

ORIGINAL ARTICLE

A new species of the genus *Cobitis* (Cypriniformes: Cobitidae) from the Northeast China

Abstract A new spined loach, *Cobitis gracilis* **sp. nov.**, is described based on specimens collected from Jilin and Heilongjiang Provinces, China. Morphological and molecular data showed that *C. gracilis* **sp. nov.** is closely related to *C. granoei* Rendahl and *C. melanoleuca* Nichols. The new species differs from its sister species in lamina circularis, suborbital spine, and pigmentation at the base of caudal fin. In addition, both morphological and molecular analyses revealed that specimens of *C. lutheri* Rendahl from China differs from those from Korea. Korean specimens are appropriately placed in a separate species. Molecular analyses revealed that *C. choui* Kim & Son is a new record in China.

Key words Loaches, taxonomy, Jilin, Heilongjiang, molecular phylogeny.

1 Introduction

Northeast China represents the northeastern of China and includes Liaoning, Jilin and Heilongjiang Provinces. The Heilongjiang River with more than 200 tributaries is the main river system of the northeast China, forming the border between the Russian Far East and Northeastern China, and flowing into the Strait of Tartary. The Tumen River forms much of the southern border of Jilin Province, North Korea, and Russia, rising on the slopes of Mount Paektu and flowing into the Sea of Japan. The Yalu River, a river on the border between North Korea and China, rises on the Mount Paektu, flowing through the Jilin and Liaoning Provinces from northeast to southwest and emptying towards the north of the Yellow Sea. The Suifen River, located in the southeast of Heilongjiang Province, enters Russian territory, and extends to the Japan Sea.

Spined loaches of the genus *Cobitis* L., 1758 (Cypriniformes, Cobitidae) are small benthic freshwater fish that are found throughout most of the Palaearctic and Oriental Regions, with the exception of the cold boreal areas and Tibetan Plateau (Sawada, 1982; Bănărescu, 1990). Recent studies on loaches of the genus *Cobitis* has shown that two species, *C. lutheri* Rendahl, 1935 and *C. granoei* Rendahl, 1935, occur in the northeast China (Chen *et al.*, 2015). *Cobitis granoei* was firstly described by Rendahl (1935) as *C. taenia granoei* based on seven specimens from Irtych River near Omsk in Siberia. Later, Nalbant *et al.* (1970) considered *C. granoei* as a separate species. Nalbant (1993) proposed *C. granoei* and *C. melanoleuca* Nichols, 1925 (Chin-ssu, Shansi Province, China) were conspecific, and names were merged as synonyms of *C. melanoleuca* that has a wide distribution from China and Russian Far East to Europe. Molecular studies of *C. melanoleuca* populations from different regions showed *C. granoei* and *C. melanoleuca* are two separate species (Chen *et al.*, 2015). *Cobitis lutheri* was firstly described by Rendahl (1935) based on five specimens from the Khanka Lake basin (Santachesa and Odarka streams) in Siberia. Kim (1980) made a detailed description of *C. lutheri* in Korea. However, recent studies on the phylogenetic relationships of the family Cobitidae indicated *C. lutheri* was a non-natural group (Perdices *et al.*, 2012).

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In this study, loaches of the genus *Cobitis* from the Northeast China were studied and a new species of *Cobitis* was described based on morphological characters and DNA sequences of the mitochondrial *cyt b* gene.

2 Materials and methods

2.1 Sampling

The examined materials were collected from the Heilongjiang and Yalu rivers in the Jilin and Heilongjiang Provinces in the northeastern of China during the autumn (in October 2015) (Figs 1–2). 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 of the Hebei University (HU), Baoding, Hebei, China.

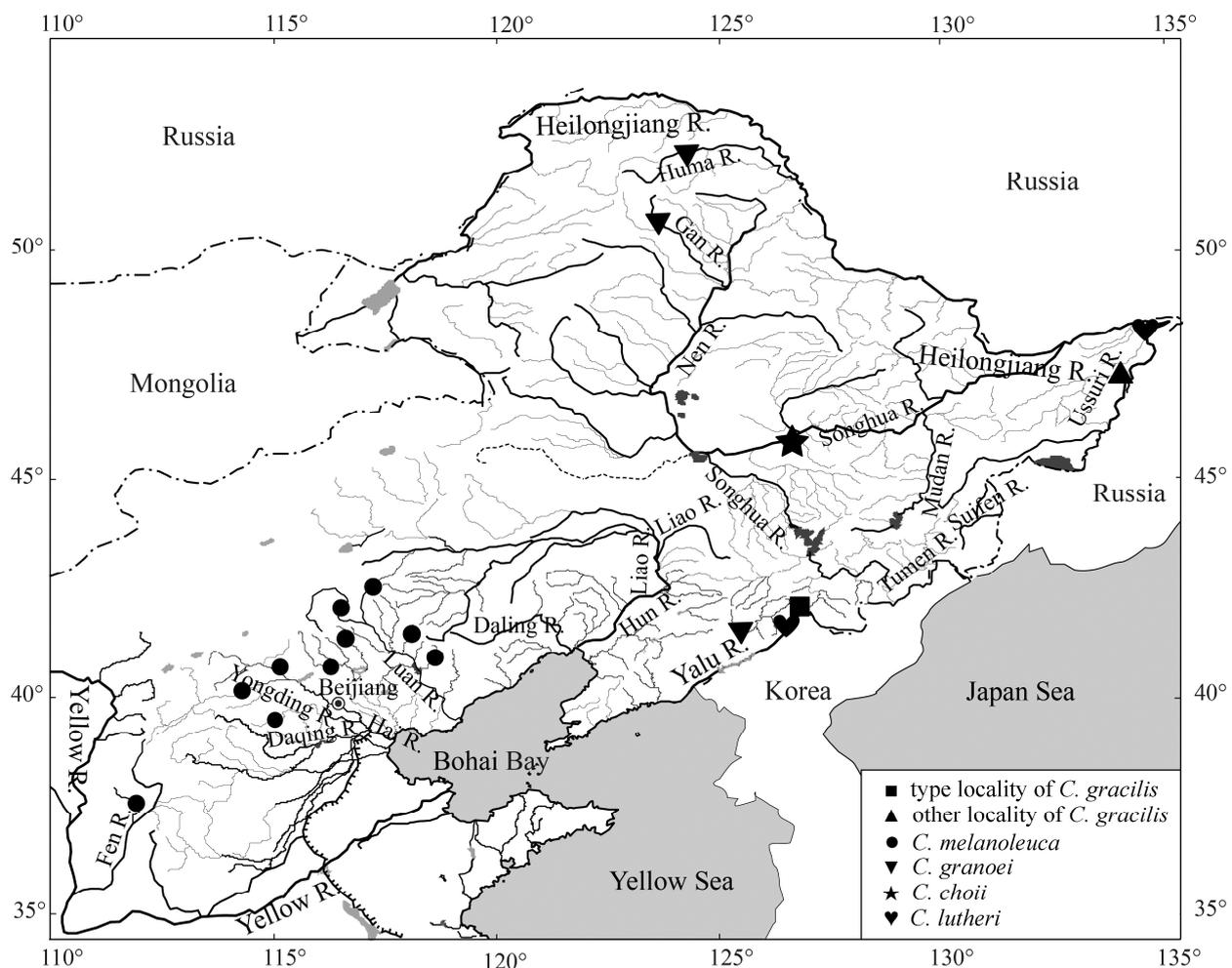


Figure 1. The collection sites (i.e. currently known localities) of 5 species of the genus *Cobitis* from north to Yellow River in China.

2.2 Morphology

Nineteen morphometric variables were measured followed Doadrio & Perdices (1997), and modified according to procedures by Chen & Chen (2011, 2013). All measurements are given in millimeters (mm). Scales were collected from the subdorsal region between the origin of the dorsal fin and lateral line, and photographed using a Leica DFC295 camera attached to a Leica M205A stereomicroscope. Notations (L_1 – L_5) describing lines and speckles on the dorsolateral side of the trunk follow those designated by Takeda & Fujie (1945).

2.3 DNA extraction, amplification and sequencing

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 AAG AC-3') (Xiao *et al.*, 2001). Amplification of the products was performed in a Biometra thermal cycler under the following conditions: 4 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, 1 minute at 72°C for extension, and a final extension at 72°C for 8 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.

2.4 Analysis of DNA sequences

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 38 individuals of 11 species of the genus *Cobitis*, and 6 individuals of 4 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 neighbor-joining (NJ) as performed using MEGA 5.05. For the NJ analyses, the substitution model was calculated by applying Kimura 2-parameter using uniform rates among sites. Nonparametric bootstrap support for internal branches was calculated for NJ 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). 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.

3 Results

3.1 Taxonomy

Cobitis gracilis sp. nov. (Figs 3–12)



Figure 2. Ecological environment of *Cobitis gracilis* sp. nov.

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 biwae</i>	Japan, Yamagata, Mogami R.	AB084395*
<i>Cobitis choii1</i>	Russia, Chita District, Shilka R.	JN858876*
<i>Cobitis choii2</i>	South Korea, Yeo Cheong, Mi Ho R.	EF508510*
<i>Cobitis choii3</i>	South Korea, Baekgok R.	NC010649*
<i>Cobitis granoei1</i>	Mongolia, Kerulen R.	KM583541*
<i>Cobitis granoei2</i>	China, Liaoning, Hunjiang R.	DQ105243*
<i>Cobitis granoei3</i>	China, Heilongjiang, Gan R.	KY009946
<i>Cobitis granoei4</i>	China, Heilongjiang, Gan R.	KY009947
<i>Cobitis granoei5</i>	China, Heilongjiang, Gan R.	KY009948
<i>Cobitis granoei6</i>	China, Heilongjiang, Huma R.	KX823423
<i>Cobitis granoei7</i>	China, Heilongjiang, Huma R.	KX823424
<i>Cobitis granoei8</i>	China, Heilongjiang, Huma R.	KX823425
<i>Cobitis granoei9</i>	China, Heilongjiang, Songhua R.	DQ105242*
<i>Cobitis gracilis 1</i>	China, Jilin, Yalu R.	KY009955
<i>Cobitis gracilis 2</i>	China, Jilin, Yalu R.	KY009954
<i>Cobitis gracilis 3</i>	China, Heilongjiang, Ussuri R.	KY009957
<i>Cobitis gracilis 4</i>	China, Heilongjiang, Ussuri R.	KY009956
<i>Cobitis lutheri1</i>	China, Jilin, Yalu R.	KY009953
<i>Cobitis lutheri2</i>	China, Heilongjiang, Heilongjiang R.	KP133112
<i>Cobitis lutheri3</i>	Russia, Primorye District, Karasik R.	JN858891*
<i>Cobitis lutheri4</i>	Russia, Primorye District, Poyma R.	JN858892*
<i>Cobitis lutheri5</i>	Russia, Khabarovsk District, Gur R.	JN858894*
<i>Cobitis lutheri6</i>	South Korea, Oa U, Jin Am R.	EF508499*
<i>Cobitis lutheri7</i>	South Korea, Tosan-dong, Yongsan R.	AB162956*
<i>Cobitis lutheri8</i>	South Korea	KM576244*
<i>Cobitis lutheri9</i>	South Korea, Mankyeong R.	KF661685*
<i>Cobitis matsubarae</i>	Japan, Saba River	AB039348*
<i>Cobitis melanoleuca1</i>	China, Hebei, Juma R.	KY009949
<i>Cobitis melanoleuca2</i>	China, Hebei, Juma R.	KY009950
<i>Cobitis melanoleuca3</i>	China, Hebei, Tang R.	KY009951
<i>Cobitis melanoleuca4</i>	China, Hebei, Bai R.	KY009952
<i>Cobitis melanoleuca5</i>	China, Hebei, Yang R.	KX823426
<i>Cobitis melanoleuca6</i>	China, Hebei, Yang R.	KX823427
<i>Cobitis melanoleuca7</i>	China, Hebei, Yang R.	KX823428
<i>Cobitis pacifica</i>	South Korea, Cheon Jin, Cheon Jin R.	EF508505*
<i>Cobitis striata</i>	Japan	NC004695*
<i>Cobitis takatsuensis</i>	Japan, Misumi R.	AB039337*
<i>Cobitis tetralineata</i>	Korea, Gokseong, Boseong R.	KC524528*
<i>Iksookimia yongdokensis</i>	South Korea, Yong Pyeong, Dae Seo R.	EF508516*
<i>Iksookimia longicorpa1</i>	South Korea, Gye San, Seon Jin R.	EF508513*
<i>Iksookimia longicorpa2</i>	South Korea, Gye San, Seon Jin R.	EF508514*
<i>Iksookimia longicorpa3</i>	South Korea, Doekcheon R.	EU670753*
<i>Iksookimia koreensis</i>	South Korea, Cheon Gon, Han Tan R.	EF508511*
<i>Iksookimia pumila</i>	Korea, Buan Dam	EF508515*
<i>Sabanejewia balcanica</i>	Georgia, Caucasus, R. Rione	AF499190*

*Retrieved from GenBank.



Figures 3–8. *Cobitis gracilis* sp. nov., Yalu River, Linjiang, Jilin, China. 3. Holotype, HU 1505143, lateral view. 4. HU 1505134, lateral view. 5. Lamina circularis in the pectoral fin of male, dorsal view. 6. Mouth characters, front view. 7. Suborbital spine, left dorsal view, right interior view. 8. Subdorsal scales, dorsal view. Scale bars: 3–4 = 1 cm; 5–7 = 1 mm; 8 = 100 μ m.

Holotype. ♂, HU 1600062, 74.4 mm TL, 64.2 mm SL, the Yalu River, Linjiang (41°81'N, 126°92'E), Jilin, China, October 2015, collected from the Linjiang farm product market by Yongxia Chen.

Paratypes. HU 1505136, 1600037, 1600056, 3♀, 75.2–88.4 mm TL, 65.0–78.4 mm SL, same data as holotype; HU 1506359, 1506347, 1506354, 3♀, 81.5–86.4 mm TL, 71.0–74.3 mm SL, the Ussuri River, Raohe (46°80'N, 134°02'E), Heilongjiang, China, October 2015, collected from the Raohe farm product market by Yongxia Chen.

Diagnosis. The new species is most similar to *C. granoei* and *C. melanoleuca*, but distinguished from *C. granoei* and *C. melanoleuca* in the upper jet black spot at the base of caudal fin inconspicuous or absent (Figs 3–4, 9–10) (vs. jet black spot conspicuous in *C. melanoleuca* (Figs 19–20) and *C. granoei* (Figs 13–14)); suborbital spine is slender and straight, with long processus latero-caudalis, less than one-second of the processus medio-caudalis (Fig. 7) (vs. suborbital spine thick and straight with short processus latero-caudalis in *C. melanoleuca* (Fig. 23); suborbital spine slender and curved with long processus latero-caudalis in *C. granoei* (Fig. 17)); males with a small meniscus lamina circularis at the base of the first branched pectoral fin ray (Fig. 5) (vs. an knife lamina circularis in *C. melanoleuca* (Fig. 21); a larger semilunar lamina circularis in *C. granoei* (Fig. 15)); 15–20 blotches on L_5 (vs. 10–16 blotches on L_5 in *C. melanoleuca*; 11–16 large blotches on L_5 in *C. granoei*).

Description. General appearance and morphometric data of holotype and paratypes are given in Figs 3–12 and Table 2, respectively. D. III–7; A. III–5; V. I–6; P. I–7–8; C. IV–14–16–IV. Body slender, depth 8.7 in SL in males and 8.9–11.1 (mean 9.8) in females. Head small, with a length of 5.3 in SL in males and 5.2–5.6 (mean 5.5) in females. Snout rounded. Preorbital part of head shorter than postorbital part of head. Mouth small, with three pairs of short barbels. Length of maxillo-mandibular barbels shorter than diameter of eye. Maxillary barbels not reach under anterior border of eye. Mental lobes undeveloped, two superficial longitudinal lobes short, and lower tip bluntly rounded (Fig. 6). Suborbital spine slender and straight, with long processus latero-caudalis, less than one-second of processus medio-caudalis (Fig. 7). Subdorsal scales small and oval, with a large focal area, 19–22 radial grooves, and 3–5 supplementary ones (Fig. 8).



Figures 9–12. *Cobitis gracilis* sp. nov., color in life. 9, 11. Lateral view. 10, 12. Dorsal view. Scale bars = 1 cm.

Dorsal fin inserted midway between posterior nasal and base of caudal fin. Length of predorsal 1.9 in SL in males and 1.8–1.9 (mean 1.8) in females. In males, pectoral fins longer than those in females; first branched pectoral fin ray longest. Length of first branched pectoral fin ray 7.1 in SL. In females, second branched pectoral fin ray longest with length of second branched pectoral fin ray 8.6–11.0 (mean 9.8) in SL. Ventral fins approximately at same level as dorsal fin. Anal fin located in far behind dorsal extremity and not reach caudal fin. Anal orifice close to anal fin. Caudal fin emarginated tip.

Pigmentation pattern. Body color whitish with a variable dark brown pigmentation pattern organized in L₁–L₅ (Figs 3–4, 9–12). Color patterns characteristic of sexual dimorphism not obvious. L₁ consisted of a row of 7–9 rectangular blotches before dorsal fin that became less regular behind head; 2 on dorsal fin and 7–10 behind dorsal fin. Gap of rectangular blotches narrower than width of blotches. L₂ comprised a line of irregularly small dots that not intermingle with gap of L₁, and diminished towards end of caudal fin. L₃ comprised a row of horizontally elongated or rounded spots and that decreased beyond anal fin. L₄ spotted with one line of dots and that diminished towards end of caudal fin. L₅ comprised 15–20 oval blotches that together formed an irregular small blotch near head and caudal fin. At base of caudal fin, one inconspicuous

jet-black spot found in upper region. In a few individuals, spot absent. Five or six striations on dorsal and caudal fins. Head sprinkled with many black spots on dorsal side, and a black stripe extended from occiput through eye to insertion of rostral barbels.

Sexual dimorphism. Males smaller than females with proportionally longer pectoral, ventral, and anal fins. In males, first branched pectoral-fin ray thickened and elongated, with a small meniscus lamina circularis at base (Fig. 5). In females, second branched pectoral fin ray elongated.

Distribution. This new species occurs in the Yalu and Heilongjiang rivers in Jilin and Heilongjiang Provinces in northeast of China (Figs 1–2).

Etymology. The specific name derives from the Latin *gracilis*, meaning slender, in reference to the slender body.

Table 2. Morphometric and meristic characters for *Cobitis gracilis* sp. nov., all measurements given in millimeters (mm).

Variable	Male (<i>n</i> = 1)	Females (<i>n</i> = 6)		
	Holotype	Range	Mean	SD
Standard Length (SL)	64.2	65.0–79.4	72.2	5.01
SL/ Head Length (HL)	5.3	5.2–5.6	5.5	0.16
SL/ Body Depth	8.7	8.9–11.1	9.8	0.85
SL/ Pectoral-Ventral Length	3.4	2.9–3.1	3.0	0.09
SL/ Caudal Fin Length (CPL)	6.4	5.3–6.1	5.7	0.28
SL/ Caudal Peduncle Depth (CPD)	12.2	12.8–14.5	13.9	0.60
SL/ Dorsal Fin Length	6.6	6.7–9.4	7.9	0.98
SL/ Dorsal Fin Bases Length	12.8	12.2–15.1	13.4	1.00
SL/ Pectoral Fin Length	7.1	8.6–11.0	9.8	0.94
SL/ Ventral Fin Length	9.0	10.1–12.1	10.8	0.75
SL/ Anal Fin Length	8.4	8.5–9.9	9.1	0.53
SL/ Anal Fin Bases Length	18.2	15.1–17.6	16.2	0.90
SL/ Caudal Peduncle Length	6.3	5.9–7.9	6.6	0.73
SL/ Predorsal Length	1.9	1.8–1.9	1.8	0.05
SL/ Preventral Length	1.9	1.8–1.9	1.8	0.05
SL/ Preanal Length	1.3	1.3–1.3	1.3	0.01
HL/ Preorbital Length	2.3	2.2–2.4	2.4	0.10
HL/ Eye Diameter	6.7	5.9–7.8	6.8	0.81
HL/ Interorbital Width	6.4	6.2–8.6	7.2	0.96
CPL/CPD	1.9	2.3–2.6	2.4	0.14

3.2 Species delimitation with DNA sequences

Analyses of the mitochondrial data showed the sequence divergence (K2P) between all specimens of *Cobitis* ranging from 1.76% (between *C. granoei* and *C. melanoleuca*) to 19.20% (between *C. lutheri* and *C. takatsuensis* Mizuno, 1970), and the sequence divergence levels of *C. gracilis* sp. nov. with other species of *Cobitis* ranged from 6.51% to 16.00% (Table 3). Similar low genetic distance was observed between *Iksookimia koreensis* (Kim, 1975) and *Iksookimia pumila* (Kim & Lee, 1987) (1.73%).

The NJ tree which was recovered from the *cyt b* gene sequences was shown in Fig. 25. The topology obtained from Bayesian inferences was similar to the NJ tree. The new species, *C. gracilis* sp. nov., conspecific individuals co-clustered with strong support (Bayesian values=1.00 and bootstrap values=100), indicating good resolution at the level of species. *Cobitis gracilis* sp. nov. has a closer relationship with *C. granoei* with a genetic distance of 6.51%, and *C. melanoleuca* with a genetic distance of 6.73%.

4 Discussion

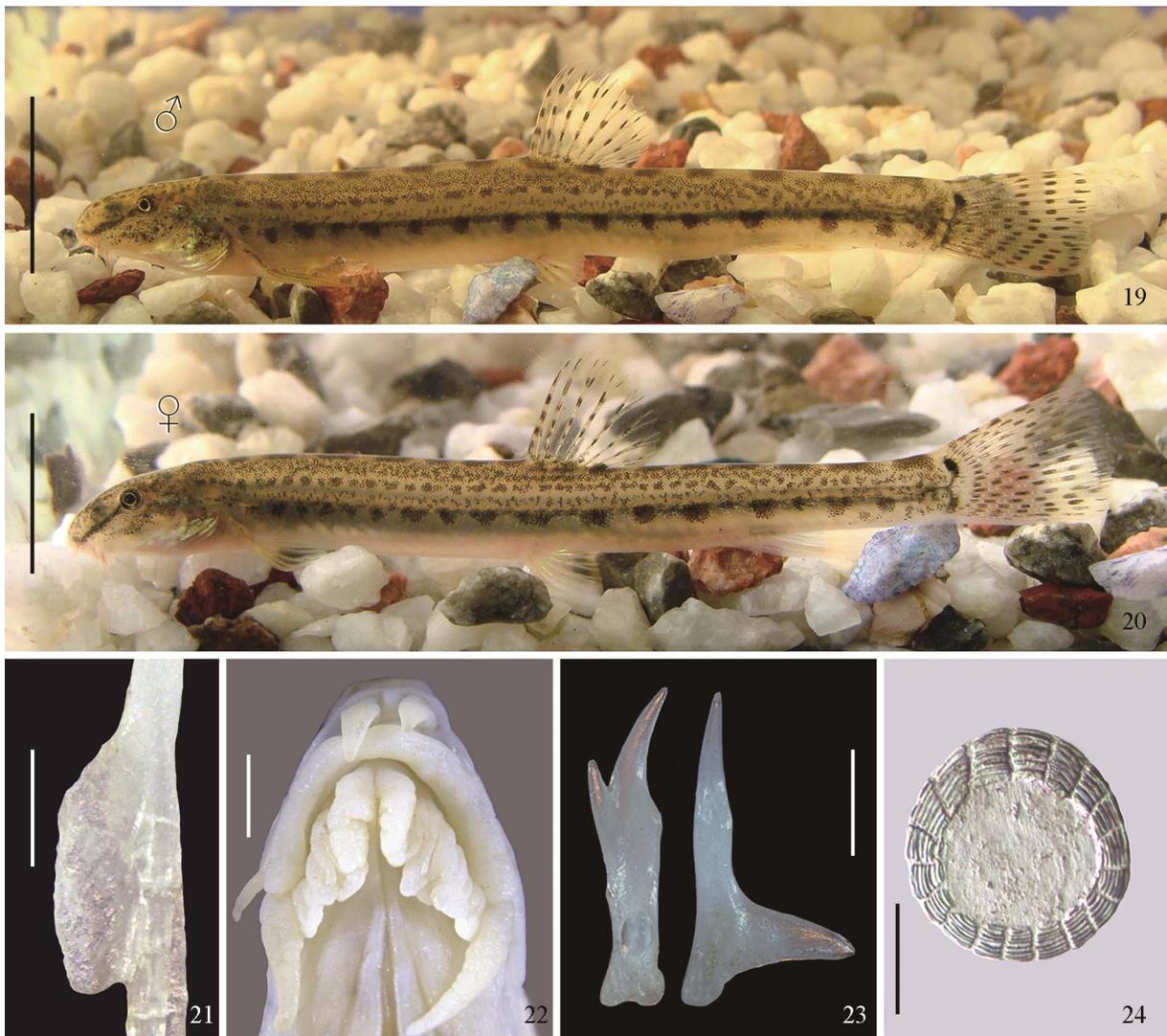
Phenotypic and reproductive plasticity within *Cobitis* group make taxonomic identification difficult (Nalbant, 1993;



Figures 13–18. *Cobitis granoei*, Huma River, Tahe, Heilongjiang, China. 13–14. Color in life, lateral view. 15. Lamina circularis in the pectoral fin of male, dorsal view. 16. Mouth characters, front view. 17. Suborbital spine, left dorsal view, right interior view. 18. Subdorsal scales, dorsal view. Scale bars: 13–14=1 cm; 15–17=1 mm; 18=100 μ m.

Janko *et al.*, 2007), especially in *C. melanoleuca*, *C. granoei*, and *C. gracilis* **sp. nov.** These three species are morphologically poorly identifiable, morphological characters, e.g., mental lobes (Figs 6, 16, 22), scale (Figs 8, 18, 24), and pigmentation on the body (Figs 3–4, 9–14, 19–20) are indistinguishable, but that are different in lamina circularis, suborbital spine, and pigmentation at the base of caudal fin (see diagnosis section) (Table 4). According to mitochondrial DNA data, these three species are well differentiated. Although, there was a low level of sequence divergence between them (1.76%, between *C. granoei* and *C. melanoleuca*). Although genetic distances cannot be utilized as accurate diagnostic data for species identification, they can provide an approximation of species status (Perdices *et al.*, 2015). Hebert *et al.* (2003) considered that intraspecific divergences were rarely greater than 2%, with most less than 1%. Similarly, Geiger *et al.* (2014) concluded that 11.3% of all Mediterranean freshwater fishes with a K2P interspecific distances below 2%. In the present case, *C. granoei* and *C. melanoleuca* were considered as distinct species. *C. melanoleuca* is distributed in the Yellow, Hai and Luan Rivers. *C. granoei* is distributed from the northeast China across Siberia to the European Don, Volga, and Kuban drainages. *C. gracilis* **sp. nov.** occurs in the Heilongjiang River and Yalu River.

The molecular analysis shows *C. lutheri* from different localities as a non-natural group. The molecular results support two well-differentiated molecular lineages. One lineage related all *C. lutheri* specimens from the northeast China and Far East of Russia with low genetic variability. The second lineage was recovered with Korean specimens identified as *C. lutheri* by Šlechtová *et al.* (2008) and Kitagawa *et al.* (2005) (Table 1) that phylogenetically related to *C. tetralineata* Kim, Park & Nalbant, 1999 from Korea (Fig. 25). Kim (1980) first record of this species in Korea and Kim *et al.* (1999) made detailed diagnosis characteristics of this species based on specimens from South Korea. Although, Korean material does not differ from that of described and illustrated by Rendahl (1935). Chinese specimens can be distinguished from Korean specimens by the mental lobes of the lower lip short and bluntly rounded (Chen & Chen, 2005: Fig. 3E) (vs. mental lobes of the lower lip being pointed with a slightly filiform tip) (Kim *et al.*, 1999: Fig.7B); subdorsal scales with a large focal area (Chen & Chen, 2005: Fig. 5E) (vs. a small focal area) (Kim *et al.*, 1999: Fig.7E); suborbital spine with short processus



Figures 19–24. *Cobitis melanoleuca*, Juma River, Yixian, Hebei, China. 19–20. Color in life, lateral view. 21. Lamina circularis in the pectoral fin of male, dorsal view. 22. Mouth characters, front view. 23. Suborbital spine, left dorsal view, right interior view. 24. Subdorsal scales, dorsal view. Scale bars: 19–20=1 cm; 21–23=1 mm; 24=100 μ m.

latero-caudalis, less than one-fifth of the processus medio-caudalis (Chen & Chen, 2005: Fig. 4D) (vs. long processus latero-caudalis, less than one-third of the processus medio-caudalis) (Kim *et al.*, 1999: Fig.7D); usually two spots on caudal base, the upper one jet black, the lower one jet black or inconspicuous (Fig. 26) (vs. one jet black spot on the upper) (Kim, 1980: Fig.1; Kim *et al.*, 1999: Fig.7A). Therefore, both morphological and molecular analyses revealed that Korean specimens are appropriately placed in a separate species within the *Cobitis*. This conclusion was also supported in previous studies about the non-conspicuity of Korean spined loaches identified as *C. lutheri* and *C. lutheri* described from Khanka Lake (Vasil'ev & Vasil'eva, 2008; Perdices *et al.*, 2012).

The specimen of *C. granoei* (9) from the Heilongjiang Province is not a member of the species *C. granoei*. *C. granoei* (9) specimen and all *C. choii* Kim & Son, 1984 individuals from Korea and Russia were grouped in a monophyletic clade with low genetic divergences (Fig. 25). The genetic distance between them are from 0.43% to 0.85%, which are consistent with the intraspecific differentiation value of <1% (Hebert *et al.*, 2003). According to mitochondrial DNA data, *C. choii* is a new record in China. However, the specimen from the Heilongjiang Province should be taxonomically revised. *C. melanoleuca*, *C. granoei*, and *C. gracilis* **sp. nov.** differ from *C. choii* by having a plate or medium slender and long lamina circularis (vs. slender and long with serrate lamina circularis (Kim & Son, 1984)); slender body, length of depth more than 8.0 in SL both in males and females (vs. sturdy body, less than 8.0 in SL both in males and females (Kim & Son, 1984)). *C. melanoleuca*, *C. granoei*, and *C. gracilis* **sp. nov.** differ from the other Korean *Cobitis* species (*C. tetralineata* Kim, Park & Nalbant, 1999, *C. pacifica* Kim, Park & Nalbant, 1999 and *C. hankugensis* Kim, Park, Son & Nalbant, 2003) by the slender body (vs. sturdy

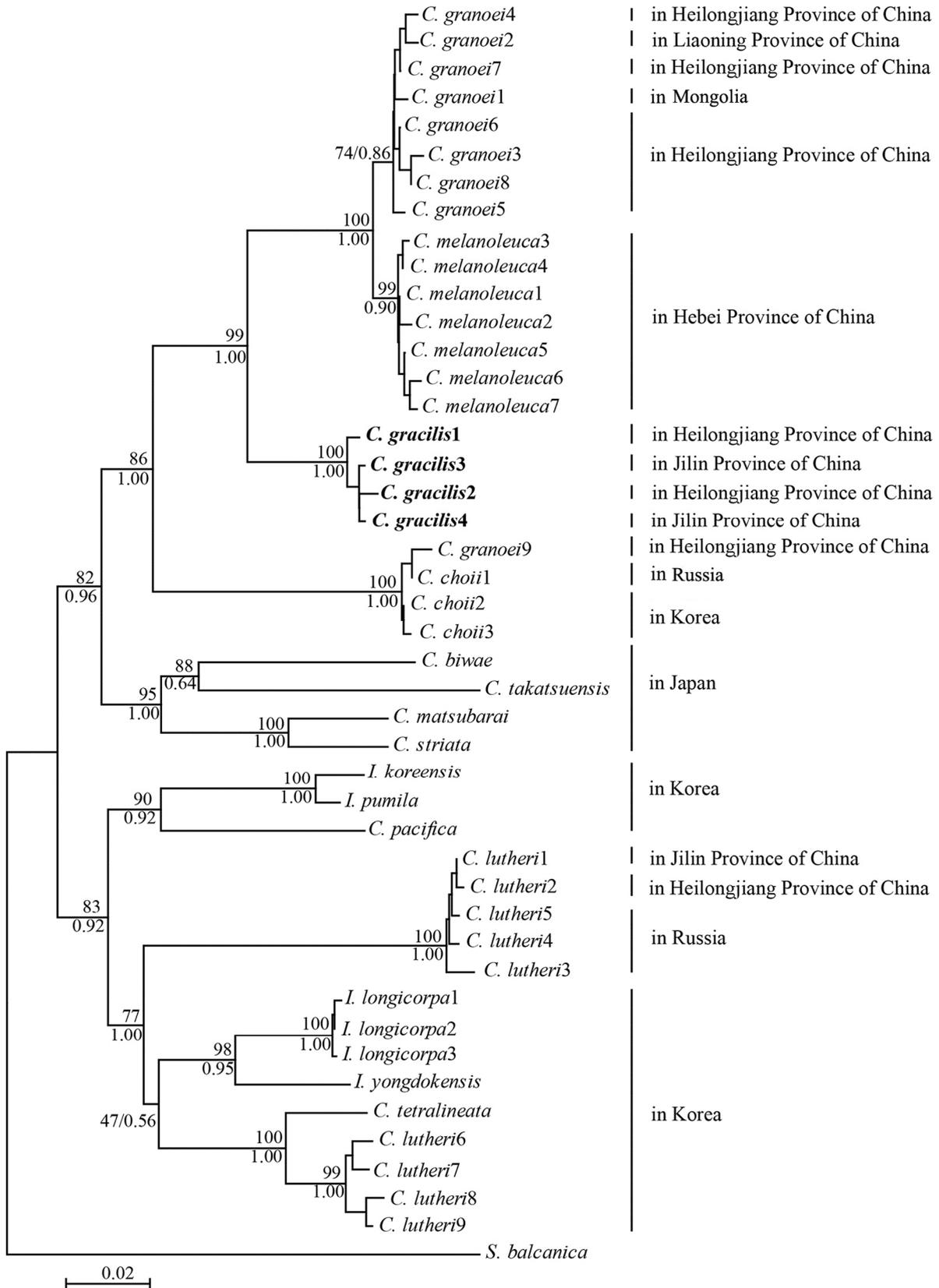
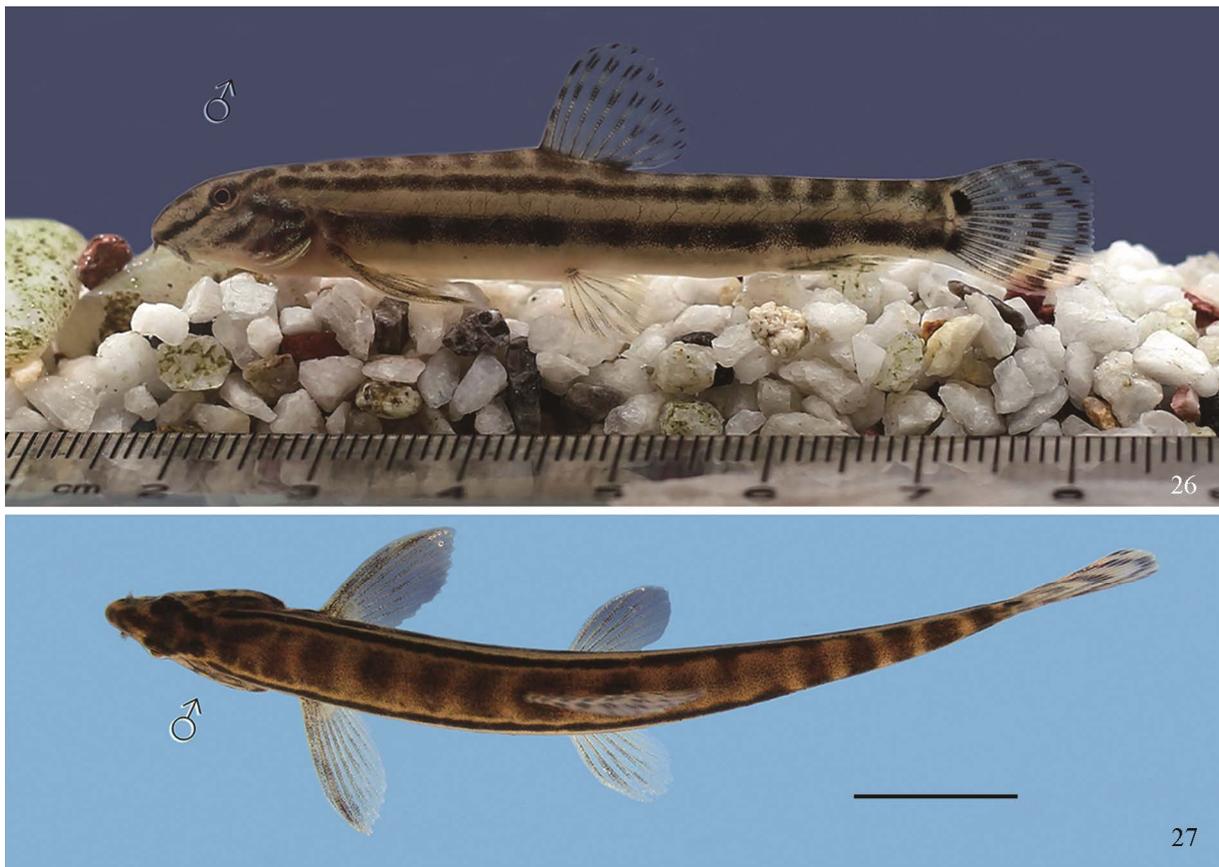


Figure 25. Neighbor-joining tree for *Cobitis* spp. and *Iksookimia* spp. inferred from mitochondrial cytochrome *b* gene sequences based on Kimura 2-parameter model. Clade credibility values are given for nodes with bootstrap support for NJ (above branch) and posterior probability for Bayesian inferences (below branch). The lineages are numbered as in Table 1.



Figures 26–27. *Cobitis lutheri*, color in life, Yalu River, Linjiang, Jilin, China. 26. Lateral view. 27. Dorsal view. Scale bars = 1 cm.

body, length of depth less than 8.0 in SL both in males and females (Kim *et al.*, 2003)). *C. gracilis* **sp. nov.** further differs from *C. tetralineata*, *C. pacifica* and *C. hankugensis* by having 15–20 small oval blotches on L₅ (vs. a dark stripe on L₅ in *C. tetralineata*; 9–13 cordiform or chevron shaped blotches on L₅ in *pacifica*; 8–13 ovoid or rectangular blotches on L₅ in *C. hankugensis* (Kim *et al.*, 2003)); a small meniscus lamina circularis (vs. round lamina circulari in *C. tetralineata* and *C. hankugensis*; triangle lamina circulari in *C. pacifica* (Kim *et al.*, 2003)).

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

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
<i>C. biwae</i>															
<i>C. choii</i>	15.25														
<i>C. granoiei</i>	14.84	12.19													
<i>C. gracilis</i>	13.78	11.28	6.51												
<i>C. lutheri</i> (China)	18.04	16.75	18.44	15.97											
<i>C. lutheri</i> (Korea)	16.43	16.66	15.40	14.67	14.05										
<i>C. matsubarai</i>	11.46	14.00	13.82	13.55	14.82	15.27									
<i>C. melanoleuca</i>	14.39	12.28	1.76	6.73	18.53	15.33	14.40								
<i>C. pacifica</i>	16.62	15.63	15.91	14.52	14.35	13.15	13.21	15.56							
<i>C. striata</i>	11.46	14.96	13.67	13.40	16.96	16.27	4.73	14.09	13.91						
<i>C. takatsuensis</i>	11.85	18.07	15.50	16.00	19.20	19.07	13.16	16.50	17.91	12.80					
<i>C. tetralineata</i>	16.75	15.52	15.87	14.52	13.49	4.04	15.17	15.21	12.38	16.64	18.86				
<i>I. yongdokensis</i>	15.70	15.55	15.79	13.70	10.93	10.13	12.87	15.16	11.36	13.92	17.93	9.70			
<i>I. longicorpa</i>	15.46	14.46	15.48	13.53	12.19	9.26	12.65	14.74	11.02	14.24	17.11	8.67	5.19		
<i>I. koreensis</i>	15.19	14.68	15.99	14.49	14.65	13.49	15.49	15.67	9.58	14.89	16.83	12.09	11.24	11.72	
<i>I. pumila</i>	15.19	14.49	15.05	13.94	13.55	13.49	14.74	14.73	9.25	14.71	16.07	12.80	11.59	11.37	1.73

Table 4. Comparison of *C. gracilis* with 4 related *Cobitis* species.

Characters	<i>C. gracilis</i>	<i>C. melanoleuca</i>	<i>C. granoei</i>	<i>C. lutheri</i>	<i>C. choii</i>
Body depth	Slenderer, length of depth 8.7 in SL in male and 8.9–11.1 (9.8) in females	Slender, length of depth 8.1–10.1 (8.8) in SL in males and 7.4–9.2 (8.3) in females	Slender, length of depth 7.5–8.4 (8.0) in SL in males and 6.9–9.5 (8.6) in females	Sturdier, length of depth 6.0–6.2 (6.1) in SL in males and 6.4–9.2 (7.9) in females	Sturdy, length of depth 6.1–7.6 (6.7) in SL both in males and females
Caudal peduncle	Long, length of caudal peduncle 6.4 in SL in male and 5.3–6.1 (5.7) in females	Long, length of caudal peduncle 4.5–6.1 (5.2) in SL in males and 4.5–5.9 (5.3) in females	Long, length of caudal peduncle 5.3–5.9 (5.6) in SL in males and 5.9–6.4 (5.9) in females	Shorter, length of caudal peduncle 8.7–10.2 (9.5) in SL in males and 8.4–10.4 (9.6) in females	Short, length of caudal peduncle 5.7–6.8 (6.3) in SL both in males and females
Lamina circularis	Smaller, meniscus	Small, knife	Small, semicircle	Larger, kidney-shaped	Long, serrated
Spots on the caudal base	One inconspicuous	One jet black	One jet black	Two jet black	One jet black
Spots/blotches on L ₅	15–20 small spots	10–16 small spots	11–16 large spots	A dark stripe	12–17 small spots
Suborbital spine	Slender and straight, with long processus latero-caudalis	Thick and straight, with short processus latero-caudalis	Slender and curved, with long processus latero-caudalis	Thick and straight, with short processus latero-caudalis	Slender and curved, with short processus latero-caudalis

Key to the five species of *Cobitis* in or north to the Yellow River in China.

- Lamina circularis slender and long, with serrae (Songhua River in China, central South Korea and Amur drainage in Russia) *C. choii* Kim & Son, 1984
Lamina circularis plate or medium slender and long, without serrae 2
- Two large spots on the caudal base, the upper one jet black and the lower one jet black or inconspicuous; a dark stripe on L₅ in males (Heilongjiang and Yalu Rivers in China, and Amur drainage in Russia) *C. lutheri* Rendahl, 1935
One jet black or inconspicuous small spot on the caudal base, the lower spot inconspicuous or absent; a row of small blotches on L₅ both in males and females 3
- Barbels long, longer than eye diameter; lamina circularis large and width (Liao, Heilongjiang and Ertix Rivers in China, Amur drainage in Russia and Kherlen River in Mongolia) *C. granoei* Rendahl, 1935
Barbels short, equal to or shorter than eye diameter; lamina circularis small, medium slender and long 4
- One jet black spot on the caudal base; 10–16 blotches on L₅; lamina circularis knife (Yellow, Hai and Luan Rivers) *C. melanoleuca* Nichols, 1925
One inconspicuous spot on the caudal base; 15–20 blotches on L₅; lamina circularis meniscus (Ussuri and Yalu Rivers) *C. gracilis* sp. nov.

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References

- Akaike, H. 1973. Information theory and an extension of the maximum likelihood principle. In: Petrov, B.N., Csaiikl, F. (eds.), *Second International Symposium on Information Theory*. Akadémiai Kiadó, Budapest. pp. 267–281.
- Bănărescu, P. 1990. *Zoogeography of Fresh Waters. Vol. 1, General Distribution and Dispersal of Freshwater Animals*. AULA-Verlag, Wiesbaden. 511pp.
- Chen, Y.F., Chen, Y.X. 2005. Secondary sexual characters, pigmentary zones of Gambetta and taxonomical revision the genus *Cobitis* from China (Pisces: Cobitidae: Cobitinae). *Acta Zootaxonomica Sinica*, 30: 647–658.

- Chen, Y.X., Chen, Y.F. 2011. Two new species of cobitid fish (Teleostei, Cobitidae) from the River Nanliu and River Beiliu, China. *Folia Zoologica*, 60: 143–152.
- Chen, Y.X., Chen, Y.F. 2013. Three new species of cobitid fish (Teleostei, Cobitidae) from the River Xinjiang and the River Le'anjiang, tributaries of the Lake Poyang of China, with remarks on their classification. *Folia Zoologica*, 62: 83–95.
- Chen, Y.X., Liang, N., Li, X., Pan, X.R. Wu, D.Y. 2015. Taxonomic status of three subspecies of *Cobitis melanoleuca* based on sequences of mitochondrial cytochrome b gene. *Journal of Tianjin Normal University (Natural Science Edition)*, 35 (3): 1–6.
- Doadrio, I., Perdices, A. 1997. Taxonomic study of the Iberian *Cobitis* (Osteichthyes, Cobitidae), with description of a new species. *Zoological Journal of the Linnean Society*, 119(1): 51–67.
- Geiger, M.F., Herder, F., Monaghan, M.T., Almada, V., Barbieri, R., Bariche, M., Berrebi, P., Bohlen, J., Casal-Lopez, M., Delmastro, G.B., Denys, G.P.J., Dettal, A., Doadrio, I., Kalogianni, E., Kärst, H., Kottelat, M., Kovačić, M., Laporte, M., Lorenzoni, M., Marčić, Z., Özuluğ, M., Perdices, A., Perea, S., Persat, H., Porcelotti, S., Puzzi, C., Robalo, J., Šanda, R., Schneider, M., Šlechtová, V., Stoumboudi, M., Walter, S., Freyhof, J. 2014. Spatial heterogeneity in the Mediterranean Biodiversity hotspot affects barcoding accuracy of its freshwater fishes. *Molecular Ecology Resources*, 14(6): 1210–1221. doi: 10.1111/1755-0998.12257.
- Hebert, P.D.N., Ratnasingham, S., Dewaard, J.R. 2003. Barcoding animal life: Cytochrome c oxidase subunit 1 divergences among closely related species. *Proceeding of Royal Society of London, Series B: Biological Sciences*, (Suppl.): S96–S99.
- Huelsenbeck, J.P., Ronquist, F. 2001. MrBayes: Bayesian inference of phylogeny. *Bioinformatics*, 17: 754–755.
- Janko, K., Flajshanš, M., Choleva, L., Bohlen, J., Šlechtová, V., Rábová, M., Lajbner, Z., Šlechta, V., Ivanova, P., Dobrovolov, I., Culling, M., Persat, H., Kotusz, J., Ráb, P. 2007. Diversity of European spined loaches (genus *Cobitis* L.): an update of the geographic distribution of the *Cobitis taenia* hybrid complex with a description of new molecular tools for species and hybrid determination. *Journal of Fish Biology*, 71 (Supplement): 387–408.
- Kim, I.S. 1980. Systematic studies on the fishes of the family Cobitidae (Order Cypriniformes) in Korea. I. Three unrecorded species and subspecies of the genus *Cobitis* from Korea. *Korean Journal of Zoology*, 34 (4): 239–250.
- Kim, I.S., Park, J.Y., Nalbant, T.T. 1999. The far-east species of the genus *Cobitis* with the description of three new taxa (Pisces: Ostariophysi: Cobitidae). *Travaux du Museum D'histoire Naturelle "Grigore Antipa"*, 41: 373–391.
- Kim, I.S., Park, J.Y., Son, Y.M., Nalbant, T.T. 2003. A review of the loaches, genus *Cobitis* (Teleostomi: Cobitidae) from Korea, with the description of a new species *Cobitis hankugensis*. *Korean Journal of Zoology*, 15 (1): 1–11.
- Kim, I.S., Son, Y.M. 1984. *Cobitis choii*, a new cobitid fish from Korea. *Korean Journal of Zoology*, 27 (1): 49–55.
- Kitagawa, T., Jeon, S. R., Kitagawa, E., Yoshioka, M., Kashiwagi, M., Okazaki, T. 2005. Genetic relationships among the Japanese and Korean striated spined loach complex (Cobitidae: *Cobitis*) and their phylogenetic positions. *Ichthyological Research*, 52: 111–122.
- Nalbant, T.T. 1993. Some problems in the systematics of the genus *Cobitis* and its relatives (Pisces, Ostariophysi, Cobitidae). *Revue Roumaine de Biologie: Serie Biologie Animale*, 38: 101–110.
- Nalbant, T.T., Holěřk, J., Pivnička, K. 1970. A new loach, *Cobitis granoei olivai*, ssp. n., from Mongolia, with some remarks on the *Cobitis elongata-belseli-macrostigma* group (Pisces, Ostariophysi, Cobitidae). *Věstník Československé Společnosti Zoologické*, 34: 121–128.
- Perdices, A., Vasil'ev, V., Vasil'eva, E. 2012. Molecular phylogeny and intraspecific structure of loaches (genera *Cobitis* and *Misgurnus*) from the Far East region of Russia and some conclusions on their systematics. *Ichthyological Research*, 59: 113–123.
- Perdices, A., Vasil'eva, E., Vasil'ev, V. 2015. From Asia to Europe across Siberia: phylogeography of the Siberian spined loach (Teleostei, Cobitidae). *Zoologica Scripta*, 44(1): 29–40.
- Posada, D. 2008. jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution*, 25: 1253–1256.
- Rendahl, H. 1935. Ein paar neue Unterarten von *Cobitis taenia*. *Menor. Soc. Proc. Fauna et Flora Fennica*, 10: 330–332.
- Sambrook, J., Fritsch, E.F., Maniatis, T. 1989. *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York. 1626 pp.
- Sawada, Y. 1982. Phylogeny and zoogeography of the superfamily Cobitoidea (Cyprinoidei, Cypriniformes). *Reprinted from Memoirs of the Faculty of Fisheries Hokkaido University*, 28: 199.
- Šlechtová V., Bohlen, J., Perdices, A. 2008. Molecular phylogeny of the freshwater fish family Cobitidae (Cyriniformes: Teleostei): delimitation of genera, mitochondrial introgression and evolution of sexual dimorphism. *Molecular Phylogenetics and Evolution*, 47: 812–831.
- Takeda, R., Fujie, K. 1945. Distribution of some colour pattern types of *Cobitis taenia* (Tokyo). *Zoology Magnetism*, Tokyo, 56 (11–12): 1–5.
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S. 2011. MEGA5: Molecular Evolutionary Genetics Analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, 28: 2731–2739.
- Vasil'ev, V.P., Vasil'eva, E.D. 2008. Comparative karyological analysis of mud loach and spined loach species (genera *Misgurnus* and *Cobitis*) from the Far East region of Russia. *Folia Zoologica*, 57: 51–59.
- Xiao, W., Zhang, Y., Liu, H. 2001. Molecular systematics of Xenocyprinae (Teleostei: Cyprinidae): taxonomy, biogeography, and coevolution of a special group restricted in East Asia. *Molecular Phylogenetics and Evolution*, 18: 163–173.