IHB 0604022-24, three males, 66.4-88.2 mm SL; IHB 0604010-20, 0504025-6, 13 females, 66.8-97.1 mm SL, the same locality as holotype, April 2006, collected by Chen Yongxia and Zhu Xiufang.

Diagnosis: *C. leptosoma* is distinguishable from its congeners by the combination of following characteristics: undeveloped mental lobes, with

Table 2. Morphometric and meristic characters for *Cobitis leptosoma*, *Cobitis wumिंगensis* and *Cobitis obtusirostra*.

| Variable | <i>Cobitis leptosoma</i> | | | | | <i>Cobitis wumिंगensis</i> | | | | | <i>Cobitis obtusirostra</i> | | | | | |
|----------|--------------------------|-------------|-----------------|------|----------|----------------------------|------|------------------|-----------|----------|-----------------------------|------|------------------|------------|-------|------|
| | Males (n = 5) | | Females (n = 8) | | Holotype | Males (n = 19) | | Females (n = 23) | | Holotype | Males (n = 2) | | Females (n = 10) | | | |
| | Mean | Range | Mean | SD | | Mean | SD | Mean | SD | | Mean | SD | Mean | SD | | |
| SL | 83.2 | 84.5-117.4 | 97.4 | 9.91 | 65.6 | 51.7-68.8 | 59.3 | 5.15 | 78.3-56.0 | 75.1 | 6.82 | 76.0 | 77.2 | 92.7-110.7 | 102.0 | 5.46 |
| SL/HL | 4.8 | 5.0-5.3 | 5.2 | 0.14 | 4.9 | 4.6-5.2 | 4.9 | 0.14 | 4.7-5.3 | 5.0 | 0.16 | 5.2 | 5.2 | 5.0-5.9 | 5.3 | 0.24 |
| SL/BD | 6.6 | 6.3-7.8 | 7.1 | 0.55 | 6.4 | 5.8-6.8 | 6.4 | 0.25 | 5.7-7.5 | 6.4 | 0.43 | 6.7 | 6.6 | 5.3-7.9 | 6.6 | 0.92 |
| SL/PVL | 3.0 | 2.9-3.2 | 3.0 | 0.08 | 3.1 | 3.0-3.4 | 3.2 | 0.15 | 2.8-3.3 | 3.1 | 0.14 | 2.8 | 2.9 | 2.7-3.1 | 2.9 | 0.12 |
| SL/CPL | 8.4 | 6.9-7.9 | 7.4 | 0.37 | 7.0 | 6.5-8.1 | 7.2 | 0.44 | 6.3-8.4 | 7.1 | 0.58 | 9.7 | 9.2 | 6.9-10.4 | 7.9 | 1.00 |
| SL/CPD | 10.0 | 10.5-12.6 | 11.4 | 0.78 | 11.6 | 10.0-11.6 | 11.0 | 0.42 | 10.7-12.5 | 11.5 | 0.47 | 10.2 | 10.0 | 10.8-12.5 | 11.4 | 0.50 |
| SL/DFL | 6.5 | 6.4-7.3 | 7.0 | 0.27 | 6.0 | 5.3-6.4 | 5.8 | 0.31 | 5.7-6.8 | 6.1 | 0.30 | 7.1 | 6.6 | 6.2-7.3 | 6.8 | 0.42 |
| SL/DBL | 10.8 | 9.8-13.7 | 11.5 | 1.10 | 10.5 | 9.6-12.0 | 10.5 | 0.73 | 9.6-11.7 | 10.7 | 0.61 | 10.7 | 10.6 | 9.2-11.6 | 10.3 | 0.68 |
| SL/PFL | 4.9 | 7.7-9.1 | 8.5 | 0.52 | 4.9 | 4.6-5.6 | 5.1 | 0.28 | 7.0-8.4 | 7.7 | 0.39 | 5.3 | 5.2 | 8.0-8.9 | 8.5 | 0.32 |
| SL/VFL | 8.5 | 8.6-10.0 | 9.2 | 0.39 | 7.8 | 6.9-8.2 | 7.7 | 0.43 | 8.0-10.0 | 8.9 | 0.45 | 8.3 | 8.2 | 9.1-10.5 | 9.8 | 0.54 |
| SL/AFL | 8.2 | 7.5-10.1 | 8.9 | 0.73 | 8.2 | 7.3-9.0 | 7.9 | 0.52 | 7.4-10.3 | 8.3 | 0.61 | 8.3 | 7.7 | 8.1-9.3 | 8.4 | 0.34 |
| SL/ABL | 16.1 | 14.8-20.5 | 16.6 | 1.81 | 15.3 | 12.4-15.8 | 14.2 | 1.07 | 13.4-16.6 | 15.0 | 0.88 | 14.1 | 14.2 | 13.3-15.7 | 14.1 | 0.72 |
| SL/CFL | 5.2 | 5.1-5.7 | 5.4 | 0.19 | 5.6 | 4.8-6.2 | 11.0 | 0.42 | 5.0-5.9 | 5.5 | 0.24 | 5.4 | 5.1 | 5.1-6.0 | 5.4 | 0.24 |
| SL/PrDL | 1.8 | 1.8-1.9 | 1.9 | 0.04 | 1.8 | 1.8-2.0 | 1.9 | 0.03 | 1.8-2.0 | 1.9 | 0.04 | 1.8 | 1.8 | 1.8-1.9 | 1.8 | 0.03 |
| SL/PrVL | 1.7 | 1.7-1.8 | 1.8 | 0.03 | 1.7 | 1.7-1.8 | 1.7 | 0.03 | 1.7-1.9 | 1.8 | 0.04 | 1.7 | 1.7 | 1.7-1.8 | 1.8 | 0.04 |
| SL/PrAL | 1.2 | 1.2-1.3 | 1.2 | 0.02 | 1.3 | 1.2-1.3 | 1.2 | 0.02 | 1.2-1.4 | 1.3 | 0.03 | 1.2 | 1.2 | 1.1-1.3 | 1.2 | 0.02 |
| HL/PrOL | 2.2 | 2.0-2.2 | 2.1 | 0.10 | 2.3 | 2.3-2.7 | 2.4 | 0.11 | 2.2-2.5 | 2.3 | 0.10 | 2.1 | 2.2 | 2.2-2.3 | 2.3 | 0.19 |
| HL/ED | 8.7 | 7.9-6.3-7.7 | 6.9 | 0.49 | 5.9 | 4.8-6.6 | 5.5 | 0.40 | 5.0-6.4 | 5.8 | 0.41 | 6.3 | 6.6 | 5.9-6.8 | 6.5 | 0.31 |
| HL/IW | 8.9 | 7.1-9.1 | 7.9 | 0.72 | 7.2 | 5.2-7.8 | 6.3 | 0.64 | 5.8-8.7 | 7.2 | 0.88 | 7.1 | 7.0 | 6.5-9.2 | 7.6 | 0.85 |
| CPL/CPD | 1.2 | 1.2-1.3-1.8 | 1.5 | 0.17 | 1.7 | 1.4-1.8 | 1.5 | 0.09 | 1.4-1.8 | 1.6 | 0.13 | 1.0 | 1.1 | 1.1-1.8 | 1.5 | 0.19 |

ABL, Anal fin bases length; AFL, Anal fin length; BD, Body depth; CFL, Caudal fin length; CPD, Caudal peduncle depth; CPL, Caudal peduncle length; DBL, Dorsal fin bases length; DFL, Dorsal fin length; ED, Eye diameter; HL, Head length; IW, Interorbital width; PFL, Pectoral fin length; PrAL, Preanal length; PrDL, Predorsal length; PrOL, Preorbital length; PrVL, Preventral length; PVL, Pectoral-ventral length; SL, Standard length; VFL, Ventral fin length. All measurements given in millimeters.

bluntly rounded tips (Fig. 3D); round subdorsal scales, with a slightly large focal area, and 19-25 radial grooves (Fig. 3E); males with a long knife-shaped lamina circularis at the bases of the second pectoral-fin ray, and a sharp and nearly filamentous tip of the pectoral-fin ray (Fig. 3B-C); suborbital spine is bicuspid, with a long processus latero-caudalis (Fig. 3A); four Gambetta pigment lines, absence of L_2 , L_5 consisting of 10-12 oval blotches; and a round or oblong spot smaller than or equal to the eye diameter on the upper part of the caudal peduncle (Fig. 2).

Description: General appearance and morphometric data of holotype and paratypes are given in Figs. 2-3 and Table 2, respectively. D. III-7; A. III-5; V. I-6; P. I-7-8; C. IV-14-16-IV. Vertebrae 4 + 37 – 39 + 1. The body is elongated and laterally compressed. The depth of the body is homogenous between the nape and dorsal-fin base and it is slightly decreases towards the caudal-fin base. Head is small, with a length of 4.8 in SL in males and 5.0-5.3 (mean 5.2 ± 0.14) in females. The snout is rounded. The preorbital part of the head is slightly longer than the postorbital part of the head. The mouth is small, inferior, with three pairs of slight long barbels. The length of the maxillo-mandibular barbels is equal to the diameter of the eye. The maxillary barbels do not reach under the anterior border of the eye, with maxillo-mandibular length 7.0 in HL in males and 6.2-8.8 (mean 7.6 ± 1.98) in females. The mental lobes of the lower tip is bluntly rounded (Fig. 3D). The interorbital width is equal to or slightly shorter than the diameter of the eye. The suborbital spine is bicuspid, with long processus latero-caudalis (Fig. 3A). The subdorsal scales are small and round, with a slightly large and excentric (closer to the base) focal area, 19-25 radial grooves, and 6-11 supplementary ones (Fig. 3E).

The dorsal fin is moderately long, it is located in middle of the posterior naris to caudal-fin base distance. The length of the predorsal is 1.8-1.9 (mean 1.9 ± 0.04) in SL both in males and females. In males, the pectoral fins are longer than those in females. The first branched pectoral-fin is the longest, with a sharp and nearly filamentous tip of the pectoral-fin ray and length of the first branched pectoral-fin ray 5.0 in SL. In females, the third pectoral-fin ray is the longest, with the length of the third pectoral-fin ray 7.7-9.1 (mean 8.5 ± 0.52) in SL. The ventral fins are short, and approximately at the same level as the third branched dorsal-fin ray. The anal fin is small, and located in far behind dorsal extremity. The anus orifice is close to the anal fin. The caudal fin is long, with an emarginated tip. Caudal peduncle with a reduced ventral adipose crest.

Pigmentation pattern: L_1 is composed of a line of 12-15 large, rectangular blotches, and the gaps between the rectangular blotches are narrower than their widths. L_2 is usually absent, though some specimens of L_2 are present, which are characterized by a line of minute black dots, the sizes of which diminished toward the end of the caudal fin. L_2 is merged with L_1 . L_3 is variable in females, merging into a dark stripe from the level of the caudal end of the anal fin to the caudal end of the head. In males, it merges into a dark stripe from the level of the caudal end of the pectoral fin to the caudal end of the head, and then a row of irregular blotches diminished toward the end of the caudal fin. L_4 is spotted with one line of dots, which are sometimes reduced to a few spots behind the dorsal fin. L_5 consists of 10-12 oval or rectangular blotches, often with 1-3 small spots forming a longitudinal line before the pectoral fin. No sexually dimorphic colour patterns could be observed. At the level of the base of the caudal fin, one large round or oblong spot is seen on the dorsal side, which is smaller than or as large as the eye diameter. There are four or five striations on the dorsal and caudal fins. The head is sprinkled with many black spots on the dorsal side, and a black stripe extended from the occiput through the eye to the insertion of the rostral barbels.

Sexual dimorphism: Males are smaller than females with proportionally longer pectoral, dorsal, ventral, anal, and caudal fins. The base of dorsal fin in males is also longer than those in females, as well as barbels. Anal, dorsal and pelvic fins are inserted from more posterior part in females than those in males. In males, the caudal peduncle is higher than those in females. In males, the first branched pectoral-fin ray is thickened and elongated, with a long knife-shaped lamina circularis at the base (Fig. 3B-C). In females, the second branched pectoral-fin ray is elongated.

Distribution: This new species occurs in the River Lijiang, a tributary of the River Xijiang, belonging to the River Pearl basin in the Lingchuan County in Guangxi in southern China (Fig. 1).

Etymology: The species name- *leptosoma* derives from the Greeks *leptos*, meaning slender, and *sōma*, meaning body, in reference to the slender body.

Cobitis wumingensis sp. nov. (Figs. 4-5)

Holotype: IHB 0605153, male, 65.6 mm SL; China: Guangxi, Wuming County, the River Wuming, a tributary of the River Youjiang in the middle part of the River Xijiang, belonging to the River Pearl basin, 23°16' N, 108°26' E, April, 2006, collected by Chen Yongxia and Zhu Xiufang.

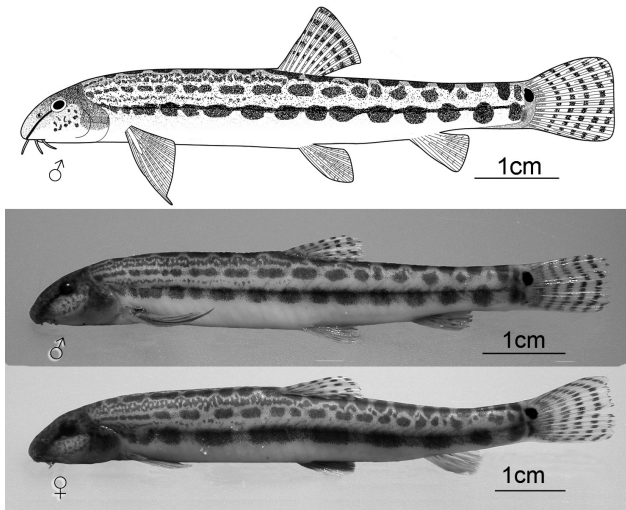


Fig. 4. *C. wumingensis* sp. nov. male, holotype, IHB 0605153, 65.6 mm SL, female, IHB 0605154, 74.6 mm SL. The River Wuming, Wuming, Guangxi, China.

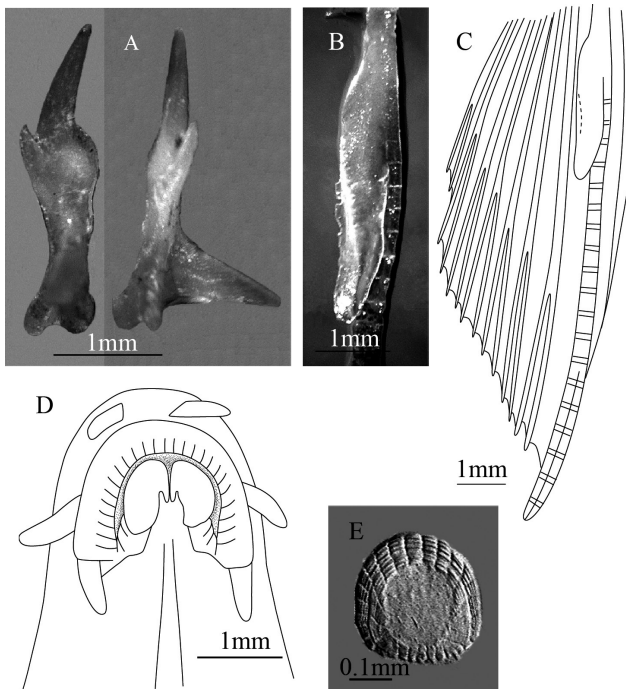


Fig. 5. *C. wumingensis* sp. nov. A, suborbital spine; B-C, lamina circularis in the pectoral fin of male; D, mouth characters; E, subdorsal scales.

Paratypes: IHB 0605060-66, seven males, 55.5-65.8 mm SL; IHB 0605091-98, eight females, 56.0-78.3 mm SL, the same data as holotype.

Diagnosis: *C. wumingensis* is distinguishable from its congeners by the combination of following characteristics: undeveloped mental lobes, with bluntly rounded tips; small barbels, maxillo-mandibular barbels shorter than eye diameter, maxillary barbels not reaching vertically to nostrils, maxillo-mandibular

barbels reaching vertically to nostrils (Fig. 5D); small scales, round, with a large focal area, and 20-24 radial grooves (Fig. 5E); males with a long finger-like lamina circularis, and a sharp and nearly filamentous tip of the pectoral-fin ray (Fig. 5B-C); suborbital spine is bicuspid, with a short processus latero-caudalis (Fig. 5A); five Gambetta pigment lines, L_2 characterized by two rows of irregular spots that melted into two bands, the thickness of which are reduced towards the end of the length of the pectoral fin. L_5 showing sexual dimorphism; a deeper bluish band pigment in females; a surface pigment with 13-14 blotches in males; and a conspicuous jet-black roundish spot smaller than or equal to the eye diameter on the upper part of the caudal peduncle (Fig. 4).

Description: General appearance and morphometric data of holotype and paratypes are given in Figs. 4-5 and Table 2, respectively. D. III-7; A. III-5; V. I-6; P. I-7-8; C. VI-16-IV. Vertebrae 4 + 36 – 39 + 1. The body is laterally compressed. Abdomen is laterally rounded; dorsal surface and abdomen are almost parallel. Head is small, with a length of 4.6-5.2 (mean 4.9 ± 0.14) in SL in males and 4.7-5.3 (mean 5.0 ± 0.16) in females. Preorbital part of the head is slightly shorter than the postorbital part. Barbels are small, maxillo-mandibular barbels are shorter than eye diameter, not reaching the anterior border of the eye. Mental lobes are undeveloped, with bluntly rounded tips (Fig. 5D). Interorbital width is narrower than the eye diameter. Suborbital spine is bicuspid, with the processus latero-caudalis extending posteriorly to beneath the front edge of the eyes (Fig. 5A). Body scales are small, round, with a large focal area situated closer to the base, 20-24 radial grooves (Fig. 5E). Lateral line is short, not exceeding the length of the pectoral fin.

The dorsal fin is moderately long; it is 1.0-1.4 (mean 1.2 ± 0.30) in HL both in males and females; it is located in caudal half of the body. In males, the pectoral fins longer than those in females, the first branched pectoral-fin ray is the longest; it is 4.6-5.6 (mean 5.1 ± 0.28) in SL. In females, the second branched pectoral-fin ray is the longest, and it is 7.0-8.4 (mean 7.7 ± 0.39) in SL. The ventral fins are short and approximately at the same level as the third dorsal-fin ray. The anal fin is small, and located in half of the space between the ventral and caudal fins. The caudal fin is long, with an emarginated tip. Caudal peduncle with a reduced ventral adipose crest.

Pigmentation pattern: L_1 is composed of a line of 11-14 rectangular brownish blotches, and the gaps between the rectangular blotches are narrower than

their lengths. L_2 is characterized by two rows of irregular spots melted into two bands, the sizes of which are diminished toward the end of the length of the pectoral fin. The two rows of spots merge into a band and are diminished toward the end of the dorsal fin. L_2 do not merge with L_1 . L_3 has a row of small rectangular blotches, often with some small spots forming a longitudinal line behind the head. L_3 could be traced beyond the caudal fin. L_4 consists of a line of minute dots and is usually present beyond the dorsal fin. L_5 has sexual dimorphism in females, and the deeper pigment is a bluish band with a surface pigment of 9-10 longitudinal rectangular blotches. In males, the deeper pigment is inconspicuous with a row of 13-14 roundish blotches on the surface layer. At the level of the base of the caudal fin, one conspicuous black roundish spot is seen on the dorsal side, and the spot is smaller than or as large as the eye diameter. There are five or six striations on the dorsal and caudal fins. The head is sprinkled with many dots, and a black stripe extended from the occiput through the eye to the insertion of the rostral barbels.

Sexual dimorphism: Males are smaller than females with proportionally longer pectoral, anal and ventral fins. In males, the first branched pectoral-fin ray is thickened and elongated, with a long finger-shaped lamina circularis at the base of the second pectoral-fin ray (Fig. 5B-C), whereas in females, the second branched pectoral-fin ray is elongated.

Distribution: This new species occurs in the River Wuming, a tributary of the River Youjiang in the middle part of the River Xijiang, belonging to the River Pearl basin in the Wuming County in Guangxi in southern China (Fig. 1).

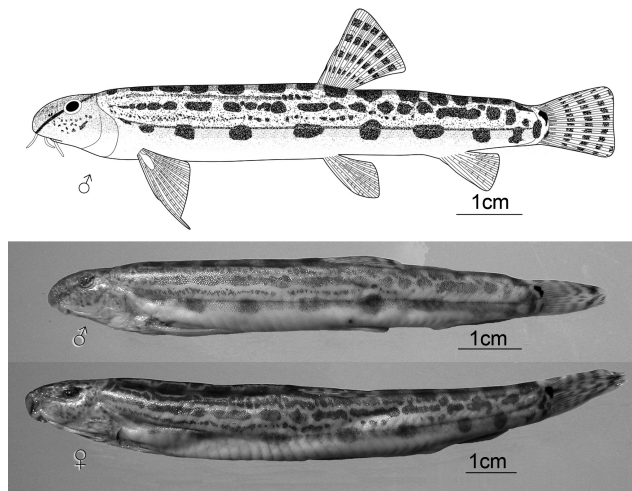


Fig. 6. *C. obtusirostra* sp. nov. male, holotype, IHB 76iv6670, 76.0 mm SL, female, IHB 76iv6669, 102.5 mm SL. The River Lianjiang, Lianxian City, Guangdong Province, China.

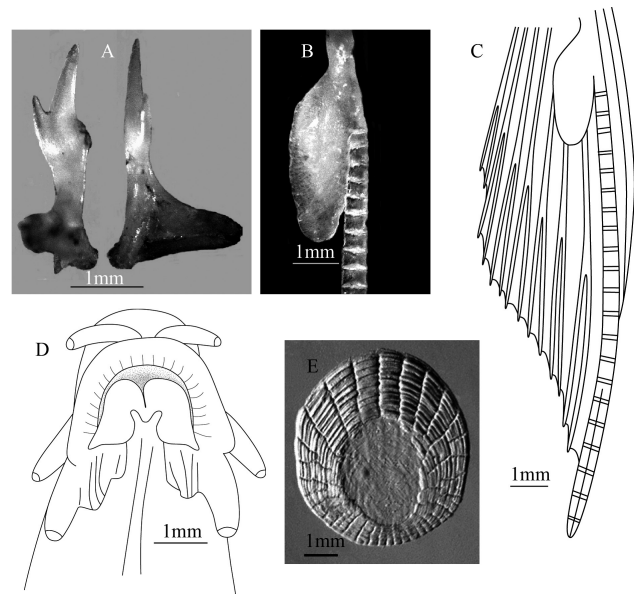


Fig. 7. *C. obtusirostra* sp. nov. A, suborbital spine; B-C, lamina circularis in the pectoral fin of male; D, mouth characters; E, subdorsal scales.

Etymology: The species name- *wumingensis* refers to the type locality in the River Wuming in southern China.

Cobitis obtusirostra sp. nov. (Figs. 6-7)

Holotype: IHB 76IV6670, male, 76.0 mm SL; China: Guangdong Province, Lianxian City, the River Lianjiang, a tributary of the River Beijiang, belonging to the River Pearl basin, 24°78' N, 112°38' E, April, 1976.

Paratypes: IHB II28660009, one male, 78.4 mm SL; IHB 76IV6665-9, 76IV6672-4, 76IV6676-7, IHB II28660008, III14660269, 12 females, 92.7-110.7 mm SL; the same data as holotype.

Diagnosis: *C. obtusirostra* is distinguished from its congeners by the combination of following characteristic: undeveloped mental lobes, with pointed tips (Fig. 7D); slightly large scales, prolonged, with a slightly large, excentric and closer to the base focal area, and 26-33 radial grooves (Fig. 7E); males with a cystiform lamina circularis, and a sharp and nearly filamentous tip of the pectoral-fin ray (Figs. 7B-C); five Gambetta pigment lines, L_2 showing a row of irregularly minute spots that merged into a band, the thickness of which was reduced towards the end of the dorsal fin; L_5 consists of 8-10 rounded blotches; and a conspicuous jet-black arcuate spot, smaller than the eye diameter on the upper part of the caudal peduncle (Fig. 6).

Description: General appearance and morphometric data of holotype and paratypes are given in Figs. 6-7

and Table 2, respectively. D. III-7; A. III-5; V. I-6; P. I-7-8; C. VI-16-IV. Vertebrae 4 + 35 – 37 + 1. Body is slightly round and small. Head is small and short, and the snout is bluntly rounded. Preorbital part of the head is slightly longer than the postorbital part. Barbels are slightly long; length of maxillo-mandibular barbels was almost equal to the eye diameter. Mental lobes are undeveloped, with pointed tips (Fig. 7D). Interorbital width is equal to or slightly narrower than the eye diameter. Suborbital spine is bicuspid, extending posteriorly to beneath the front edge of the eyes, with a short processus latero-caudalis (Fig. 7A). Body scales are minute, the subdorsal scales are slightly large, prolonged, with a slightly large, excentric and closer to the base focal area, 26-33 radial grooves, and 12-14 supplementary grooves (Fig. 7E). Lateral line is very short, merely reaching the base of the pectoral fin.

The dorsal fin is moderately long, and located in middle or caudal half of the anterior eye and the caudal-fin base. The length of the predorsal is 1.8-1.9 (mean 1.8 ± 0.04) in SL both in males and females. The dorsal fin length shorter than head length. In males, pectoral fins longer than those in females; the first branched pectoral-fin ray is the longest, with a length that is 5.2-5.3 in SL. In females, the second branched pectoral-fin ray is the longest, with a length that is 8.0-8.9 (mean 8.5 ± 0.32) in SL. The ventral fins are short and approximately at the same level as the second dorsal-fin ray. The anal fin is small, and located in half of the space between the ventral and caudal fins. The caudal fin is long, with an emarginated tip. The anal orifice is close to anal fin. Caudal peduncle with a reduced ventral adipose crest.

Pigmentation pattern: L_1 consists of a row of 13-18 rectangular brownish blotches, and the gaps between the rectangular blotches are equal to or slightly narrower than their lengths. L_2 has a row of irregularly minute spots that merge into a band, the size of which diminished toward the end of the dorsal fin. Some specimens are traced beyond the anal fin. L_3 has a row of rounded blotches, and some fuse into large rectangular blotches. L_3 could be traced beyond the caudal fin. L_4 is characterized by a line of black dots, which in some cases join into a band, and their size diminished toward the end of the anal orifice. The surface pigment layer of L_5 consists of 8-10 large rounded blotches. Pigments on the deeper layer of L_5 are lighter. No sexually dimorphic colour patterns could be observed. At the level of the base of the caudal fins, one conspicuous black arcuate spot is seen on the dorsal side, and the spot is smaller than the eye diameter. There are four or six striations on

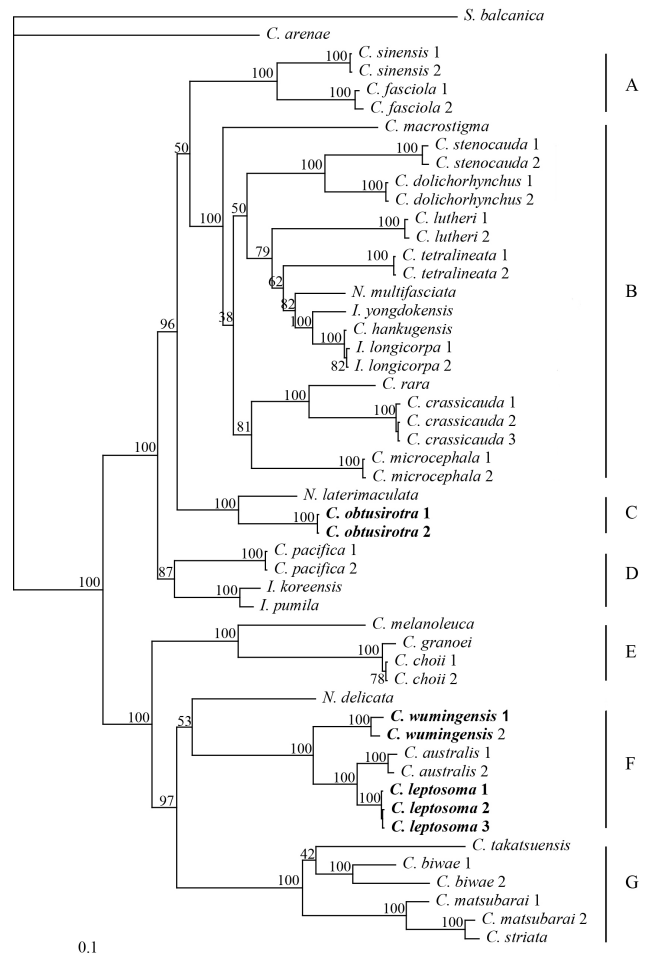


Fig. 8. Bayesian phylogeny of 42 mitochondrial cytochrome *b* lineages of *Cobitis* spp. three of *Niwaëlla* spp., five of *Iksookimia* spp. and one lineage *Sabanejewia balcanica* used as out-groups. The lineages are numbered as in Table 1. Upper values at the branches correspond to Bayesian posterior probabilities.

the dorsal and caudal fins. The head is sprinkled with many dots, and a black stripe extends from the occiput through the eye to the insertion of the rostral barbels. **Sexual dimorphism:** Males are smaller than females with proportionally longer pectoral, anal and ventral fins. The caudal peduncle in males is higher than those in females. Ventral fins are inserted from more posterior part in females than those in males. In males, the first branched pectoral-fin ray is thickened and elongated, with an elongate cystiform lamina circularis at the base (Fig. 7B-C). In females, the second branched pectoral-fin ray is elongated.

Distribution: This new species is found in the Lianjiang and Wengjiang Rivers, tributaries of the River Beijiang of the River Pearl basin in Guangdong Province in southern China (Fig. 1). *C. obtusirotra* co-occurs with *C. arenae* in the River Lianjiang. The populations of *C. arenae* are apparently declining, since all the specimens were collected from the River Lianjiang before the

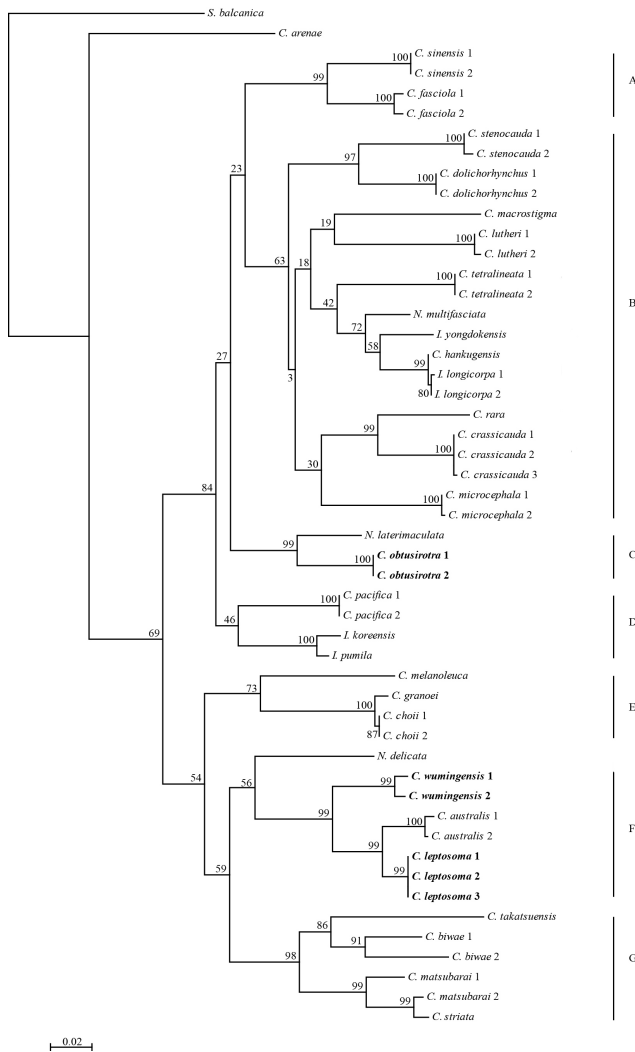


Fig. 9. Maximum likelihood tree based on Tamura-Nei model calculated in MEGA 5.05 based on entire cytochrome *b* sequences. Bootstrap percentages are shown on branches.

1980s, and no specimens of *C. arenae* were obtained in recent sampling collection trips.

Etymology: The species name- *obtusirostra* stems from the Latin *obtusus*, meaning blunt, and *rostrum*, meaning beak, in reference to the bluntly rounded snout.

Phylogenetic analysis of cytochrome *b* sequences

Topologies of the trees recovered by two phylogenetic methods were shown in Fig. 8 (BI) and Fig. 9 (ML). In the two types of phylogenetic trees provided by BI and ML analyses, based on mitochondrial cytochrome *b* gene data, the three new species *C. leptosoma*, *C. wumingensis*, and *C. obtusirostra* conspecific individuals co-clustered with strong support (Byesian values > 100 % and bootstrap values > 99 %), indicating good resolution at the level of species. The trees provided by BI and ML analyses were slightly

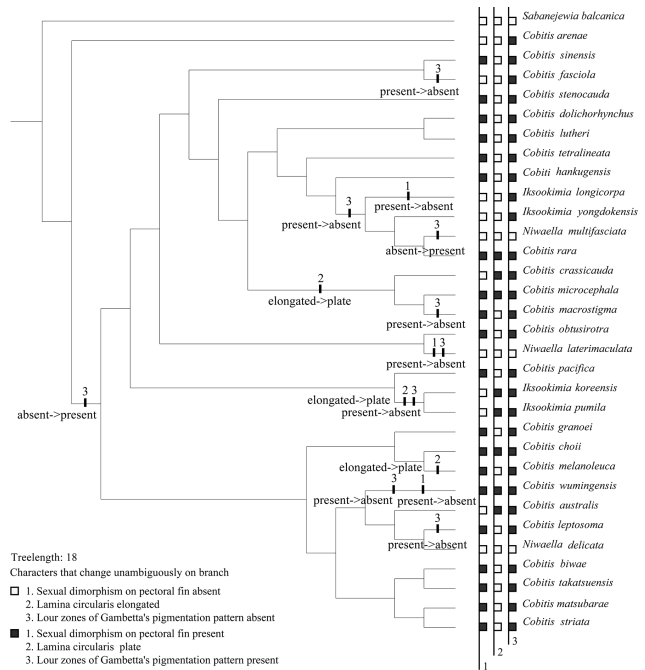


Fig. 10. Character evolution cladogram of sexual dimorphism on pectoral fin, lamina circularis, and pigmentation pattern. Based on the phylogeny recovered with the *cyt b* gene.

incongruent. The topology from the BI analysis differed from the ML topology regarding the relative positions of *C. macrostigma* Dabry, 1872. The three new species *C. leptosoma*, *C. wumingensis*, and *C. obtusirostra* belonged to clearly different clades, with strong high Bayesian values (100 %) (Fig. 8) and bootstrap values (> 99 %) (Fig. 9). *C. leptosoma* and *C. australis* clustered together (clade F in Figs. 8-9), with a genetic distance of 3.4-3.7 % (Table 3). They had a sister relationship with the new species *C. wumingensis*, with a genetic distance ranging between 6.8 % (*C. wumingensis* vs. *C. leptosoma*) and 8.4 % (*C. wumingensis* vs. *C. australis*), and then a sister relationship with *N. delicata* Niwa, 1937 of Japan, with a genetic distance ranging between 11.2 % (*N. delicata* vs. *C. leptosoma*) and 12.2 % (*N. delicata* vs. *C. leptosoma*). The new species *C. obtusirostra* had a sister relationship with *N. laterimaculata* (Yang & Zheng, 1984) (clade C in Figs. 8-9), with a genetic distance of 6.6 %.

Mapping the evolution of the sexual dimorphism on the pectoral fin, lamina circularis, and the pigmentation pattern on the molecular phylogeny suggested that none of these characteristics were synapomorphic to all species studied here (Fig. 10).

Discussion

The morphology of the lamina circularis in males and the pigmentation pattern on the body sides have


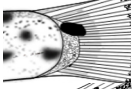


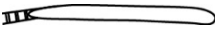

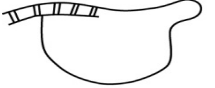
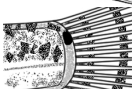
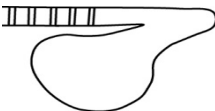
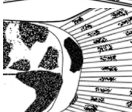
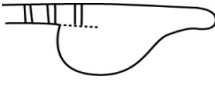
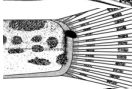
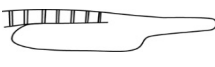
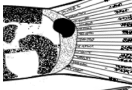


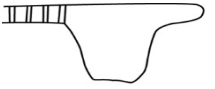

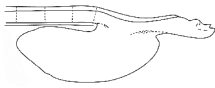

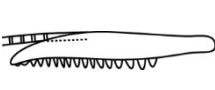



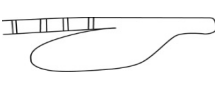


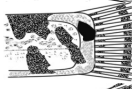








Table 3. The sequence divergence (in percentage) between species of *Cobitis*, *Niwaella* and *Iksookimia* based on the *cyt b* gene under the Kimura-2 parameter model.

| Species | <i>C. leptosoma</i> | <i>C. obtusirostra</i> | <i>C. wumingensis</i> |
|---------------------------|---------------------|------------------------|-----------------------|
| <i>C. arenae</i> | 18.6 | 17.6 | 16.5-17.5 |
| <i>C. australis</i> | 3.4-3.7 | 14.2 | 7.7-8.4 |
| <i>C. biwae</i> | 13.3-13.9 | 14.0-15. | 14.1-14.6 |
| <i>C. choii</i> | 15.0 | 14.3 | 14.3-14.7 |
| <i>C. crassicauda</i> | 15.2-15.4 | 12.0-12.1 | 14.1-14.5 |
| <i>C. dolichorhynchus</i> | 16.0 | 13.3 | 16.1-16.3 |
| <i>C. fasciola</i> | 14.4-14.9 | 11.5 | 14.3-14.9 |
| <i>C. granoei</i> | 15.0 | 14.2 | 14.5-14.8 |
| <i>C. hankugensis</i> | 15.0 | 10.1 | 15.3-15.8 |
| <i>C. leptosoma</i> | - | 14.2 | 6.8-7.1 |
| <i>C. lutheri</i> | 16.7-17.1 | 15.3-15.7 | 14.0-15.2 |
| <i>C. macrostigma</i> | 16.1 | 14.9 | 14.9-15.7 |
| <i>C. matsubarai</i> | 14.1-14.6 | 13.9-15.7 | 13.3-16.2 |
| <i>C. melanoleuca</i> | 13.1 | 14.6 | 14.2-14.6 |
| <i>C. microcephala</i> | 17.3 | 13.5-13.6 | 14.9-16.3 |
| <i>C. obtusirostra</i> | 14.2 | - | 14.3-14.5 |
| <i>C. pacifica</i> | 15.0 | 12.6 | 13.7-14.6 |
| <i>C. rara</i> | 15.6 | 11.6 | 15.8-16.0 |
| <i>C. sinensis</i> | 14.8 | 12.3 | 14.9 |
| <i>C. stenocauda</i> | 17.9-18.0 | 14.6-14.7 | 15.3-16.5 |
| <i>C. striata</i> | 15.0 | 15.5 | 15.8-16.8 |
| <i>C. takatsuensis</i> | 16.7 | 16.7 | 16.8-17.4 |
| <i>C. tetralineata</i> | 15.1 | 11.8 | 15.2-15.4 |
| <i>C. wumingensis</i> | 6.8-7.1 | 14.3-14.5 | - |
| <i>I. koreensis</i> | 15.0 | 10.1 | 13.2-13.7 |
| <i>I. longicorpa</i> | 14.8-15.0 | 10.0-10.1 | 15.1-15.8 |
| <i>I. pumila</i> | 14.3 | 9.9 | 12.5-13.0 |
| <i>I. yongdokensis</i> | 15.9 | 10.6 | 14.9-15.8 |
| <i>N. delicata</i> | 12.2 | 15.3 | 11.2-11.7 |
| <i>N. laterimaculata</i> | 13.9 | 6.6 | 13.8-14.0 |
| <i>N. multifasciata</i> | 16.5 | 11.4 | 15.8-15.9 |

been considered the most important characteristics for identifying the species of *Cobitis*. The main distinguishing characteristics between the 18 species of *Cobitis* from China are summarized in Table 4. The pigmentation pattern of *Cobitis* could be divided into two types. In one type, the Gambetta pigmentation pattern is absent. This type is characteristic of *C. arenae*, *C. australis*, *C. crassicauda* Chen & Chen, 2013, *C. fasciola* Chen & Chen, 2013 and *C. multimaculata*. *C. australis* and *C. crassicauda* have an elongated lamina circularis on the second pectoral-fin ray in males. Other three species, *C. arenae*, *C. fasciola*, and *C. multimaculata* have a plate lamina circularis in males. *C. australis*, *C. multimaculata*,

and *C. arenae* are distributed in and to the south of the River Pearl basin, whereas *C. fasciola* and *C. crassicauda* in the River Yangtze basin. In the second type of colouration, the Gambetta pigmentation pattern was observed. This type is characteristic of all other species, i.e. *C. sinensis* Sauvage & Dabry, 1874, *C. dolichorhynchus* Nichols, 1918, *C. macrostigma*, *C. melanoleuca* Nichols, 1925, *C. microcephala*, *C. lutheri* Rendahl, 1935, *C. granoei* Rendahl, 1935, *C. rara* (= *C. rarus*) Chen, 1981, *C. zhejiangensis* Son & He, 2005, *C. stenocauda* Chen & Chen, 2013, *C. leptosoma*, *C. obtusirostra* and *C. wumingensis*. Within this group, males of *C. microcephala*, *C. rara*, *C. wumingensis*, *C. leptosoma*, *C. obtusirostra*, and

Table 4. Eighteen species of *Cobitis* from China distinguished on the basis of their main diagnostic characters and their distributions.

| Species | Gambetta zones | Lamina circularis | Caudal spot | River | Drainage |
|---------------------------|----------------|---|---|---------------------------------|-------------------------------------|
| <i>C. arenae</i> | absent |  |  | Nandujiang and Pearl Rivers | |
| <i>C. australis</i> | absent |  |  | River Pearl | Yongjiang and Yujiang drainages |
| <i>C. crassicauda</i> | absent |  |  | River Yangtze | Xinjiang drainage |
| <i>C. dolichorhynchus</i> | present |  |  | Jiulongjiang and Mulanxi Rivers | |
| <i>C. fasciola</i> | absent |  |  | River Yangtze | Xinjiang and Le'anjiang drainages |
| <i>C. granoiei</i> | present |  |  | River Heilongjiang | |
| <i>C. leptosoma</i> | present |  |  | River Pearl | Lijiang drainage |
| <i>C. lutheri</i> | present |  |  | Heilongjiang and Liaohe Rivers | |
| <i>C. macrostigma</i> | present |  |  | River Yangtze | Poyang and Dongting Lakes |
| <i>C. melanoleuca</i> | present |  |  | Yellow, Haihe and Liaohe Rivers | |
| <i>C. microcephala</i> | present |  |  | Nanliu and Pearl Rivers | Beiliu drainage |
| <i>C. multimaculata</i> | absent |  |  | River Nanliu | |
| <i>C. obtusirostra</i> | present |  |  | River Pearl | Lianjiang and Wengjiang drainages |
| <i>C. rara</i> | present |  |  | River Yangtze | Jialingjiang and Hanjiang drainages |
| <i>C. sinensis</i> | present |  |  | River Yangtze | Yuanjiang drainage |
| <i>C. stenocauda</i> | present |  |  | River Yangtze | Xinjiang drainage |
| <i>C. wumingensis</i> | present |  |  | River Pearl | Youjiang drainage |
| <i>C. zhejiangensis</i> | present |  |  | River Ling | |

C. melanoleuca have an elongated lamina circularis on the second pectoral-fin ray. *C. rara* is distributed in the River Yangtze basin, *C. melanoleuca* occurs in the River Yellow basin. *C. microcephala*, *C. wumingensis*, *C. leptosoma*, and *C. obtusirostra* are distributed in and to the south of the River Pearl basin. All other seven species have a plate lamina circularis in males. *C. macrostigma*, *C. sinensis*, and *C. stenocauda* were reported in the River Yangtze basin, *C. granoei* and *C. lutheri* in the River Haihe basin (including the Heilongjiang, Liaohe, and Huangshui Rivers), *C. dolichorhynchus* in the River Jiulongjiang, and *C. zhejiangensis* in the River Ling.

In the second group, males of *C. leptosoma*, *C. obtusirostra*, and *C. melanoleuca*, with an elongated but not finger-shaped lamina circularis, are rather similar to *C. pacifica* (Kim, 1980) from Korea and *C. biwae* Jordan & Snyder, 1901 from Japan. *C. leptosoma* and *C. obtusirostra* have a conspicuous jet-black spot at the upper part of the caudal fin, whereas no black spot or one small faded spot can be observed in *C. melanoleuca* and *C. pacifica* (Kim et al. 1999: Figs. 11A, 12A). *C. leptosoma* and *C. obtusirostra* have scales with a smaller focal area compared to *C. melanoleuca* and *C. pacifica* (Kim et al. 1999: Figs. 11E, 12E). *C. leptosoma* differs from *C. obtusirostra* and *C. biwae* by the following characteristics: a knife-shaped lamina circularis; vs. a cystiform in *C. obtusirostra* and an oblong oval in *C. biwae* (Sawada & Aizawa 1983: Fig. 2, Takahama et al. 2012: Fig. 4A); a roundish spot smaller than or as large as the eye diameter on the upper part of the caudal peduncle (vs. an arcuate spot smaller than the eye diameter in *C. obtusirostra* and an oblong spot smaller than eye diameter in *C. biwae*); L_5 having 10-12 blotches; vs. 8-10 blotches both in *C. obtusirostra* and *C. biwae* (Nakajima et al. 2008: Fig. 2B, Takahama et al. 2012: Fig. 3A). Our molecular genetic data showed that *C. leptosoma*, *C. obtusirostra*, *C. melanoleuca*, *C. pacifica*, and *C. biwae* belong to distinctly divergent clades, and no reciprocal sister group relationships were revealed. Molecular results placed *C. leptosoma* as the sister group of *C. australis*, with a genetic distance of 3.5-3.8 %. *C. leptosoma* was easily distinguished from *C. australis* by a colour pattern showing five Gambetta pigment lines, vs. the absence of the Gambetta pigment line (Chen et al. 2013: Figs. 3, 9-10); and a knife-shaped lamina circularis; vs. a finger-shaped lamina circularis (Chen et al. 2013: Figs. 5-6).

Cobitis wumingensis, *C. rara*, and *C. microcephala* have a long and slender lamina circularis in males

(Table 4). *C. wumingensis* can be distinguished from *C. microcephala* by the finger-shaped lamina circularis (vs. serrated). *C. wumingensis* can be distinguished from *C. rara* by the sharp and nearly filamentous tip of the pectoral-fin ray in males; vs. not sharp and slightly oval (Chen & Chen 2005: Fig. 6H); L_5 having 9-10 longitudinal rectangular blotches in females and 13-14 in males; vs. 6-9 vertical elongated blotches (Chen & Chen 2005: Fig. 2H); a caudal fin with 5-6 narrow bars; vs. 3-4 broad bars (Chen & Chen 2005: Fig. 2H); barbel lengths shorter than eye diameter; vs. longer than eye diameter (Chen & Chen 2005). Petrtyl et al. (2010) found *C. laoensis* (Sauvage, 1878) in the Vietnam part of the River Pearl basin and expected that the species could also be found in China. The morphology of the lamina circularis of *C. wumingensis* is also similar to that of *C. laoensis* from Vietnam. The former species can be distinguished from the latter one by the two superficial longitudinal mental lobes posteriorly not ending in a single filiform tip (Fig. 5D); vs. the presence of a filiform tip (Freyhof & Serov 2000: Fig. 1); barbel lengths shorter than eye diameter, with maxillo-mandibular barbels reaching to the vertical area of the nostrils; vs. longer than eye diameter, with maxillo-mandibular barbels reaching to the vertical area of the front border or in the middle of the eye (Freyhof & Serov 2000); sexually dimorphic colour patterns observed in L_5 , L_5 having 9-10 longitudinal rectangular blotches in females and 13-14 in males; vs. no sexually dimorphic colour patterns (Freyhof & Serov 2000); a dorsal fin near the caudal-fin, the dorsal fin situated in the middle of the anterior eyes and base of the caudal fin; vs. equidistant from the base of the caudal fin and end of the snout (Freyhof & Serov 2000).

In this study, the phylogenetic relationships were investigated by comparing the DNA sequences of cytochrome *b* gene from 31 species, including 24 *Cobitis* species, three *Niwaëlla* and four *Iksookimia* species. Results of the phylogenetic analyses were not congruent with traditional systematic views. Our analyses showed that the genera *Cobitis*, *Niwaëlla*, and *Iksookimia* were paraphyletic. MtDNA phylogeny revealed three *Niwaëlla*, three *Iksookimia*, and 13 *Cobitis* lineages that do not correspond to the defined morphological groups based on characteristics associated with secondary sexual dimorphism and pigmentation pattern. The genera *Cobitis* and *Iksookimia* exhibit a lamina circularis on the pectoral fin in males, which is absent in genus *Niwaëlla*. Morphologically defined genera do not correspond to natural groups, as suggested in other studies (Tang et

al. 2006, Šlechtová et al. 2008, Perdices et al. 2012). Mapping these characteristics into our molecular cladogram (Fig. 10) showed that none of them were synapomorphous to all species studied here, thereby reflecting parallel evolution. Although the lamina circularis and pigmentation pattern appear to be good characteristics to identify all species of spined loaches, the above evidence suggests that they are inappropriate to define the monophyletic lineages included in the genera *Cobitis*, *Niwaëlla*, and *Iksookimia*.

Our phylogenetic analysis confirms *C. arenae* as the sister lineage of all other *Cobitis*, *Iksookimia*, and *Niwaëlla*. *C. arenae* displays unusual characteristics that do not exactly fit into the proper definition of *Cobitis*. Firstly, the lamina circularis of *C. arenae* is not at the base of the second pectoral-fin ray that differs from other *Cobitis* species (Chen & Chen 2011: Fig. 5B). Secondly, *C. arenae* does not share the body shape and colour pattern of *Cobitis*, but closely resembles *Acantopsis* species, lacking the lamina circularis. Thirdly, *C. arenae* has four barbel-like fringes in mental lobes, which differs from other *Cobitis* species with smaller mental lobes (Chen & Chen 2011: Fig. 5D). Lastly, the scales of *C. arenae* are small with a very large focal area, which differs from other *Cobitis* species (Chen & Chen 2011: Fig. 5C). The most recent study of *C. arenae* also suggested no affiliation of this species to the genera *Cobitis* or *Acantopsis* (Kottelat 2012). The basal position and morphological singularity of *C. arenae* exclude its inclusion in *Cobitis*, and its proper taxonomic level needs a more inclusive study.

Our phylogenetic relationships are partially congruent with geographical patterns, and clearly relate the Chinese *Cobitis* species with the Japanese and Korean species. Chinese *Cobitis* species can be currently divided into seven different lineages: the first group includes the southern lineage (*C. leptosoma*, *C. australis*, and *C. wumingensis*), the second group includes the northern lineage (*C. granoei* and *C. melanoleuca*), the third group includes the southern lineage (*C. obtusirostra*), the fourth group includes the southern lineage (*C. microcephala*) and the central lineage (*C. crassicauda* and *C. rara*), the fifth group includes the northern lineage (*C. lutheri*) and the central lineage (*C. macrostigma*), the sixth group includes the central lineage (*C. dolichorhynchus* and *C. stenocauda*), and the seventh group includes the central lineage (*C. fasciola* and *C. sinensis*). The Chinese *Cobitis* species are not closely related even in groups with geographical proximity. The southern lineage (*C. leptosome*, *C. australis*, and *C.*

wumingensis) showed closer affinities with Japanese, northern lineage (*C. granoei* and *C. melanoleuca*), and Korean (*C. choii* Kim & Son, 1984) species than with other southern species (*C. obtusirostra* and *C. microcephala*). Molecular studies identified *C. lutheri* as a sister group of the Korean species, and this species was not included within the northern lineage (*C. granoei* and *C. melanoleuca*) and Korean *C. choii*. Our results suggested that Chinese *Cobitis* are not monophyletic, thereby indicating that the northern and southern Chinese species are related to two routes of dispersal. However, these relationships were not strongly supported. Therefore, a more comprehensive analysis, with different molecular markers and more taxa, is necessary to revise the relationships of the *Cobitis* species from East Asia.

Comparative material

Cobitis arenae: IHB 82077 (1), male, 80.1 mm SL; China: from the River Pearl basin in the Huaiji County, in Guangdong Province; IHB 863-867, 6294-6296 (8), females, 61-78.8 mm SL; China: from the River Pearl basin in the Lingyun County and Guiping City in Guangxi; IHB 0509404 (1), male, China: from the River Nanduijiang in the Ding'an County in Hainan Province.

Cobitis macrostigma: IHB 0509147, 0509149, 0509153-6, 0509157-8 (8), males, 94.4-109.2 mm SL; IHB 0509139-146, 0509148, 0509151-2 (11), females, 111.9-137.4 mm SL; China: from the Lake Dongting in the Anxiang County in Hubei Province.

Cobitis dolichorhynchus: IHB 74v0402, 74v0409, 74v0436, 74v0439-40, 74v0443-4, 74v0448, 74v0627 (9), males, 56.1-66.0 mm SL; IHB 74v0401, 74v0408, 74v0432, 74v0413-6, 74v0437, 74v0445, 74v0448, 74v0612-3, 74v0615, 74v0617-20, 74v0621-5, 74v0629-30, 74v0641 (25), females, 83.1-107.8 mm SL; China: from the River Jiulongjiang in Zhangzhou City in the Fujian Province.

Cobitis melanoleuca: IHB 800742, 900044 (2), females, 53.3-74.5 mm SL; China: from the Liujiaxia reservoir in the Gansu Province.

Cobitis granoei: IHB 6331, 6335, 6354, 6356-8, 6360-1 (8), males, 47.4-51.9 mm SL; IHB 6329-30, 6336-8, 6340-1, 6343, 6344-6, 6366 (12), females, 51.9-62.5 mm SL; China: from the River Huangshui in Xining City in the Qinghai Province.

Cobitis rara: IHB 80vi0905, 80vi0909-10, 80vi0913, 80vi0915-6, 80vi1070, 80vi1072, 80vi1074-5, 80vi1058 (11), males, 73.2-80.8 mm SL; IHB 80vi0473, 80vi0475-6, 80vi0694, 80vi0904, 80vi0911-2, 80vi0914, 80vi1055, 80vi1057,

80vi1059, 80vi1063, 80vi1065, 80vi1067, 80vi1194, 80vi1201 (16), females, 76.2-99.6 mm SL; China: from the River Jialingjiang in the Shaxi Province.

Cobitis sinensis: IHB 8840712, 8840702 (2) males, 78.9-85.3 mm SL; IHB 701, 703-8, 710, 713 (9), females, 75.4-113.9 mm SL; China: from the River Yuangjiang in the Songtao County in the Guizhou Province.

Cobitis lutheri: IHB 58908, 58924, 58927, 58932-5, 58937 (8), males, 40.8-61.8 mm SL; IHB 58903, 58910-4, 58917, 58919-20, 58930-1, 58935, 58939 (13), females, 52.5-64.4 mm SL; China: from the River Haila'er in the Neimenggu Province; IHB 6031-2, 6035-6, 6038, 6040, 6046-8, 6052 (10), males, 43.2-56.8 mm SL; China: from the River Fabiela in the Aihui County in the Heilongjiang Province.

Cobitis multimaculata: IHB 75v3203, 75v3188, 75v3190, 75v3192, 75v3194-5, 75v3198, 75v3202, 75v3205, (9) males, 60.3-68.8 mm SL; IHB 75v3100, 75v3186, 75v3189, 75v3193, 75v3196-7, 75v3204, (7) females, 68.4-92.0 mm SL; China: from the River Nanliu in the Bobai County in Guangxi.

Cobitis microcephala: IHB 0605135, 0605138, (2) male, 47.9-51.8 mm SL; IHB 0605205-210, (6)

females, 50.4-59.8 mm SL; China: from the River Nanliu in the Bobai County in Guangxi.

Cobitis stenocauda: IHB 9607073 (1), male, 62.8 mm SL; IHB 0509377-379, 0509372-374 (6), females, 92.0-77.4 mm SL; China: from the River Xinjiang in the Guixi County in the Jiangxi Province.

Cobitis australis: IHB 0605157 (1), male, 80.8 mm SL; China: from the River Yongjiang in Nanning City in Guangxi; IHB 0605158 (1), female, 83.1 mm SL; China: from the River Yujiang in Guiping City in Guangxi.

Cobitis fasciola: IHB 9607001-10 (11), males, 70.9-84.4 mm SL; IHB 9607012-50 (39), females, 81.9-103.1 mm SL; China: from the River Xinjiang in the Yujiang County in the Jiangxi Province.

Cobitis crassicauda: IHB 9607027, 9607076-7 (3), males, 63.5-68.1 mm SL; IHB 9607078-80 (3), females, 79.8-81.6 mm SL; China: from the River Xinjiang in the Yujiang County in the Jiangxi Province.

Acknowledgements

This research was financially supported by the National Natural Science Foundation of China (Grant No. 31372166). We thank the reviewers for giving lots of valuable suggestions on both science and language.

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