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ORIGINAL ARTICLE

Two new species of the genus *Cobitis* (Cypriniformes: Cobitidae) from South China

Yongxia Chen[1], Hao Chen[1], Dekui He[2], Yifeng Chen[2 *]

[1]*College of Life Sciences, Hebei University, Baoding 071002, Hebei, China; E-mail: chenyongxia@hbu.edu.cn*
[2]*Laboratory of Biological Invasion and Adaptive Evolution, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, Hubei, China*
[*]*Corresponding author, E-mail: chenyf@ihb.ac.cn*

Abstract Two spined loaches, *Cobitis oxycephala* Chen YX & Chen YF, **sp. nov.** and *C. brachysoma* Chen YX & Chen YF, **sp. nov.**, are described based on specimens collected from Guangdong and Hainan Provinces, South China. Morphology features and sequences of mitochondrial cytochrome *b* gene of these two new species were analyzed. Morphological and molecular data show that *C. oxycephala* Chen YX & Chen YF, **sp. nov.** is closely related to *C. dolichorhynchus* Nichols, and *C. brachysoma* Chen YX & Chen YF, **sp. nov.** is closely related to *C. microcephala* Chen & Chen. These two new species can be distinguished from their congeners by the combination of characteristics of body shape, color pattern, lamina circularis, mental lobes, suborbital spine, subdorsal scales, and caudal peduncle.

Key words Loaches, taxonomy, mt DNA, molecular phylogeny.

1 Introduction

The spined loaches genus, *Cobitis* Linnaeus, 1758, is one of the most diverse and widely distributed genera within the family Cobitidae (Osteichthyes, Cypriniformes). It is mostly distributed in Palaearctic and Oriental Regions, except the cold boreal areas and Tibetan Plateau (Sawada, 1982; Bănărescu, 1990), with the widest distributional range of all cobitine genera. *Cobitis* species are adapted to flowing mountain streams and rivers with sandy bottoms. As a consequence of strong adaptation to benthic habitats, they have an elongated, compressed body, with highly variable color patterns. Phenotypic and reproductive plasticity within this group make taxonomic identification difficult (Nalbant, 1993; Janko *et al*., 2007). Froese & Pauly (2017) listed 84 valid species of *Cobitis* in the world. In China, 21 species of *Cobitis* were found totally (Chen *et al*., 2015, 2016; Chen & Chen, 2016).

From these, nine species are known from the Pear River basin south to the rivers of Hainan Island. Of these, *C. arenae* (Lin) has a wide range of geographical distribution, known from the Pearl River basin and the Nandujiang River in Hainan; *C. baishagensis* Chen, Sui, Liang & Chen is known from the rivers of Hainan; *C. multimaculata* Chen & Chen is known from the Nanliu River in Guangxi; *C. hereromacula* Chen, Sui, Liang & Chen is known from the Luohe River in Guangdong; the remaining five species are known from the Pear River basin. More specifically, *C. australis* Chen, Chen & He, *C. leptosoma* Chen, Sui, He & Chen, *C. microcephala* Chen & Chen and *C. wumingensis* Chen, Sui, He & Chen are known from the Xijiang River of the Pearl River, while *C. obtusirostra* Chen, Sui, He & Chen is known from the Beijiang River of the Pearl River.

During July 2015 to June 2016, ichthyological surveys were done in Guangdong and Hainan Provinces, South China

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and dozens of *Cobitis* specimens were collected. Based on morphological and molecular analyses, two species of *Cobitis* were described as new to science here.

2 Materials and methods

2.1 Sampling

A total of 62 specimens from China were checked in this study, with 17 (14♂3♀) from the Nandujiang River (Ding'an County, Hainan; Fig. 1), 21 (6♂15♀) from the Suijiang River (a tributary of the Pearl River, Guangning County, Guangdong), 11 (5♂6♀) from the Nalongjiang River (a tributary of the Moyangjiang River, Yangjiang County, Guangdong), and 13 (5♂9♀) from the Zengjiang River (a tributary of the Pearl River, Longmen County, Guangdong), respectively.

Materials used in morphology were preserved in 10% formaldehyde solution, and specimens for molecular analyses were preserved in 95% ethanol. Type specimens were deposited in the College of Life Science, the Hebei University (HU), Baoding, Hebei, China and the Freshwater Fish Museum, the Institute of Hydrobiology (IHB), the Chinese Academy of Sciences, Wuhan, Hubei, China.

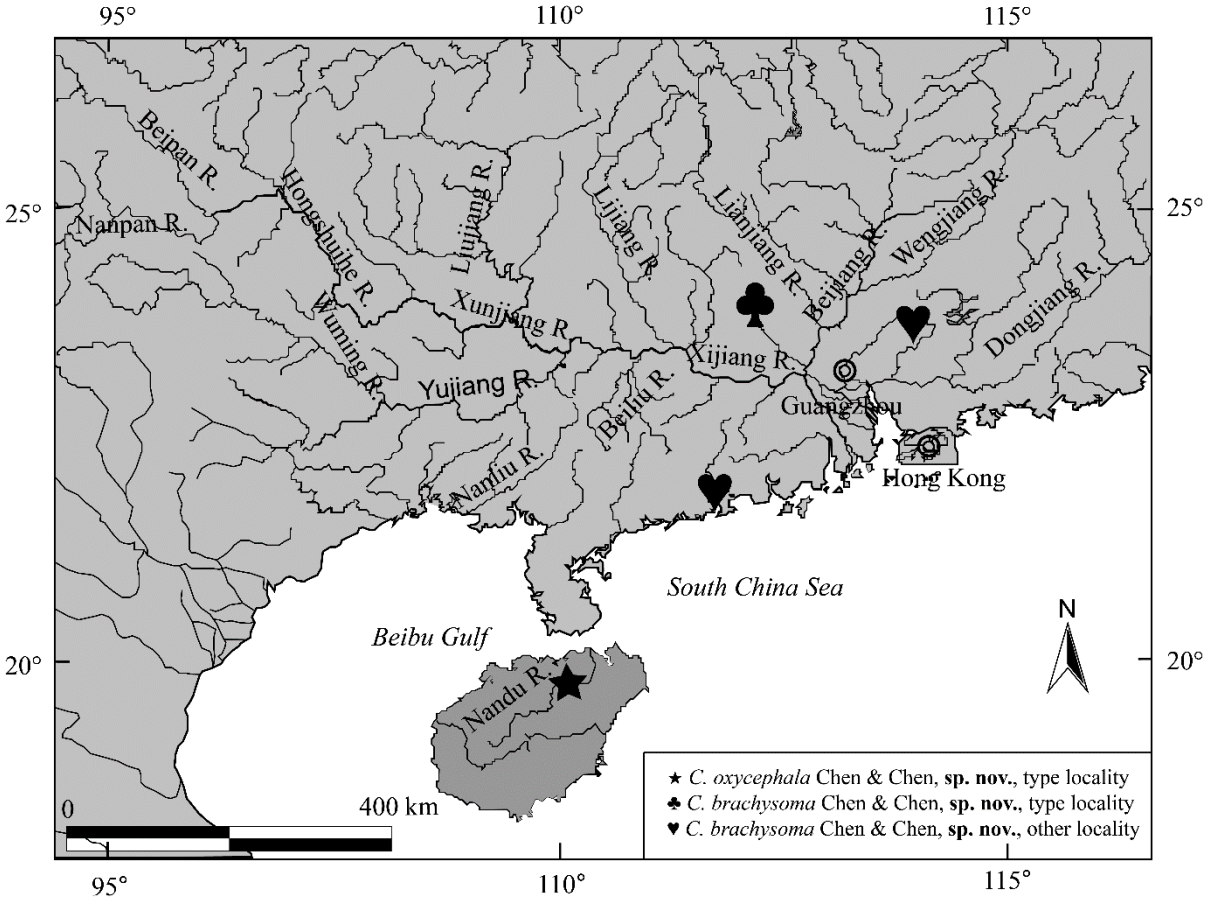


Figure 1. Map showing the collection sites of the two new species of the genus *Cobitis* in Guangdong and Hainan Provinces of China.

2.2 Morphology

Seventeen morphometric variables were measured followed Chen & Chen (2011). Measurements were made to the nearest 0.1 mm using digital calipers. Scales were collected from the subdorsal region between the origin of the dorsal fin and lateral line, and photographed using a Leica M205A stereomicroscope with Leica DFC295 camera, and edited using Adobe Photoshop CS5.1®. Color photos were taken using a Canon Powershot digital camera. Map was constructed using Adobe Illustrator CS5.1®. Terminology of mouth, suborbital spine, lamina circularis and subdorsal scales structures were

adopted from Nalbant (1963). Notations (L_1–L_5) describing lines and speckles on the dorsolateral side of the trunk followed those designated by Takeda & Fujie (1945).

2.3 DNA extraction, amplification and sequencing

Total genomic DNA was extracted from muscle tissue or fin clips using the standard phenol-chloroform method (Sambrook *et al*., 1989). The mitochondrial cytochrome *b* gene (cyt *b*) was amplified and sequenced adopting the methods of He & Chen (2009). A total of 12 individuals of the two new species from four locations were sequenced, including two individuals of *C. oxycephala* Chen YX & Chen YF, **sp. nov.**, ten of *C. brachysoma* Chen YX & Chen YF, **sp. nov.**

2.4 Analysis of DNA sequences

Electropherograms were visually checked using Chromas 2.22. Sequences were aligned using Clustal X 2.0 (Larkin *et al*., 2007). 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 (K2P) model using MEGA 5.05 (Tamura *et al*., 2011). Forty-four cyt *b* sequences of *Cobitis*, five of *Iksookimia*, and two of *Sabanejewia* were downloaded from GenBank (Table 1).

Phylogenetic analyses were inferred by Maximum Likelihood (ML) method and Bayesian inferences (BI), using programs of RAxML version 8.0.2 (Stamatakis, 2014) and MrBayes v3.2.3 (Ronquist *et al*., 2012), respectively. The best-fit model (GTR + G+ I) of nucleotide substitution was selected using the Akaike Criterion (AIC) in jModelTest 0.1.1 (Posada, 2008). For ML analysis, the GTRGAMMAIX model was selected, and the credibility of each node was evaluated by bootstrap analysis with 1000 replicates. For Bayesian analyses, four Monte Carlo Markov Chains were run simultaneously with 1,000,000 trees, sampling 1/100 trees, with a burn-in value of 1,000. The remaining trees were used to build a 50 % majority rule consensus tree, and statistical support of each node was assessed by posterior probabilities.

Abbreviations used are as following:

A—anal-fin;
C—caudal-fin;
D—dorsal-fin;
P—pectoral-fin;
SL—stander length;
TL—total length;
V—ventral-fin.

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

Scientific name in source	Locality	Accession Nos.
Cobitis arenae	China, Hainan, R. Nandujiang	JX888905
*Cobitis australis*1	China, Guangxi, R. Yongjiang	KC753352
*Cobitis australis*2	China, Guangxi, R. Yujiang	KC753353
*Cobitis australis*3	China, Guangxi, R. Liujiang	MF004247
Cobitis baishagensis	China, Hainan, R. Nandujiang	KX823432
*Cobitis biwae*1	Japan, Yamagata, R. Mogami	AB084395*
*Cobitis biwae*2	Japan, Akita, R. Yoneshiro	AB084407*
*Cobitis biwae*3	Japan, Tokushima, R. Anabuki	AB084399*
*Cobitis biwae*4	Japan, Shizuoka, Fujieda, R. Asahina	AB084404*
Cobitis brachysoma Chen YX & Chen YF, **sp. nov.**1	China, Guangdong, R. Nalongjiang	MF004251
Cobitis brachysoma Chen YX & Chen YF, **sp. nov.**2	China, Guangdong, R. Nalongjiang	MF004252
Cobitis brachysoma Chen YX & Chen YF, **sp. nov.**3	China, Guangdong, R. Suijiang	MF004253
Cobitis brachysoma Chen YX & Chen YF, **sp. nov.**4	China, Guangdong, R. Suijiang	MF004254
Cobitis brachysoma Chen YX & Chen YF, **sp. nov.**5	China, Guangdong, R. Suijiang	MF004255
Cobitis brachysoma Chen YX & Chen YF, **sp. nov.**6	China, Guangdong, R. Zengjiang	MF004256
*Cobitis choii*1	Korea, R. Baekgok	EU656112*
*Cobitis choii*2	Russia, R. Arsenyevka, R. Ussury	JN858872*
Cobitis crassicauda	China, Jiangxi, R. Xinjiang	JX888909

Table 1 (continued)

Scientific name in source	Locality	Accession Nos.
*Cobitis dolichorhynchus*1	China, Fujian, R. Minjiang	KX823435
*Cobitis dolichorhynchus*2	China, Fujian, R. Minjiang	KX823436
*Cobitis dolichorhynchus*3	China, Fujian, R. Aojiang	MF004249
*Cobitis dolichorhynchus*4	China, Fujian, R. Aojiang	MF004248
*Cobitis dolichorhynchus*5	China, Fujian, R. Aojiang	MF004250
*Cobitis fasciola*1	China, Jiangxi, R. Xinjiang	JX888910
*Cobitis fasciola*2	China, Jiangxi, R. Xinjiang	KP133115
*Cobitis gracilis*1	China, Jilin, R. Yalu	KY009955
*Cobitis gracilis*2	China, Jilin, R. Yalu	KY009954
*Cobitis granoei*1	China, Liaoning, R. Hunjiang	DQ105243*
*Cobitis granoei*2	China, Heilongjiang, R. Panguhe	KX823423
*Cobitis granoei*3	China, Heilongjiang, R. Panguhe	KX823425
*Cobitis granoei*4	China, Heilongjiang, R. Panguhe	KX823424
Cobitis hankugensis	Korea, R. Geum Cheon	KP161086*
*Cobitis hereromacula*1	China, Guangdong, R. Longjin	KX823433
*Cobitis hereromacula*2	China, Guangdong, R. Longjin	KX823434
Cobitis laoensis	Vietnam, Central Vietnam, R. unknown	KP161089*
Cobitis lebedevi	Mongolia, Khentii, R. Dund Bayen	KP161090*
Cobitis leptosoma	China, Guagnxi, R. Lijiang	KP133117
*Cobitis lutheri*1	China, Heilongjiang, R. Heilongjiang	JX888906
*Cobitis lutheri*2	China, Heilongjiang, R. Heilongjiang	KP133112
Cobitis macrostigma	China, Jiangxi, L. Poyang	JX888904
Cobitis matsubarae	Japan, Saga, R. Matuura	AB091225*
Cobitis magnostriata	Japan, Shiga	LC097340*
*Cobitis melanoleuca*1	China, Hebei, R. Yanghe	KX823426
*Cobitis melanoleuca*2	China, Hebei, R. Yanghe	KX823427
*Cobitis melanoleuca*3	China, Hebei, R. Yanghe	KX823428
*Cobitis microcephala*1	China, Guangxi, R. Nanliu	JX888907
Cobitis minamorii tokaiensis	Japan	AP013305*
Cobitis minamorii oumiensis	Japan, Shiga	LC097366*
Cobitis minamorii minamorii	Japan	AP013309*
Cobitis obtusirostra	China, Guangdong, R. Lianjiang	KP133118
Cobitis oxycephala Chen YX & Chen YF, **sp. nov.**	China, Hainan, R. Nandujiang	MF004260
Cobitis pacifica	Korea, R. Doekcheon	KP161126*
Cobitis rara	China	EF508507*
Cobitis sinensis	China, Guizhou, R. Yuangjiang	JX888902
*Cobitis stenocauda*1	China, Jiangxi, R. Xinjiang	JX888903
*Cobitis stenocauda*2	China, Jiangxi, R. Xinjiang	KP133111
*Cobitis takatsuensis*1	Japan, R. Tsutsuga	AB039338*
*Cobitis takatsuensis*2	Japan, R. Shigenobu	AB039341*
Cobitis tetralineata	Korea, Gokseong, R. Boseong	KC524528*
*Cobitis wumingensis*1	China, Guangxi, R. Heishui	MF004257
*Cobitis wumingensis*2	China, Guangxi, R. Wuming	KP133116
*Cobitis wumingensis*3	China, Guangxi, R. Wuming	KX823429
*Cobitis wumingensis*4	China, Guangxi, R. Jinchengjiang	AM711122*
*Cobitis wumingensis*5	China, Guangxi, R. Chengjiang	MF004258

Table 1 (continued)

Scientific name in source	Locality	Accession Nos.
*Cobitis wumingensis*6	China, Guangxi, R. Chengjiang	MF004259
*Cobitis zhejiangensis*1	China, Zhejiang, R. Lingjiang	KX962073
*Cobitis zhejiangensis*2	China, Zhejiang, R. Lingjiang	KX823430
*Cobitis zhejiangensis*3	China, Zhejiang, R. Lingjiang	KX823431
*Cobitis zhejiangensis*4	China, Zhejiang, R. Nanxi	KX962065
*Cobitis zhejiangensis*5	China, Zhejiang, R. Nanxi	KX962066
Cobitis sp.1	China, Guangdong, R. Pearl	AM937070*
Cobitis sp.1	China, Guangdong, R. Pearl	AM937071*
Cobitis sp.1	China, Guangdong, R. Pearl	AM937073*
Cobitis sp.2	China, Guangxi, R. Beilun	AM937067*
Cobitis sp.2	China, Guangxi, R. Beilun	AM937068*
Cobitis sp.3	China, Guangdong, R. Jianjiang	AM921774*
Cobitis sp.3	China, Guangdong, R. Jianjiang	AM921775*
Cobitis sp.3	China, Guangxi, R. Fangchengjiang	AM921770*
Cobitis sp.3	China, Guangxi, R. Fangchengjiang	AM921771*
Cobitis sp.3	China, Guangxi, R. Beilun	AM937069*
Cobitis sp.4	China, Guangdong, R. Huanggang	AM930409*
Cobitis sp.4	China, Guangdong, R. Huanggang	AM930410*
Cobitis sp.5	China, Guangdong, R. Hanjiang	AM921759*
Cobitis sp.5	China, Guangdong, R. Hanjiang	AM921760*
Cobitis sp.5	China, Guangdong, R. Hanjiang	AM921762*
Cobitis sp.5	China, Guangdong, R. Hanjiang	AM921764*
Cobitis sp.5	China, Guangdong, R. Rongjiang	AM921772*
Cobitis sp.5	China, Guangdong, R. Rongjiang	AM921773*
Cobitis sp.5	China, Guangdong, R. Rongjiang	AM930259*
Cobitis sp.5	China, Guangdong, R. Rongjiang	AM930262*
Cobitis sp.5	China, Guangdong, R. Rongjiang	AM930263*
Cobitis sp.5	China, Guangdong, R. Pearl	AM930566*
Cobitis sp.5	China, Guangdong, R. Pearl	AM930567*
Iksookimia hugowolfeldi	Korea, Daedong Dam	EU670758*
Iksookimia koreensis	Korea, R. Jeongcheon	EU670759*
Iksookimia longicorpa	Korea, R. Seomjin	EU670760*
Iksookimia pumila	Korea, Buan Dam	EU670761*
Iksookimia yongdokensis	Korea, R. Gase	EU670762*
Sabanejewia balcanica	Slovakia, R. Ipel	DQ996499
Sabanejewia aurata	Georgia, Caucasus, R. Rione	AF499190*

*Retrieved from GenBank.

3 Results

3.1 Taxonomy

***Cobitis oxycephala* Chen YX & Chen YF, sp. nov.** (Figs 2–7)

Cobitis sinensis Chen, 1986 (nec. Sauvage & Dabry, 1874): 145 (Hainan, fig. 81).

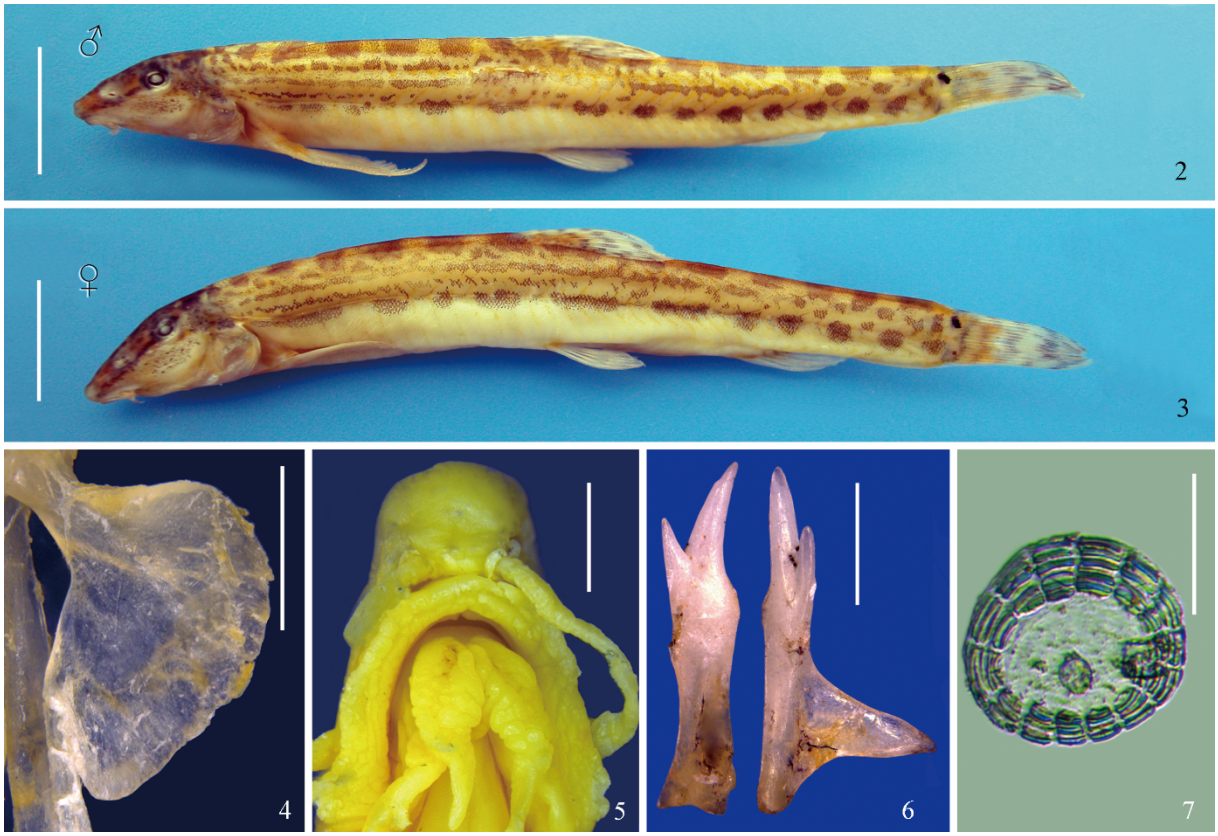
Cobitis taenia dolichorhynchus Nichols & Popo, 1927 (nec. Nichols, 1918): 335 (Hainan, fig. 8); Nichols, 1943: 197 (Fukien, Kwangtung, Hainan, fig. 81).

Holotype. ♂, IHB 0509273, 67.7 mm TL, 57.9 mm SL, China, Hainan, Ding'an County (19°68′N, 110°36′E; elev. 65 m), the Nandujiang River, July 2005, leg. Kun Li. Paratypes. IHB 0509392–9, 0509401–5, 13♂, 63.1–70.3 mm TL, 51.8–59.1 mm SL, IHB 509400, 0509406–7, 3♀, 74.6–84.7 mm TL, 61.6–71.6 mm SL, same data as holotype.

Diagnosis. The new species can be distinguished from its congeners by possessing the following combination of characteristics: 13–15 large rectangular blotches on L_1; 10–14 elongated blotches on L_5; snout sharp (Figs 2–3); males with a semicircular lamina circularis at the base of the first branched pectoral fin ray (Fig. 4); mental lobes undeveloped, three superficial longitudinal lobes short and bluntly rounded (Fig. 5); suborbital spine thick and curved, with a short processus medio-caudalis (Fig. 6). *Cobitis oxycephala* Chen YX & Chen YF, **sp. nov.** is similar to *C. sinensis*, *C. dolichorhynchus* and *C. zhejiangensis* in color pattern (with L_1–L_5 line on the body) and lamina circularis morphology (plate-like), but differs from them in having snout sharp (vs. rounded); 10–14 horizontally elongated blotches and without the deeper faint dusky band on L_5 (vs. 11–12 rectangular and vertically elongate spots in *C. sinensis* (Son & Kim, 2002); a row of more or less oval blotches and with the deeper faint dusky band on the mid-lateral line in *C. dolichorhynchus* (Nichols, 1918); 14–16 short vertical spots in *C. zhejiangensis*). It further differs from *C. dolichorhynchus* and *C. zhejiangensis* in body slender, depth 7.6 in SL in male and 7.7 in female (vs. sturdy, depth 5.8 in SL in male in *C. dolichorhynchus* (Nichols, 1918); 5.6–6.7 (mean 6.0) in males and 4.3–5.0 (mean 4.7) in females in *C. zhejiangensis*); 13–15 large rectangular blotches on L_1 (vs. irregular dark cross blotches in *C. dolichorhynchus* (Nichols, 1918); 13–19 rectangular blotches in *C. zhejiangensis*).

Description (Figs 2–7, Table 2). D. III–7; A. III–5; V. I–6; P. I–6–7; C. IV–14–16–IV. Body moderately slender, compressed. Head small. Snout sharp. Eyes located on upper part and middle of head. Preorbital part of head equal to or longer than postorbital part of head. Mouth small, inferior, with three pairs of short barbels. Length of maxillo-mandibular barbels shorter than diameter of eye. Mental lobes undeveloped, three superficial longitudinal lobes short and bluntly rounded (Fig. 5) Suborbital spine thick and curved, with a short processus medio-caudalis. Processus latero-caudalis long, less than one-third of processus medio-caudalis (Fig. 6). Subdorsal scales small, oval, with a moderately large focal area, 18–20 radial grooves, and 3–5 supplementary ones (Fig. 7).

Dorsal fin moderately long, inserted midway between nostril and base of caudal fin. In males, pectoral fins long, second



Figures 2–7. *Cobitis oxycephala* Chen YX & Chen YF, **sp. nov.**, Nandujiang River, Ding'an, Hainan, China. 2. Holotype, IHB 0509273, lateral view. 3. IHB 0509274, lateral view. 4. Lamina circularis in the pectoral in of male, dorsal view. 5. Mouth characters, front view. 6. Suborbital spine, left dorsal view, right interior view. 7. Subdorsal scales, dorsal view. Scale bars: 2–3 = 1 cm; 4–6 = 1 mm; 7 = 100 μm.

pectoral fin ray longest (Fig. 2). In females, pectoral fins slightly short, third pectoral fin ray longest (Fig. 3). Ventral fins small and short, approximately at same level as second or third branched dorsal-fin ray. Anal fin short, located on half of space between ventral and caudal fins. Anal orifice close to anal fin. Caudal fin long, emarginated tip. Caudal peduncle with ventral adipose crest. Lateral line long, not exceeding length of pectoral fins in males, and exceeding in females.

Pigmentation pattern. Color characterized by pigmentation pattern with five longitudinal lines of dark speckles on dorsolateral sides of body (L_1–L_5 from dorsal to ventral) (Figs 2–3). Color pattern characteristic of sexual dimorphism not observed. Head sprinkled with many black dots, and a black stripe extended from insertion of rostral barbels through eye to occiput. L_1 consisted of a row of 5–6 large rectangular blotches before dorsal fin; 2 on dorsal fin and 6–7 behind dorsal fin. Gap of rectangular blotches smaller than width of blotches. L_2 composed of a line of irregularly small and solitary spots or blotches and not intermingle with gap of large rectangular blotches, and reaching beyond dorsal fin. L_3 composed a narrow stripe beyond anal fin and a row of rounded blotches behind anal fin. L_4 composed of a line of minute black dots, which fused to a line and diminished towards end of ventral fin. L_5 consisted of a row of 10–14 horizontally elongated blotches, without deeper faint dusky band. One small oblique vertical jet blotch at upper base of caudal fin base and far smaller than eye diameter. 4–5 narrow rows of dark dots on dorsal and caudal fins.

Sexual dimorphism. Males smaller than females with proportionally longer pectoral fins. In males, second pectoral fin ray thickened and elongated, a semicircular lamina circularis at base of first branched pectoral fin ray. In females, third pectoral fin ray elongated.

Distribution. This new species occurs in the Nandujiang River, Hainan, China (Fig. 1).

Etymology. The species name is derived from the Greek *oxys*, meaning sharp, and *kephale* meaning head, in reference to the pointed head of the species.

Table 2. Morphometric and meristic characters for the two new species of *Cobitis*, all measurements given in millimeters (mm).

Variable	*C. oxycephala* Chen YX & Chen YF, **sp. nov.**		*C. brachysoma* Chen YX & Chen YF, **sp. nov.**		
	Male (*n*=1)	Female (*n*=1)	Males (*n*=6)		Females (*n*=15)
	Holotype	Mean	Holotype	Range	Range
Standard Length (SL)	57.5	65.5	47.1	43.1–58.6 (48.3)	44.7–61.5 (50.6)
SL/Head Length (HL)	5.1	4.8	5.3	5.0–5.4 (5.3)	4.6–6.2 (5.3)
SL/Body Depth	7.6	7.7	6.1	6.1–6.6 (6.4)	5.5–8.6 (6.8)
SL/Pectoral-Ventral Length	3.2	3.3	3.1	2.7–3.3 (3.0)	2.7–3.6 (3.0)
SL/Caudal Peduncle Length (CPL)	5.6	5.7	9.2	7.3–9.2 (8.4)	7.0–9.9 (8.5)
SL/Caudal Peduncle Depth (CPD)	14.3	13.3	10.9	9.9–11.3 (10.8)	10.3–12.5 (11.6)
SL/Dorsal Fin Length	6.2	6.4	6.0	5.1–6.0 (5.6)	5.2–6.6 (5.7)
SL/Pectoral Fin Length	5.1	7.7	6.4	5.9–7.9 (6.6)	6.2–9.2 (7.6)
SL/Ventral Fin Length	9.2	9.6	7.8	6.5–8.0 (7.5)	6.9–9.9 (8.2)
SL/Anal Fin Length	9.6	9.0	6.7	6.6–7.2 (6.9)	6.1–7.6 (6.8)
SL/Caudal Fin Length	5.8	5.8	7.7	5.0–7.7 (5.9)	5.1–7.4 (6.0)
SL/Predorsal Length	2.0	1.9	2.0	1.9–2.1 (2.0)	1.9–2.2 (2.0)
SL/Preventral Length	1.8	1.9	1.7	1.7–1.9 (1.8)	1.7–1.9 (1.8)
SL/Preanal Length	1.3	1.3	1.3	1.3–1.3 (1.3)	1.2–1.3 (1.3)
HL/Preorbital Length	2.3	2.2	2.5	1.9–2.5 (2.3)	1.8–2.9 (2.3)
HL/ Eye Diameter	6.6	6.3	6.6	5.3–6.7 (6.0)	4.0–8.0 (6.0)
HL/ Interorbital Width	5.9	4.6	6.5	4.1–6.5 (5.1)	3.4–5.8 (4.8)
CPL/CPD	2.5	2.3	1.2	1.2–1.5 (1.3)	1.1–1.7 (1.4)

***Cobitis brachysoma* Chen YX & Chen YF, sp. nov.** (Figs 8–17)

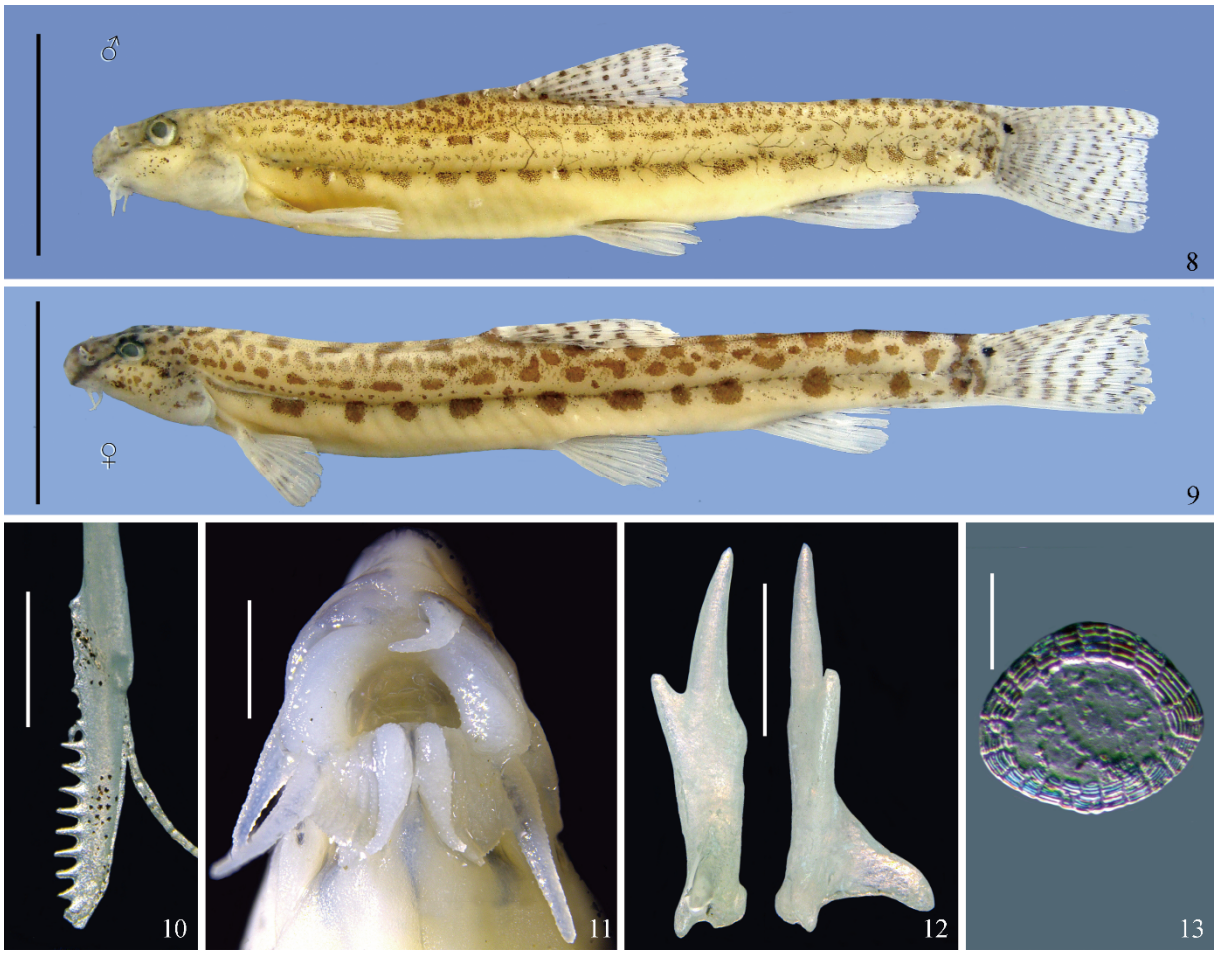
Cobitis sinensis Ye, 1991(nec. Sauvage & Dabry, 1874): 253 (Guangdong); Jia *et al*., 2013: 1492 (Zengjiang River, Guangdong).

Holotype. ♂, HU 1506334, 55.2 mm TL, 47.1 mm SL, China: Guangdong, Guangning County (23°64′N, 112°44′E; elev. 65 m), the Suijiang River (a tributary of the Pearl River), April 2015, leg. Yongxia Chen. Paratypes. HU 1505204, 1505207, 1506320, 1506326, 1506330, 5♂, 50.5–71.4 mm TL, 43.1–58.6 mm SL, HU 1505209, 1505289, 1505292, 1505296, 1506290, 1506302, 1506307, 1506315, 1506322, 1506325, 1506328, 1506331–3, 1506336, 15♀, 53.1–74.2 mm TL, 44.7–

61.5 mm SL, same data as holotype. Other material examined. HU 1506360, 1506369, 1600080, 1600083, 1600086, 5♂, 47.4–74.4 mm TL, 39.5–63.6 mm SL, HU 1506341–2, 1506349, 1600081, 1600084–5, 6♀, 51.2–61.6 mm TL, 42.3–52.9 mm SL, China: Guangdong, Yangjiang County (21°86′N, 111°98′E; elev. 65 m), the Nalongjiang River (a tributary of the Moyangjiang River), April 2015, leg. Yongxia Chen. HU 1608048–9, 1608052–4, 5♂, 50.4–60.8 mm TL, 42.9–52.4 mm SL, HU 1608040–1, 1608043–7, 1608050–1, 9♀, 59.4–92.3 mm TL, 51.3–78.7 mm SL, China: Guangdong, Longmen County (23°73′N, 114°25′E; elev. 65 m), the Zengjiang River (a tributary of the Pearl River), September 2016. leg. Yongxia Chen.

Diagnosis. The new species can be distinguished from its congeners by possessing the following combination of characteristics: small body size, SL mean 50 mm in males and 60 mm in females; a small jet spot far smaller than the eye diameter on the upper part of the caudal peduncle; 7–8 narrow rows of dark spots on the caudal fin (Figs 8–9, 14–17); males with a slender and finely serrated lamina circularis at the base of the first branched pectoral fin ray (Fig. 10); mental lobes undeveloped, two superficial longitudinal lobes short and bluntly rounded (Fig. 11); suborbital spine straight, with long processus medio-caudalis (Fig. 12). *Cobitis brachysoma* Chen YX & Chen YF, **sp. nov.** is similar to *C. microcephala* in body size and shape of lamina circularis, but differs from it in having long processus medio-caudalis of suborbital spine (vs. short); caudal peduncle short, length of caudal peduncle 7.3–9.2 (mean 8.4) in SL in males and 7.0–9.9 (mean 8.5) in females (vs. caudal peduncle long, length of caudal peduncle 5.9–6.6 (mean 6.3) in SL in males and 5.3–7.2 (mean 6.3) in females); body slender, depth 6.1–6.6 (mean 6.4) in SL in males and 5.5–8.6 (mean 6.8) in females (vs. sturdy, depth 5.8–6.2 (mean 5.9) in SL in males and 5.0–6.5 (mean 5.6) in females).

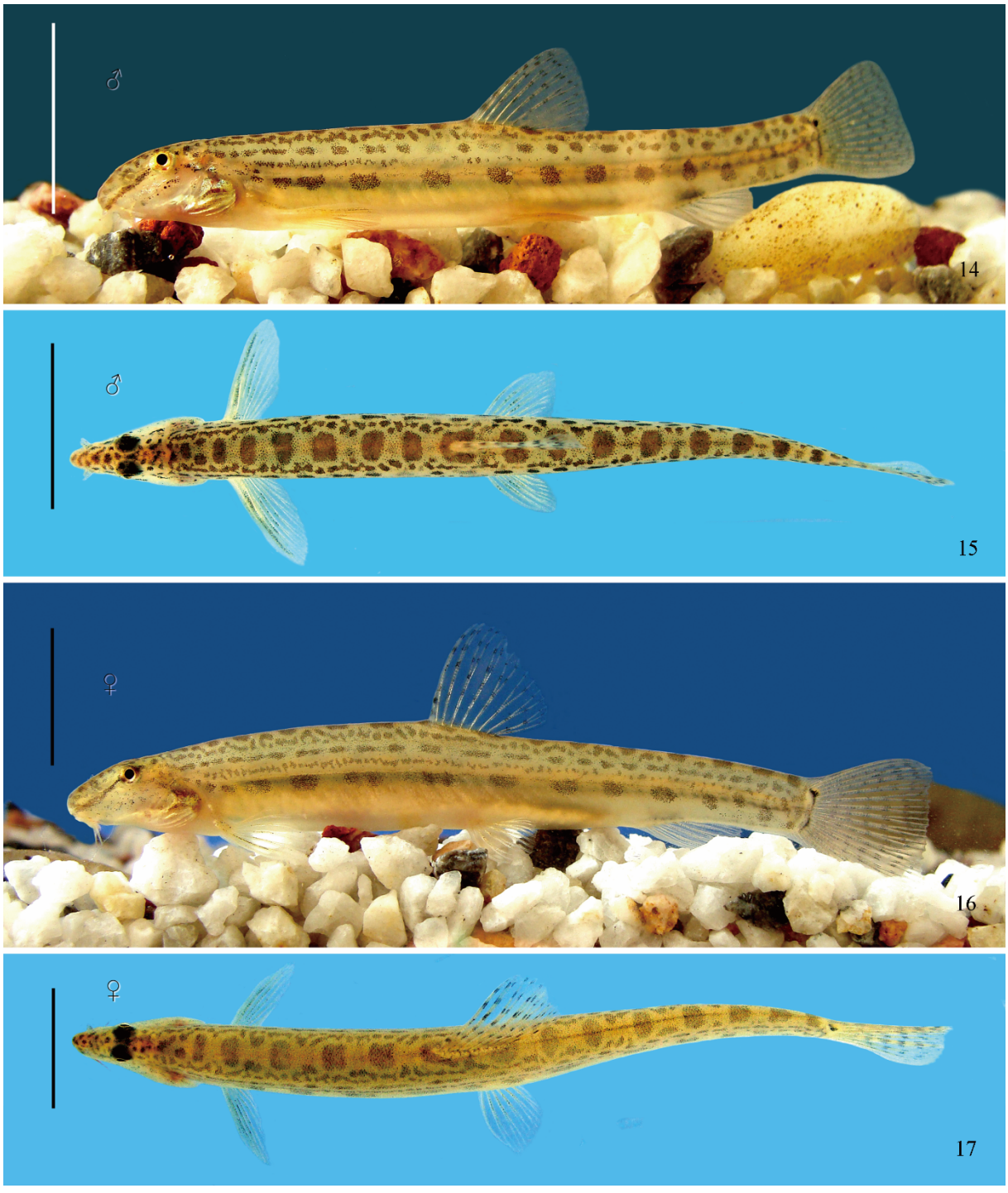
Description (Figs 8–17, Table 2). D. III–7; A. III–5; V. I–6; P. I–7–8; C. IV–14–16–IV. Body small, elongated, and compressed. Head small and compressed. Snout rounded. Eyes located on upper part and middle of head. Preorbital part of head shorter than postorbital part of head. Mouth small, inferior, with three pairs of short barbels. Length of maxillo-



Figures 8–13. *Cobitis brachysoma* Chen YX & Chen YF, **sp. nov.**, Suijiang River, Guangning, Guangdong, China. 8. Holotype, HU 1506334, lateral view. 9. HU 1506307, lateral view. 10. Lamina circularis in the pectoral in of male, dorsal view. 11. Mouth characters, front view. 12. Suborbital spine, left dorsal view, right interior view. 13. Subdorsal scales, dorsal view. Scale bars: 8–9 = 1 cm; 10–12 = 1 mm; 13 = 100 μm.

mandibular barbels longer than diameter of eye. Mental lobes undeveloped, two superficial longitudinal lobes short and bluntly rounded (Fig. 11). Suborbital spine bifid, relatively slender and straight, with a long processus medio-caudalis. Processus latero-caudalis short, less than one-fifth of processus medio-caudalis (Fig. 12). Subdorsal scales small and oval, with a larger focal area, 17–21 radial grooves, and 4–8 supplementary ones (Fig. 13).

Dorsal fin long, inserted midway of body; dorsal fin slightly shorter than head length. In males, pectoral fins moderately long, second pectoral fin ray longest (Fig. 15). In females, pectoral fins slightly shorter, third pectoral fin ray longest (Fig. 17). Ventral fins short, small, and approximately at same level as third branched dorsal-fin ray. Anal fin moderately long, located on half of space between ventral and caudal fins and not reach caudal fin. Anal orifice close to anal fin. Caudal fin



Figures 14–17. *Cobitis brachysoma* Chen YX & Chen YF, **sp. nov.**, color in life. 14, 16. Lateral view. 15, 17. Dorsal view. Scale bars = 1 cm.

emarginated tip. Caudal peduncle with ventral adipose crest. Lateral line short, not exceeding length of pectoral fins.

Pigmentation pattern. Color pattern characteristic of sexual dimorphism not observed. Head sprinkled with many black dots, and a black stripe extended from the insertion of rostral barbels to eye. L_1 consisted of a row of 5–7 rectangular blotches before dorsal fin that became less regular behind head; 2 on dorsal fin and 6–7 behind dorsal fin. Gap of rectangular blotches larger than width of blotches. L_2 composed of a line of irregularly small spots and that diminished towards end of caudal fin. L_3 composed of a row of horizontally elongated or rounded blotches and diminished towards end of caudal fin. L_4 composed of a line of minute black dots and diminished towards end of caudal fin. L_5 consisted of a row of 11–12 horizontally elongated or rounded blotches. One small jet spot on upper half of caudal fin base and far smaller than eye diameter. 7–8 narrow rows of dark dots on dorsal and caudal fins.

Sexual dimorphism. Males smaller than females with proportionally longer pectoral, ventral and anal fins. In males, second pectoral fin ray thickened and elongated, a slender and finely serrated lamina circularis at base of first branched pectoral fin ray. In females, third pectoral fin ray elongated.

Distribution. This new species occurs in the Suijiang River (a tributary of the Pearl River) in Guangning County, the Zengjiang River (a tributary of the Pearl River) in Longmen County, and the Nalongjiang River (a tributary of the Moyangjiang River) in Yangjiang County, all from Guangdong, China (Fig. 1).

Etymology. The species name is derived from the Greek *brachys*, meaning short, and *soma* meaning body, in reference to the short body of the species.

3.2 Species delimitation with DNA sequences

In *C. oxycephala* Chen YX & Chen YF, **sp. nov.**, only one haplotypes of the mitochondrial cytochrome *b* gene (1140 bp) was identified in two specimens, while six haplotypes were identified in ten specimens of *C. brachysoma* Chen YX & Chen YF, **sp. nov.** The topologies of BI and ML methods were almost overall in agreement except the relative positions of *C. macrostigma* (Fig. 18). Of species examined in this study, the genera *Cobitis* or *Iksookimia* did not form a monophyly, respectively. But, the East Asian spined loaches (species of *Cobitis* from China, Korea, Japan, Mongolia, Russia and Vietnam and most of *Iksookimia* from Korea) formed a clade with good supports (BP = 100 %, BBP = 100 %), with the exception of *C. arenae*. These East Asian spined loaches were subdivided into two well-supported (BP > 83 %, BBP = 100 %) subgroups (Subgroups I and II). Subgroup I contained *Cobitis* from China, Korea, Japan, Mongolia, Russia and Vietnam. Subgroup II included the species of *Cobitis* (from China, Korea and Japan) and *Iksookimia* (from Korea).

The two new species *C. oxycephala* Chen YX & Chen YF, **sp. nov.** and *C. brachysoma* Chen YX & Chen YF, **sp. nov.** belonged to clearly different clades. Both species were clustered in subgroup II. *Cobitis brachysoma* Chen YX & Chen YF, **sp. nov.** conspecific individuals were clustered with strong support (BP = 99 %, BBP = 100 %), indicating good resolution in the level of species. *Cobitis brachysoma* Chen YX & Chen YF, **sp. nov.** had a closer relationship with *C. microcephala* with a genetic distance of 7.9–9.2 %, and *C. oxycephala* Chen YX & Chen YF, **sp. nov.** had a closer relationship with *C. dolichorhynchus* with a genetic distance of 4.1–4.7 %.

4 Discussion

So far, 11 species of the genus *Cobitis* were recognized from the Pear River basin south to the rivers of Hainan Island: *C. arenae*, *C. multimaculata*, *C. australis*, *C. microcephala*, *C. leptosoma*, *C. wumingensis*, *C. obtusirostra*, *C. hereromacula*, *C. baishagensis*, *C. oxycephala* Chen YX & Chen YF, **sp. nov.**, and *C. brachysoma* Chen YX & Chen YF, **sp. nov.** The new species described herein, were previously identified as *C. sinensis* by formers (e.g. Chen, 1986; Ye, 1991; Jia *et al*., 2013; Chiang *et al*., 2013). However, *C. brachysoma* Chen YX & Chen YF, **sp. nov.** easily differs from *C. sinensis* by having a slender and long lamina circularis (vs. a plate lamina circularis). *Cobitis oxycephala* Chen YX & Chen YF, **sp. nov.** is distinguished from *C. sinensis* by the characters mentioned in the diagnosis. These 11 *Cobitis* species can be divided into three morphological groups: the first group includes the species *C. arenae*, *C. multimaculata*, and *C. oxycephala* Chen YX & Chen YF, **sp. nov.**, with a plate lamina circularis in the male; the second includes the species *C. microcephala* and *C. brachysoma* Chen YX & Chen YF, **sp. nov.**, with a serrated lamina circularis in the male and a small body size (<60 mm SL); the third includes *C. australis*, *C. leptosoma*, *C. wumingensis*, *C. obtusirostra*, *C. hereromacula*, and *C. baishagensis*, with a slender and long needle-shaped or knife-shaped lamina circularis in the male. Our phylogenetic results are partially congruent with the morphological groups. With the exception of *C. arenae* (as the basal sister lineage of all other species of *Cobitis* and *Iksookimia*), and *C. multimaculata* (without molecular data), *C. microcephala* and *C. brachysoma* Chen YX &

Chen YF, **sp. nov.** have a close relationship; *C. australis*, *C. leptosoma*, *C. wumingensis*, *C. hereromacula*, and *C. baishagensis* have a close relationship; and *C. oxycephala* Chen YX & Chen YF, **sp. nov.** is closely related to *C. dolichorhynchus* by having a plate lamina circularis in the male. *Cobitis oxycephala* Chen YX & Chen YF, **sp. nov.** is distinguished from *C. dolichorhynchus* by the characters mentioned in the diagnosis. *Cobitis brachysoma* Chen YX & Chen YF, **sp. nov.** differs from *C. microcephala* by the characters mentioned in the diagnosis, and differs from the other *Cobitis* species by having a serrated lamina circularis (vs. no serrated lamina circularis).

Other species of *Cobitis* remain to be taxonomically described. Chiang *et al*. (2013) reported the genetic features of some specimens considered as *C. sinensis* from the Guangdong and Guangxi. To elucidate the phylogenetic relationship of

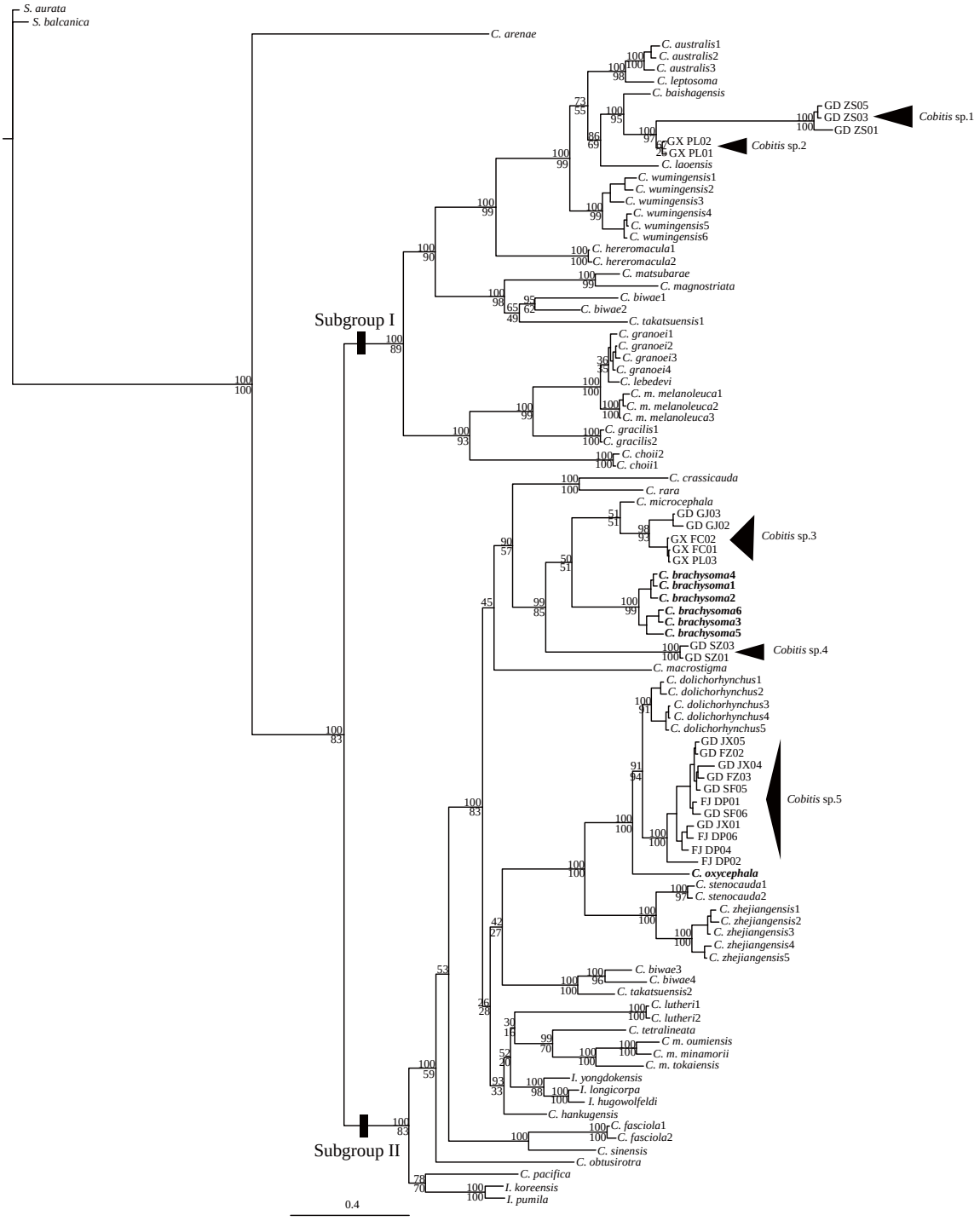


Figure 18. Bayesian tree of new species and it's closely relatives in the East Asia recovered from mitochondrial cytochrome *b* based on GTR+G+I model. Clade credibility values are given for nodes with bootstrap support for Bayesian inferences (below branch) and posterior probability for ML (above branch). The lineages are numbered as in Table 1.

the new species, we downloaded those homologous sequences and analyzed the phylogenetic characters with our own sequences. The phylogenetic analysis revealed *Cobitis* sp. 1–5 belonged to clearly different clades (Fig. 18). The mean K2P distance between *Cobitis* sp. 1–5 and other species of *Cobitis* from China ranged from 4.1–7.0 % (between *C. dolichorhynchus* and *Cobitis* sp. 5) to 21.4–22.2 % (between *C. arenae* and *Cobitis* sp. 5), that is similar to interspecific divergence levels of mitochondrial DNA sequences in the spined loaches. Those are almost certainly five undescribed species. In China, the fishes of *Cobitis* were usually identified as *C. sinensis* (Chen, 1981; Jia *et al*., 2013; Chiang *et al*., 2013). However, the type locality of *C. sinensis* was usually listed as western Szechwan of China. Based on diverse genetic features, Tang *et al.* (2008) and Chiang *et al*. (2013) also suggested *C. sinensis* included some cryptic species.

Our phylogenetic analyses showed a strongly supported bifurcation of *Cobitis* into two major subgroups (I and II). However, the phylogenetic inferences had undergone a different pattern in two subgroups. In subgroup I, the phylogenetic relationships of the species exhibited a geographical distribution pattern: species from the same drainage systems or adjacent regions often cluster together, e.g. the species from South China (Hainan, Guangxi and Guangdong) and Vietnam were clustered in a clade, and the species from North China (Hebei, Liaoning and Heilongjiang), Korea, Russia and Mongolia have close relationships, as well the species from Japan form a monophyletic lineage. While in subgroup II, the phylogenetic relationships do not match the geographical distribution of species. There are more than one monophyletic lineages for the same drainage systems or adjacent regions, suggesting multiple origins of their ichthyofauna or lineage exchanges after secondary contact.

Key to the eleven species of *Cobitis* from South China

1. Lamina circularis slender and long, needle-shaped or knife-shaped 2
   Lamina circularis plate 9
2. Lamina circularis slender and long, needle-shaped 3
   Lamina circularis moderately slender and long, knife-shaped 8
3. Color pattern with five longitudinal lines of dark speckles on the dorsolateral sides of the body (L_1–L_5) 4
   Color pattern without five longitudinal lines of dark speckles on the dorsolateral sides of the body (L_1–L_5) 7
4. Lamina circularis without serration 5
   Lamina circularis with serration 6
5. Barbels long, maxillo-mandibular barbels longer than eye diameter; 10–12 large transverse elongated blotches without a deeper dusky band on L_5 (Nandujiang River) ..... ***C. baishagensis* Chen, Sui, Liang & Chen, 2016**
   Barbels small, maxillo-mandibular barbels shorter than eye diameter; 9–14 rounded or narrow transverse elongated blotches with conspicuous deeper dusky band on L_5 (Wuming, Heishui, Chengjiang and Jinchengjiang Rivers) ..... ***C. wumingensis* Chen, Sui, He & Chen, 2015**
6. Suborbital spine with a short processus medio-caudalis; caudal peduncle long, length of caudal peduncle 5.9–6.6 (mean 6.3) in SL in males and 5.3–7.2 (mean 6.3) in females; body sturdy, body depth 5.8–6.2 (mean 5.9) in SL in males and 5.0–6.5 (mean 5.6) in females (Nanliu and Beiliu Rivers) ..... ***C. microcephala* Chen & Chen, 2011**
   Suborbital spine with a long processus medio-caudalis; caudal peduncle short, length of caudal peduncle 7.3–9.2 (mean 8.4) in SL in males and 7.0–9.9 (mean 8.5) in females; body slender, body depth 6.1–6.6 (mean 6.4) in SL in males and 5.5–8.6 (mean 6.8) in females (Suijiang, Zengjiang and Nalongjiang Rivers) ..... ***C. brachysoma* Chen YX & Chen YF, sp. nov.**
7. Mental lobes that do not end in a filiform tip; 4–5 narrow rows of dark spots on the caudal fin (Yongjiang, Yujiang and Liujiang Rivers) ..... ***C. australis* Chen, Chen & He, 2013**
   Mental lobes of the lower lip with a slightly filiform tip; 6–7 narrow rows of dark spots on the caudal fin (Longjin River) ..... ***C. hereromacula* Chen, Sui, Liang & Chen, 2016**
8. 10–12 blotches on L_5; a roundish spot as large as the eye diameter; caudal peduncle long, length of caudal peduncle 8.4 in SL in males and 6.9–7.9 (mean 7.4) in females (Lijiang River) ..... ***C. leptosoma* Chen, Sui, He & Chen, 2015**
   8–10 blotches on L_5; an arcuate spot smaller than the eye diameter; caudal peduncle short, length of caudal peduncle 9.2 in SL in males and 6.9–10.4 (mean 7.9) in females (Lianjiang and Wengjiang Rivers) ..... ***C. obtusirostra* Chen, Sui, He & Chen, 2015**
9. Color pattern without five longitudinal lines of dark speckles on the dorsolateral sides of the body (L_1–L_5) 10
   Color pattern with five longitudinal lines of dark speckles on the dorsolateral sides of the body (L_1–L_5) (Nandujiang River) ..... ***C. oxycephala* Chen YX & Chen YF, sp. nov.**
10. 20–25 spots along lateral sides; body slender, body depth more than 7.3 in SL; peduncle more than twice as long as deep (Pearl and Nandujiang Rivers) ..... ***C. arenae* (Lin, 1934)**
    17–22 oval blotches along lateral sides; body stumpy, body depth less than 7.3 in SL; peduncle less than twice as long as deep (Nanliu River) ..... ***C. multimaculata* Chen & Chen, 2011**

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