

ORIGINAL ARTICLE

Taxonomic study of the genus *Niwaella* (Cypriniformes: Cobitidae) from East China, with description of four new species

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Abstract Four loaches, *Niwaella brevipinna* Chen & Chen, **sp. nov.**, *N. fimbriata* Chen & Chen, **sp. nov.**, *N. nigrolinea* Chen & Chen, **sp. nov.** and *N. qujiangensis* Chen & Chen, **sp. nov.**, are described based on specimens collected from Zhejiang and Anhui Provinces, East China. The mitochondrial cytochrome *b* gene of 28 individuals of seven species from seven locations collected from Zhejiang, Anhui and Jiangxi Provinces were amplified and analyzed. Morphological and molecular data showed that *N. fimbriata* Chen & Chen, **sp. nov.** and *N. nigrolinea* Chen & Chen, **sp. nov.**, *N. brevipinna* Chen & Chen, **sp. nov.** and *N. laterimaculata* (Yan & Zheng), *N. qujiangensis* Chen & Chen, **sp. nov.** and *N. longibarba* Chen & Chen are closely related. The seven Chinese *Niwaella* species, *N. brevipinna* Chen & Chen, **sp. nov.**, *N. fimbriata* Chen & Chen, **sp. nov.**, *N. laterimaculata*, *N. longibarba*, *N. nigrolinea* Chen & Chen, **sp. nov.**, *N. qujiangensis* Chen & Chen, **sp. nov.**, and *N. xinjiangensis* are clearly distinguished by the combination of the color pattern, mental lobes, suborbital spine, caudal peduncle, body shape, and subdorsal scales.

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

1 Introduction

Loaches of the genus *Niwaella* Nalbant, 1963 (Cypriniformes, Cobitidae) are small benthic freshwater fishes that found in East Asia, such as China, Korea and Japan. The fishes inhabit pebble or boulder bottoms in clear, fast running mountain streams, have no sexual dimorphism and a very elongated body. So far, only 5 species of *Niwaella* are regarded as valid (Froese & Pauly, 2017): *N. delicata* (Niwa, 1937), *N. laterimaculata* (Yan & Zheng, 1984), *N. longibarba* Chen & Chen, 2005, *N. multifasciata* (Wakiya & Mori, 1929), and *N. xinjiangensis* Chen & Chen, 2005.

Niwaella delicata, distributed in Japan, was originally described under the genus *Cobitis* by Niwa (1937). Nalbant (1963) established the genus *Niwaella* for separating it from other cobitid genera on the basis of a small head and sucker-like mouth and the absence of a lamina circularis on the pectoral fin in males, and the position of the dorsal fin. Then, *Cobitis multifasciata* Wakiya & Mori, 1929, distributed in South Korea, was transferred to the genus *Niwaella* by Sawada & Kim (1977). *Niwaella laterimaculata*, *N. longibarba* and *N. xinjiangensis* are all distributed in China. *Niwaella laterimaculata* was originally described under *Cobitis* by Yan & Zheng (1984). Son & He (2001) recognized it as a species of *Niwaella*, but not examined its lectotype or topotype. Later, Chen & Chen (2005) substantiated the transfer of *laterimaculata* by

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examining its topotype, and described another two species of *Niwaella*: *N. longibarba* and *N. xinjiangensis*.

Additionally, Kim & Lee (1995) described *Niwaella brevifasciata* Kim & Lee, 1995 in South Korea. Then, the species was transferred out to erect the genus *Choia* by Kim *et al.* (1997) according to its 4 branched rays in anal fin and well developed barbels and mental lobes. The genus was soon replaced by *Kichulchoia* Kim, Park & Nalbant, 1999 later because of its junior homonym, *Choia* Walcott, 1920, in Porifera (Kim *et al.*, 1999).

In this study, loaches of the genus *Niwaella* from China were studied and four new species were 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 Fenghuajiang (a tributary of the Yongjiang River), Cao'ejiang, Qujiang (a tributary of the Qiantangjiang River), Dongshaoxi (a tributary of the Shaoxi River), Xin'anjiang (the upper reaches of the trunk stream of the Qiantangjiang River), and Xinjiang (a tributary of the Poyang Lake) rivers in the Zhejiang, Anhui and Jiangxi Provinces in east China during the spring and autumn (in 2013, 2015 and 2016) (Fig. 1). 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.

2.2 Morphology

Seventeen morphometric variables were measured followed Chen & Chen (2011). 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. Terminology of mouth, suborbital spine and subdorsal scales structures are adopted from Nalbant (1963).

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 28 individuals of seven species from seven locations collected from Zhejiang, Anhui and Jiangxi Provinces, were sequenced, including two individuals of *N. laterimaculata*, two of *N. longibarba*, three of *N. xinjiangensis*, two of *N. fimbriata* Chen & Chen, **sp. nov.**, three of *N. brevipinna* Chen & Chen, **sp. nov.**, three of *N. nigrolinea* Chen & Chen, **sp. nov.**, and 13 of *N. qujiangensis* Chen & Chen, **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 model using MEGA 5.05 (Tamura *et al.*, 2011). To elucidate the phylogenetic relationship of new species and its East Asian congeners, eight sequences of *cyt b* of *Niwaella*, 23 sequences of *cyt b* of *Cobitis*, five of *Iksookimia*, five of *Kichulchoia*, and three of *Koreocobitis* were also downloaded from GenBank. The collection sites and corresponding GenBank Accession Nos. of species in this study are listed in Table 1.

The mean K2P distance between species was calculated for each species pair in Mega 5.05. The phylogenetic analyses were inferred for unique DNA sequences of *cyt b* gene by Maximum Likelihood (ML) method and Bayesian inferences, 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;
 HL—head length;
 ll—lower lip;
 m—mandible;
 mb—maxillary barbel;
 ml—mental lobes;
 mmb—maxillo-mandibular barbel;

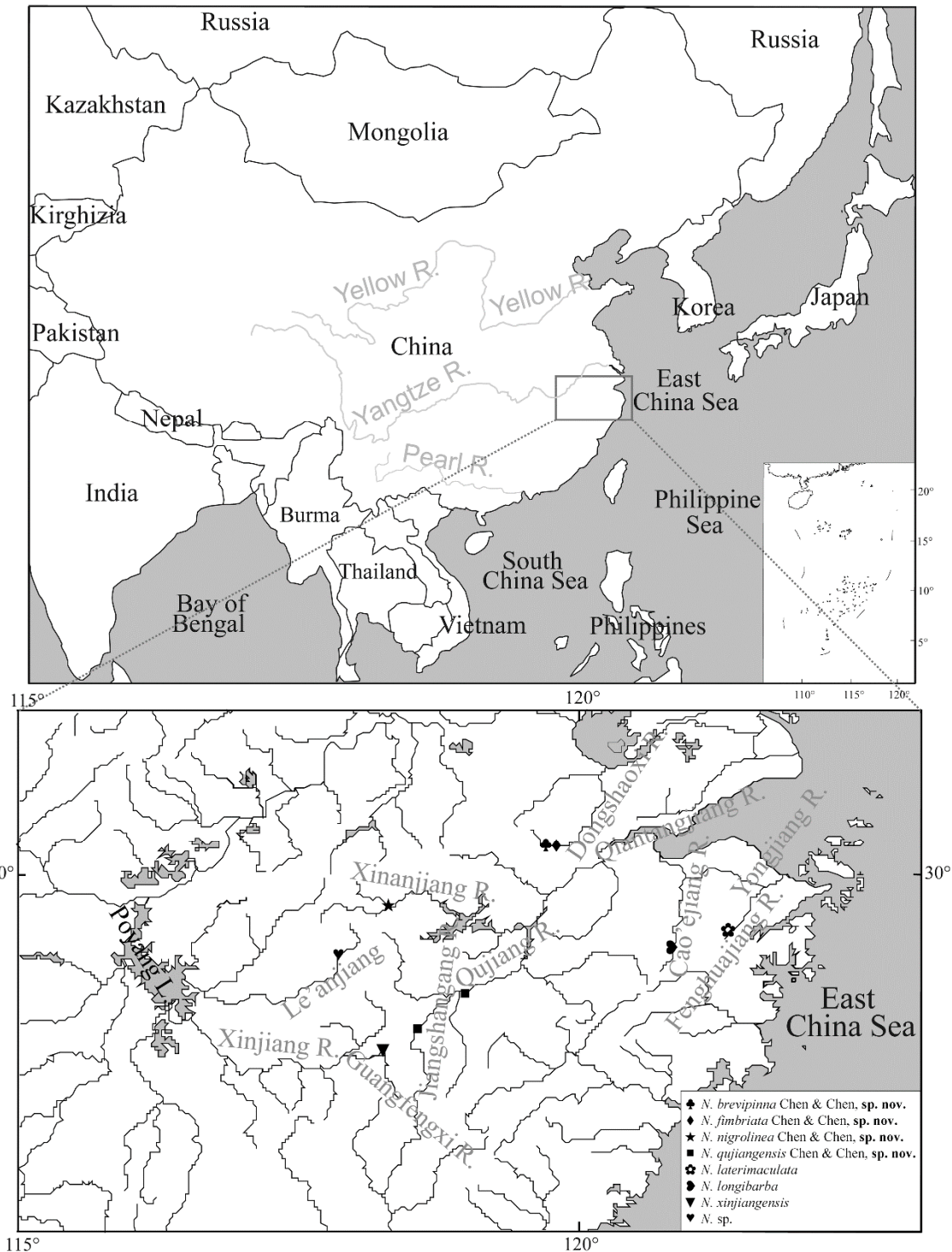


Figure 1. Currently known localities of the eight *Niwaella* species in China.

P—pectoral-fin;
 rb—rostral barbel;
 SL—stander length;
 ul—upper lip;
 V—ventral-fin.

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

Scientific name	Locality	Access. Nos.
<i>Niwaella brevipinna</i> Chen & Chen, sp. nov. 1	China, Zhejiang, R. Dongshaoxi	KY408023
<i>Niwaella brevipinna</i> Chen & Chen, sp. nov. 2	China, Zhejiang, R. Dongshaoxi	KY408024
<i>Niwaella delicata</i> 1	Japan, Gifu Pref., R. Takahara	AP009308*
<i>Niwaella delicata</i> 2	Japan, R. Hino	AB039352*
<i>Niwaella delicata</i> 3	Japan	AP011230*
<i>Niwaella delicata</i> 4	Japan, Gifu, Kuguno, R. Hida	AB195800*
<i>Niwaella fimbriata</i> Chen & Chen, sp. nov. 1	China, Zhejiang, R. Dongshaoxi	KY408021
<i>Niwaella fimbriata</i> Chen & Chen, sp. nov. 2	China, Zhejiang, R. Dongshaoxi	KY408022
<i>Niwaella laterimaculata</i>	China, Zhejiang, R. Yongjiang	KY408025
<i>Niwaella longibarba</i> 1	China, Zhejiang, R. Cao'ejiang	KY408027
<i>Niwaella longibarba</i> 2	China, Zhejiang, R. Cao'ejiang	KY408026
<i>Niwaella multifasciata</i> 1	South Korea, Daepyeong-myeon	EU670768*
<i>Niwaella multifasciata</i> 2	South Korea, Jollanam-do, Wondang-ri, R. Miryong	AB195802*
<i>Niwaella multifasciata</i> 3	South Korea	EF508573*
<i>Niwaella nigrolinea</i> Chen & Chen, sp. nov. 1	China, Anhui, R. Shuaishui	KY408014
<i>Niwaella nigrolinea</i> Chen & Chen, sp. nov. 2	China, Anhui, R. Shuaishui	KY408012
<i>Niwaella nigrolinea</i> Chen & Chen, sp. nov. 3	China, Anhui, R. Shuaishui	KY408013
<i>Niwaella qujiangensis</i> Chen & Chen, sp. nov. 1	China, Zhejiang, R. Qu'jiang	KY408016
<i>Niwaella qujiangensis</i> Chen & Chen, sp. nov. 2	China, Zhejiang, R. Qu'jiang	KY408019
<i>Niwaella qujiangensis</i> Chen & Chen, sp. nov. 3	China, Zhejiang, R. Qu'jiang	KY408015
<i>Niwaella qujiangensis</i> Chen & Chen, sp. nov. 4	China, Zhejiang, R. Qu'jiang	KY408017
<i>Niwaella qujiangensis</i> Chen & Chen, sp. nov. 5	China, Zhejiang, R. Qu'jiang	KY408018
<i>Niwaella qujiangensis</i> Chen & Chen, sp. nov. 6	China, Zhejiang, R. Qu'jiang	KY408020
<i>Niwaella xinjiangensis</i>	China, Jiangxi, R. Xinjiang	KY408011
<i>Niwaella</i> sp.	China, Jiangxi, R. Le'anjiang	DQ105236*
<i>Cobitis biwae</i> 1	Japan, Tokushima, R. Anabuki	AB084395*
<i>Cobitis biwae</i> 2	Japan, Akita, R. Yoneshiro	AB084407*
<i>Cobitis crassicauda</i>	China, Jiangxi, R. Xinjiang	JX888909
<i>Cobitis dolichorhynchus</i> 1	China, Fujiang, R. Longjiang	KX823449
<i>Cobitis dolichorhynchus</i> 2	China, Fujiang, R. Longjiang	KX823450
<i>Cobitis dolichorhynchus</i> 3	China, Fujiang, R. Longjiang	KX823451
<i>Cobitis fasciola</i> 1	China, Jiangxi, R. Xinjiang	JX888910
<i>Cobitis fasciola</i> 2	China, Jiangxi, R. Xinjiang	KP133115
<i>Cobitis hankugensis</i>	South Korea, R. Geum Cheon	KP161086*
<i>Cobitis macrostigma</i>	China, Jiangxi, L. Poyang	JX888904
<i>Cobitis matsubarae</i>	Japan, Saga, R. Matuura	AB091225*
<i>Cobitis magnostriata</i>	Japan, Shiga	LC097340*
<i>Cobitis minamorii tokaiensis</i>	Japan	AP013305*
<i>Cobitis minamorii oumiensis</i>	Japan, Shiga	LC097366*
<i>Cobitis minamorii minamorii</i>	Japan	AP013309*
<i>Cobitis pacifica</i>	South Korea, R. Doekcheon	KP161126*

Table 1 (continued)

Scientific name	Locality	Access. Nos.
<i>Cobitis takatsuensis</i>	Japan, R. Tsutsuga	AB039338*
<i>Cobitis tetralineata</i>	South Korea, Gokseong, R. Boseong	KC524528*
<i>Cobitis stenocauda</i> 1	China, Jiangxi, R. Xinjiang	JX888903
<i>Cobitis stenocauda</i> 2	China, Jiangxi, R. Xinjiang	KP133111
<i>Cobitis zhejiangensis</i> 1	China, Zhejiang, R. Ling	KX962073
<i>Cobitis zhejiangensis</i> 2	China, Zhejiang, R. Ling	KX823430
<i>Cobitis zhejiangensis</i> 3	China, Zhejiang, R. Ling	KX823431
<i>Iksookimia hugowolfeldi</i>	South Korea, Daedong Dam	EU670758*
<i>Iksookimia koreensis</i>	South Korea, R. Jeongcheon	EU670759*
<i>Iksookimia longicorpa</i>	South Korea, R. Seomjin	EU670760*
<i>Iksookimia pumila</i>	South Korea, Buan Dam	EU670761*
<i>Iksookimia yongdokensis</i>	South Korea, R. Gase	EU670762*
<i>Kichulchoia brevifasciata</i> 1	South Korea	EF508519*
<i>Kichulchoia brevifasciata</i> 2	South Korea, R. Sinpyeong	EU670763*
<i>Kichulchoia brevifasciata</i> 3	South Korea, Goheung	KC524520*
<i>Kichulchoia brevifasciata</i> 4	South Korea, Goheung	KC524521*
<i>Kichulchoia brevifasciata</i> 5	South Korea, Jollanam-do, Kohung-gun, R. Koup	AB195803*
<i>Koreocobitis rotundicaudata</i> 1	South Korea, R. Han drainage, R. Dal	KX265419*
<i>Koreocobitis rotundicaudata</i> 2	South Korea	EF508521*
<i>Koreocobitis naktongensis</i>	South Korea, Imgo-myeon, Yeongcheon-si, Gyeongsangbuk-do, R. Naktong	HM535625*

*Retrieved from GenBank

3 Results

3.1 Taxonomy

Niwaella nigrolinea Chen & Chen, *sp. nov.* (Figs 2–7)

Holotype. HU 2013112, 58.7 mm SL, the Shuaishui River (a tributary of the Xin'anjiang River), Xiuning County (29°78'N, 118°16'E), Anhui, China, October 2013, collected from the Xiuning farm product market by Yongxia Chen.

Paratypes. HU 2013113–5, 2013117–8, 5 ex., 52.0–62.2 mm SL, same data as holotype; HU 1505136–7, 2 ex., 56.4–59.5 mm SL, the Shuaishui River, Xiuning County, Anhui, China, April 2015, collected from the Xiuning farm product market by Yongxia Chen; HU 1608211, 1608209, 1608206, 3 ex., 54.9–63.3 mm SL, the Hengjiang River (a tributary of the Xin'anjiang River), Xiuning County, Anhui, China, April 2016, collected from the Xiuning farm product market by Yongxia Chen.

Diagnosis. The new species can be distinguished from its congeners by possessing the following combination of characteristics: a conspicuous black stripe from the occiput to the caudal fin on the dorsum, with 19–22 inconspicuous surface blotches (Fig. 7); short and dense vertical bars on the dorsolateral surface, and a row of 8–11 long, sparse, broad vertical stripes below the lateral midline posterior to the dorsal fin (Figs 2, 6); lower lip and mandible separated, with undeveloped mental lobes (Fig. 3); suborbital spine thick and curved, with long processus latero-caudalis, less than one-third of processus medio-caudalis (Fig. 4); caudal peduncle long, length of caudal peduncle 5.8–6.8 (mean 6.2) in SL.

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 slender, compressed, depth 9.4–10.6 (mean 9.8) in SL. Head small. Snout rounded. Preorbital part of head shorter than postorbital part of head. Mouth small, inferior, with three pairs of long barbels. Length of maxillo-mandibular barbels longer than diameter of eye. Lower lip and mandible separated, with undeveloped mental lobes (Fig. 3). Suborbital spine thick and curved, with a long processus latero-caudalis, less than one-third of processus medio-caudalis (Fig. 4). Subdorsal scales round, with a large focal area, 15–21 radial grooves, and 3–8 supplementary ones (Fig. 5).



Figures 2–5. *Niwaella nigrolinea* Chen & Chen, **sp. nov.**, the Shuaishui River, Xiuning, Anhui, China. 2. Holotype, HU 2013112, lateral view. 3. Mouth characters, front view. 4. Suborbital spine, left dorsal view, right interior view. 5. Subdorsal scales, dorsal view. Scale bars: 2 = 1 cm; 3–4 = 1 mm; 5 = 100 μ m.

Dorsal fin long, inserted the midway between the posterior margin of eye and the base of the caudal fin. Pectoral fins short. Length of the pectoral fin ray 9.0–11.9 (mean 10.5) in SL. Ventral fins short, approximately at the same level as the second branched dorsal-fin ray. Anal fin short, located in far behind dorsal extremity and not reach the caudal fin. Anal orifice close to the anal fin. Caudal fin emarginated tip.

Pigmentation pattern. Head sprinkled with many black spots, a black stripe extended from the insertion of the rostral barbels through the eye to the occiput. On the dorsum, a conspicuous longitudinal black stripe from the occiput through the dorsal fin to the caudal fin, with a surface pigment containing 19–22 small inconspicuous blotches. Short and dense vertical bars on the dorsolateral surface, and a row of 8–11 long, sparse, broad vertical stripes below the lateral midline posterior to the dorsal fin. One conspicuous large oval spot present on the upper half of the caudal fin base; the upper spot as large as the diameter of the eye. Three or four rows of brownish dots present on the dorsal and caudal fins.



Figures 6–7. *Niwaella nigrolinea* Chen & Chen, **sp. nov.**, color in life. 6. Lateral view. 7. Dorsal view. Scale bars = 1 cm.

Sexual dimorphism. Not obvious in external morphology.

Distribution. This new species occurs in the Xin'anjiang River (the upper reaches of the trunk stream of the Qiantangjiang River) in Anhui Province in east China (Fig. 1).

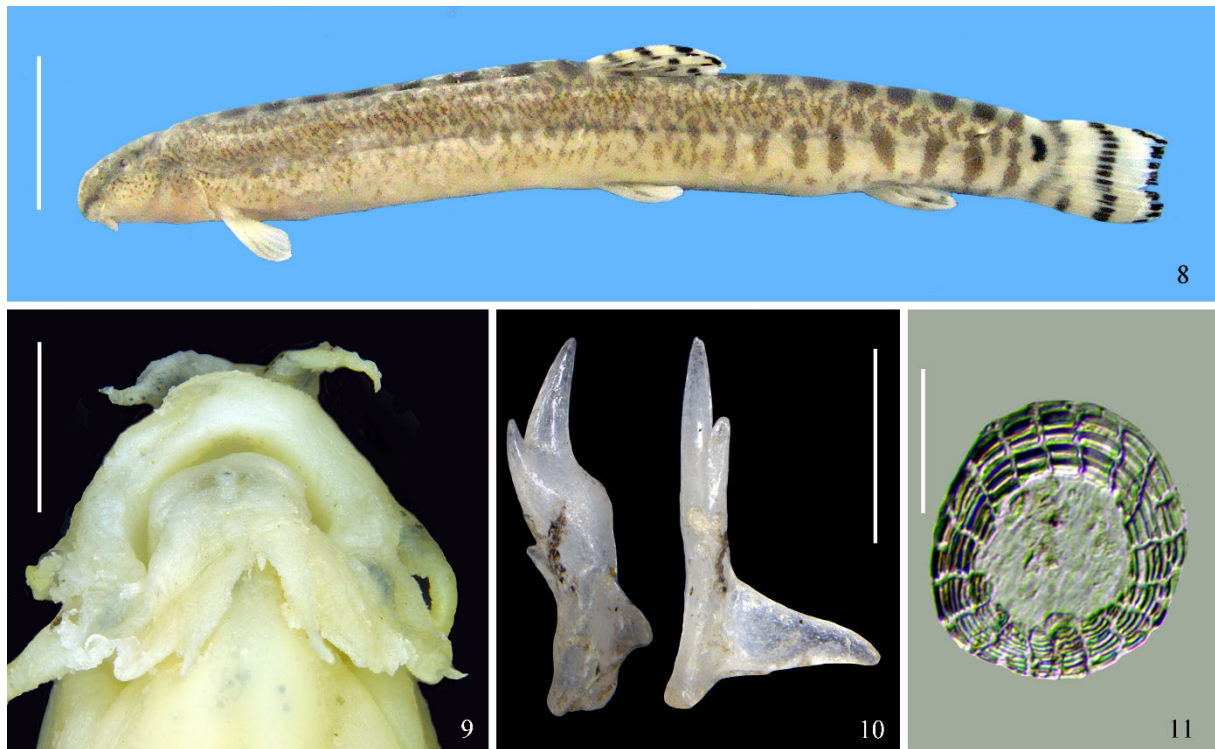
Etymology. The specific name derives from the Latin *niger*, meaning black, and Latin *linea* meaning line, in reference to the dorsum coloration of the species.

***Niwaella fimbriata* Chen & Chen, sp. nov.** (Figs 8–11)

Holotype. HU 1540121, 60.2 mm SL, the Dongshaoxi River (a tributary of the Shaoxi River), Lin'an (30°24'N, 119°68'E), Zhejiang, China, April 2015, collected from the Lin'an farm product market by Yongxia Chen.

Paratypes. HU 1540119, 1 ex., 64.1 mm SL, same data as holotype.

Diagnosis. The new species is similar to *N. nigrolinea* Chen & Chen, **sp. nov.** in color pattern, but differs by having 19–20 large, conspicuous blotches on the dorsum (vs. 19–22 small, inconspicuous blotches); suborbital spine straight, with short processus latero-caudalis, less than one-fifth of processus medio-caudalis (vs. suborbital spine curved, with long processus latero-caudalis, less than one-third of processus medio-caudalis); caudal peduncle short, length of caudal peduncle 6.5–7.5 (mean 7.0) in SL (vs. caudal peduncle long, length of caudal peduncle 5.8–6.8 (mean 6.2) in SL); body sturdy, depth 8.3–8.5 (mean 8.4) in SL (vs. slender, depth 9.4–10.6 (mean 9.8) in SL); subdorsal scales with a small focal area (vs. a large focal area).



Figures 8–11. *Niwaella fimbriata* Chen & Chen, **sp. nov.**, the Dongshaoxi River, Lin'an, Zhejiang, China. 8. Holotype, HU 1540121, lateral view. 9. Mouth characters, front view. 10. Suborbital spine, left dorsal view, right interior view. 11. Subdorsal scales, dorsal view. Scale bars: 8 = 1 cm; 9–10 = 1 mm; 11 = 100 μ m.

Description (Figs 8–11, Table 2). D. III–7; A. III–5; V. I–6; P. I–6–7; C. IV–14–16–IV. Body elongate, compressed, depth 8.3–8.5 (mean 8.4) in SL. Head small. 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. Lower lip and mandible separated, with undeveloped mental lobes (Fig. 9). Suborbital spine thick and straight, with short processus latero-caudalis, less than one-fifth of processus medio-caudalis (Fig. 10). Subdorsal scales small and oval, with a slightly small focal area, 16–19 radial grooves, and 3–5 supplementary ones (Fig. 11).

Dorsal fin inserted the midway between the eye and the base of the caudal fin. Ventral fins short, approximately at the same level as the second branched dorsal-fin ray. Anal fin located in far behind dorsal extremity and not reach the caudal fin. Anal orifice close to the anal fin. Caudal fin emarginated tip. Adipose crests developed. Lateral line short, not exceeding

length of the pectorals.

Pigmentation pattern. Head sprinkled with many black spots, a black stripe extended from the insertion of the rostral barbels through the eye to the occiput. On the dorsum, a conspicuous black stripe from the occiput through the dorsal fin to the caudal fin, with a surface pigment containing 19–20 slightly large conspicuous blotches. Short and dense vertical bars on the dorsolateral surface, and a row of 8–10 long, sparse broad vertical stripes below the lateral midline posterior to the dorsal fin. One conspicuous small half-moon-shaped spot present on the upper half of the caudal fin base; the upper spot smaller than the diameter of the eye. Three rows of brownish dots present on the dorsal and caudal fins.

Sexual dimorphism. Not obvious in external morphology.

Distribution. This new species occurs in the Dongshaoxi River (a tributary of the Shaoxi River) in Zhejiang Province in east China (Fig. 1).

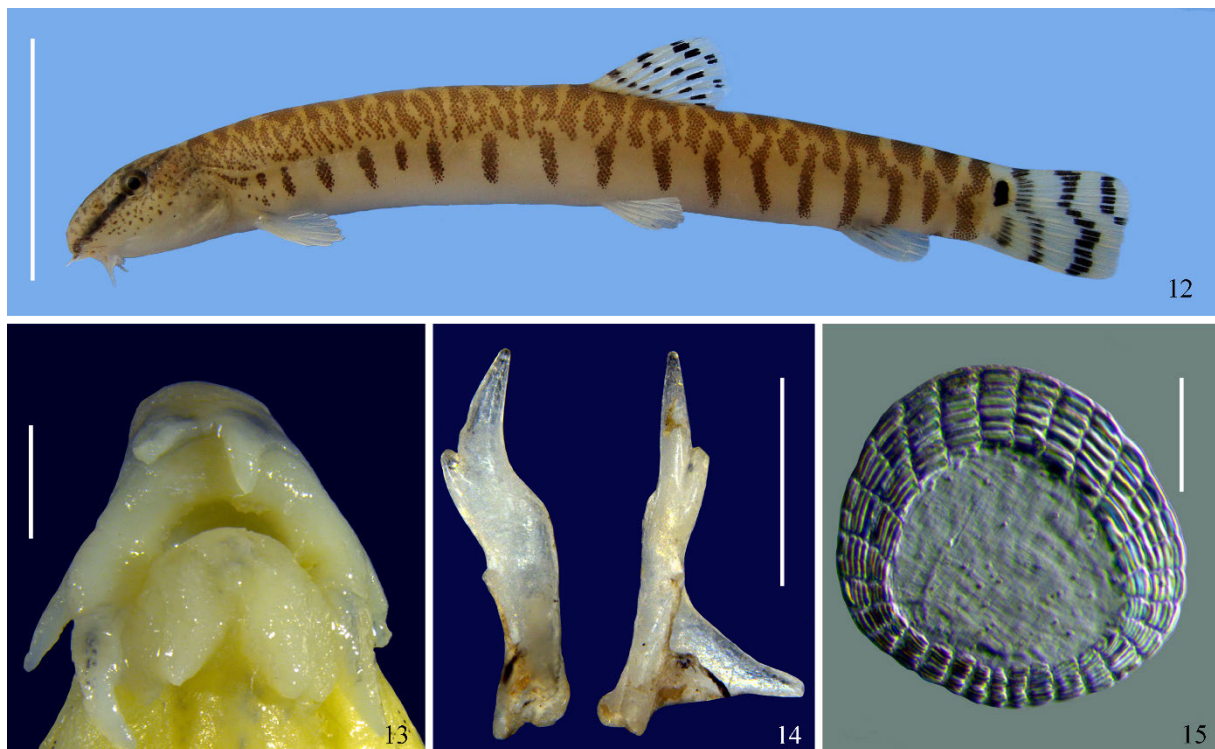
Etymology. The specific name derives from the Latin *fimbria*, meaning fringe, in reference to the lateral body coloration of the species.

Niwaella qujiangensis Chen & Chen, sp. nov. (Figs 12–17)

Holotype. HU 1505258, 54.0 mm SL, the Jiangshangang River (a tributary of the Qujiang River), Jiangshan (29°70'N, 118°62'E), Zhejiang, China, May 2015, collected from the Jiangshan farm product market by Yongxia Chen.

Paratypes. HU 1505256–7, 1505270–1, 1505275–80, 1505300, 11 ex., 45.1–63.8 mm SL, same data as holotype; HU 1608214, 1608172, 1608294–5, 1608300, 1608302, 1608304, 1608309, 8 ex., 50.9–68.5 mm SL, the Lingshangang River (a tributary of the Qujiang River), Quanwang (28°92'N, 119°14'E), Zhejiang, China, April 2016, collected from the Quanwang farm product market by Yongxia Chen.

Diagnosis. The new species can be distinguished from its congeners by possessing the following combination of characteristics: color pattern consisting of vertical stripes continuous along dorsal blotches, a row of 15–19 vertical stripes below the lateral midline (Figs. 12, 16); an inconspicuous black stripe from the occiput to the caudal fin on the dorsum, with 17–23 long, large surface blotches (Fig. 17); lower lip and mandible separated, with undeveloped mental lobes (Fig. 13); suborbital spine straight, with shorter processus latero-caudalis, more than one in 10 of processus medio-caudalis (Fig. 14); subdorsal scales round, with a large focal area, 26–28 radial grooves, and 4–8 supplementary ones (Fig. 21). It is similar to



Figures 12–15. *Niwaella qujiangensis* Chen & Chen, sp. nov., the Jiangshangang River, Jiangshan, Zhejiang, China. 12. Holotype, HU 1505258, lateral view. 13. Mouth characters, front view. 14. Suborbital spine, left dorsal view, right interior view. 15. Subdorsal scales, dorsal view. Scale bars: 12 = 1 cm; 13–14 = 1 mm; 15 = 100 μ m.

N. longibarba in color pattern, but differs from the latter by suborbital spine thick and straight, with short processus latero-caudalis, more than one in 10 of processus medio-caudalis (vs. suborbital spine slender and curved, with long processus latero-caudalis, less than one third of processus medio-caudalis); caudal peduncle long, length of caudal peduncle 6.4–8.9 (mean 7.4) in SL (vs. caudal peduncle short, length of caudal peduncle 7.1–9.0 (mean 7.9) in SL); subdorsal scales with a large focal area, 26–28 radial grooves (vs. a small focal area, 23–26 radial grooves).

Description (Figs 12–17, Table 2). D. III–7; A. III–5; V. I–6; P. I–6; C. IV–14–16–IV. Body elongate, compressed. Head moderately long, length of head 5.6–6.7 (mean 6.2) in SL. Snout bluntly rounded. Preorbital part of head shorter than postorbital part of head. Mouth small, inferior, with three pairs of long barbels. Length of maxillo-mandibular barbels longer than diameter of eye. Maxillary barbels not reach under anterior border of eye. Lower lip and mandible separated, with undeveloped mental lobes (Fig. 13). Suborbital spine thick and straight, with shorter processus latero-caudalis, more than one in 10 of processus medio-caudalis (Fig. 14). Subdorsal scales round, with a large focal area, 26–28 radial grooves, and 4–8 supplementary ones (Fig. 15).

Dorsal fin long, inserted the midway between the occiput and the base of the caudal fin. Length of the dorsal fin 6.3–7.7 (mean 6.9) in SL. Pectoral fins short, the second branched pectoral fin ray longest. Ventral fins approximately at the same level as the second dorsal-fin ray. Anal fin short, located in far behind dorsal extremity and not reach the caudal fin. Anal orifice close to the anal fin. Caudal fin emarginated tip.

Pigmentation pattern. Head sprinkled with many black spots, a black stripe extended from the insertion of the rostral barbels through the eye to the occiput. On the dorsum, an inconspicuous black stripe from the occiput through the dorsal fin to the caudal fin, with a surface pigment containing 17–23 long blotches. On the dorsolateral surface, moderately large vertical bars continuous along dorsal blotches, and a row of 15–19 vertical stripes below the lateral midline. One conspicuous small oblique square spot present on the upper half of the caudal fin base; the upper spot smaller than the diameter of the eye. Three or four rows of brownish dots present on the dorsal and caudal fins.

Sexual dimorphism. Not obvious in external morphology.

Distribution. This new species occurs in the Qujiang River (the upper reaches of the Qiantangjiang River) in Zhejiang Province in east China (Fig. 1).

Etymology. The specific name is derived from the Qujiang River in Zhejiang Province in China.



Figures 16–17. *Niwaella qujiangensis* Chen & Chen, **sp. nov.**, color in life. 16. Lateral view. 17. Dorsal view. Scale bars = 1 cm.

***Niwaella brevipinna* Chen & Chen, sp. nov.** (Figs 18–22)

Cobitis laterimaculata Nakajima *et al.* 2013 (nec. Yang & Zheng, 1984): 331 (from the East Tiaoxi River, Fig. 7h).

Holotype. HU 1608312, 55.5 mm SL, the Dongshaoxi River (a tributary of the Shaoxi River), Lin'an (30°24'N, 119°68'E), Zhejiang, China, April 2016, collected from the Lin'an farm product market by Yongxia Chen.

Paratypes. HU 1608208, 1608213, 1608215, 1608305–7, 1608299–301, 1608303, 10 ex., 38.9–58.0 mm SL, same data

as holotype.

Diagnosis. The new species is distinguishable from its congeners by the following characteristics: color pattern consisting of narrow brown vertical bars discontinuous along dorsal upper blotches; a row of 15–18 vertical stripes below the lateral midline (Figs 18, 22); lower lip wrapped the mandible, with moderately developed mental lobes (Fig. 19); suborbital spine thick and curved, with short processus latero-caudalis, less than one-fifth of processus medio-caudalis (Fig. 20); subdorsal scales small and oval, with a large focal area, 24–29 radial grooves, and 9–12 supplementary ones (Fig. 21). It is similar to *N. laterimaculata* in color pattern, but differs from it in the lower lip wrapped the mandible, with developed mental lobes (vs. lower lip and mandible separated, with undeveloped mental lobes); 4 branched rays in anal fin (vs. 5); 6 branched rays in dorsal fin (Fig. 22) (vs. 7 (Fig. 26)); 5 branched rays in ventral fin (Fig. 22) (vs. 6); suborbital spine thick, with long processus latero-caudalis, less than one-fifth of processus medio-caudalis (vs. suborbital spine slender, with short processus latero-caudalis, less than one-seventh of processus medio-caudalis); caudal peduncle long, length of caudal peduncle 6.7–8.0 (mean 7.5) in SL (vs. caudal peduncle short, length of caudal peduncle 7.3–10.7 (mean 8.9) in SL).

Description (Figs 18–22, Table 2). D. III–6; A. III–4; V. I–5; P. I–6; C. IV–14–15–IV. Body elongate, compressed. Head small. Snout bluntly rounded. Preorbital part of head shorter than postorbital part of head. Mouth small, inferior, with three pairs of short barbels. Length of maxillo-mandibular barbels equal to diameter of eye. Lips fleshy with numerous transversal wrinkles on the surface. Lower lip wrapped the mandible, with moderately developed mental lobes (Fig. 19). Suborbital spine thick and curved, with short processus latero-caudalis, less than one-fifth of processus medio-caudalis (Fig. 20). Subdorsal scales small and oval, with a large focal area, 24–29 radial grooves, and 9–12 supplementary ones (Fig. 21).

Dorsal fin short, inserted the midway between the posterior margin of eye and the base of the caudal fin, length shorter than that of head. Length of the dorsal fin 6.8–7.0 (mean 7.4) in SL. Pectoral fins short, length of the pectoral fin 9.5–11.7 (mean 10.6) in SL. Ventral fins approximately at the same level as the second branched dorsal-fin ray. Anal fin located in far behind dorsal extremity and not reach the caudal fin. Anal orifice close to the anal fin. Caudal fin emarginated tip. Adipose crests developed. Lateral line short, not exceeding length of the pectorals.

Pigmentation pattern. Head sprinkled with many black spots, a black stripe extended from the insertion of the rostral barbels through the eye to the occiput. On the dorsum, an inconspicuous black stripe with a surface pigment containing 17–24 rectangular blotches, among which 8–13 oval or rectangular blotches present from the occiput to the base of the dorsal



Figures 18–21. *Niwaella brevipinna* Chen & Chen, **sp. nov.**, the Dongshaoxi River, Lin'an, Zhejiang, China. 18. Holotype, HU 1608312, lateral view. 19. Mouth characters, front view. 20. Suborbital spine, left dorsal view, right interior view. 21. Subdorsal scales, dorsal view. Scale bars: 18=1 cm; 19–20=1 mm; 21=100 μ m.

fin, 2 on the dorsal fin, and 7–9 behind the dorsal fin, gaps between the rectangular blotches narrower than their widths. A row of 15–18 vertical stripes below the lateral midline, with the stripe being for narrower than their interspaces; many small vertical bars with irregular speckles present on the dorsolateral surface. One conspicuous, small, oblique rectangular spot present on the upper half of the caudal fin base, the upper spot smaller than the diameter of the eye. Three or four rows of brownish dots present on the dorsal and caudal fins.

Sexual dimorphism. Not obvious in external morphology.

Distribution. This new species occurs in the Dongshaoxi River (a tributary of the Shaoxi River) in Zhejiang Province in east China (Fig. 1).

Remarks. Two new species, *N. fimbriata* Chen & Chen, **sp. nov.** and *N. brevipinna* Chen & Chen, **sp. nov.** are both living in the Dongshaoxi River. Two collection trips were organized during March to April in 2015 and 2016 to obtain more samples. However, these two species were collected in different year, respectively. It is not clear whether they are sympatric species. Maybe they are spatially separated in the areas of the Dongshaoxi River. Additionally, Nakajima *et al.* (2013) wrongly recorded this species as *Cobitis laterimaculata* in the Dongshaoxi River.

Etymology. The species name is derived from the Latin *brevis*, meaning short, and *pinna*, meaning feather, in reference to with short dorsal fin.



Figures 22–23. *Niwaella brevipinna* Chen & Chen, **sp. nov.**, color in life. 22. Lateral view. 23. Dorsal view. Scale bars = 1 cm.

3.2 Species delimitation with DNA sequences

We obtained 1140 bp of the mitochondrial cytochrome *b* gene and identified five unique haplotypes in three specimens of *N. brevipinna* Chen & Chen, **sp. nov.**, two in two specimens of *N. fimbriata* Chen & Chen, **sp. nov.**, one in two specimens of *N. laterimaculata*, two in two specimens of *N. longibarba*, three in three specimens of *N. nigrolinea* Chen & Chen, **sp. nov.**, six in thirteen specimens of *N. qujiangensis* Chen & Chen, **sp. nov.**, and one in three specimens of *N. xinjiangensis*. Analyses of the mitochondrial data showed the sequence divergence (K2P) between all specimens of *Niwaella* from the China ranging from 1.8% (between *N. laterimaculata* and *N. longibarba*) to 5.7% (between *N. xinjiangensis* and *N. brevipinna* Chen & Chen, **sp. nov.**) (Figs 24–25, Table 3). The topologies of BI and ML methods were slightly incongruent on the relative positions of *Cobitis fasciola* Chen & Chen, 2013, *Cobitis hankugensis* Kim, Park, Son & Nalbant, 2003 and *N. xinjiangensis*. Of species examined in this study, the genera *Niwaella*, *Cobitis* or *Iksookimia* does not form a monophyly, respectively. But, all the Chinese *Niwaella* species formed a clade with good supports (BP = 100%, BBP = 100%).

Four new species, *N. brevipinna* Chen & Chen, **sp. nov.**, *N. fimbriata* Chen & Chen, **sp. nov.**, *N. nigrolinea* Chen & Chen, **sp. nov.** and *N. qujiangensis* Chen & Chen, **sp. nov.**, conspecific individuals cluster together with strong support (BP = 100%, BBP > 98%), indicating good resolution at the level of species. *Niwaella nigrolinea* Chen & Chen, **sp. nov.** has a closer relationship with *N. fimbriata* Chen & Chen, **sp. nov.** with a genetic distance of 3.4%. *Niwaella brevipinna* Chen & Chen, **sp. nov.**, *N. laterimaculata*, *N. longibarba* and *N. qujiangensis* Chen & Chen, **sp. nov.** have a closer relationship with

high supports (BP = 100%, BBP = 96%).

Table 2. Morphometric and meristic characters for *Niwaella*, all measurements given in millimeters (mm).

Variable*	<i>N. nigrolinea</i> Chen & Chen, sp. nov. (n=8)		<i>N. fimbriata</i> Chen & Chen, sp. nov. (n=2)		<i>N. qujiangensis</i> Chen & Chen, sp. nov. (n=19)		<i>N. brevipinna</i> Chen & Chen, sp. nov. (n=11)	
	Holotype	Range	Holotype	Range	Holotype	Range	Holotype	Range
SL	58.7	52.0–62.2 (57.3)	60.2	60.2–64.1 (62.1)	54.0	45.1–68.5 (54.5)	55.5	38.9–58.0 (51.4)
SL/HL	6.5	6.0–6.9 (6.5)	5.8	5.8–6.9 (6.3)	6.2	5.6–6.7 (6.2)	6.5	6.2–6.8 (6.6)
SL/BD	9.6	9.4–10.6 (9.8)	8.5	8.3–8.5 (8.4)	9.5	7.3–9.8 (8.4)	8.2	7.6–8.8 (8.3)
SL/PL	2.6	2.3–2.7 (2.5)	2.6	2.5–2.6 (2.5)	2.7	2.5–2.8 (2.6)	2.8	2.6–3.0 (2.8)
SL/CPL	6.6	5.8–6.8 (6.2)	7.5	6.5–7.5 (7.0)	7.1	6.4–8.9 (7.4)	7.9	6.7–8.0 (7.5)
SL/CPD	12.0	11.3–14.3 (12.5)	12.0	11.4–12.0 (11.7)	11.0	9.7–11.8 (10.6)	10.8	10.2–12.1 (11.2)
SL/DFL	7.5	6.4–8.3 (7.4)	7.3	7.3–7.4 (7.3)	7.3	6.3–7.7 (6.9)	6.8	6.8–7.0 (7.4)
SL/PFL	10.9	9.0–11.9 (10.5)	10.2	9.6–10.2 (9.9)	10.8	9.0–10.8 (9.9)	11.1	9.5–11.7 (10.6)
SL/VFL	10.4	10.4–12.7 (11.1)	10.4	10.4–12.3 (11.3)	11.1	10.1–12.4 (11.1)	11.2	10.0–11.8 (11.0)
SL/AFL	10.5	9.0–11.3 (9.8)	9.8	9.8–10.0 (9.9)	10.4	7.9–10.4 (9.2)	9.1	8.9–10.8 (9.7)
SL/CFL	7.6	7.3–8.7 (7.8)	7.0	7.0–7.1 (7.0)	7.3	6.1–8.4 (7.2)	7.5	6.7–7.8 (7.3)
SL/PDL	2.0	1.8–2.0 (1.9)	1.7	1.7–1.9 (1.8)	1.9	1.8–2.0 (1.9)	1.9	1.8–2.0 (1.9)
SL/PVL	1.9	1.7–1.9 (1.8)	1.8	1.8–1.9 (1.8)	1.9	1.8–1.9 (1.8)	1.8	1.8–2.0 (1.9)
SL/PAL	1.2	1.2–1.3 (1.2)	1.2	1.2–1.2 (1.2)	1.2	1.2–1.3 (1.2)	1.2	1.2–1.3 (1.2)
HL/POL	2.9	2.2–2.9 (2.5)	2.9	2.0–2.9 (2.5)	2.0	2.0–2.4 (2.2)	3.0	2.4–3.0 (2.7)
HL/ED	5.8	5.3–5.9 (5.6)	5.8	4.5–5.8 (5.1)	5.6	4.9–6.6 (5.7)	5.5	5.0–5.9 (5.4)
HL/EW	4.4	4.4–5.2 (4.8)	4.0	3.8–4.0 (3.9)	3.4	3.2–4.3 (3.7)	4.5	4.0–4.5 (4.5)
CPL/CPD	1.8	1.8–2.3 (2.0)	1.6	1.6–1.8 (1.7)	1.6	1.2–1.6 (1.4)	1.4	1.3–1.6 (1.5)

*Abbreviation: AFL—Anal Fin Length; BD—Body Depth; CFL—Caudal Fin Length; CPD—Caudal Peduncle Depth (CPD); CPL—Caudal Peduncle Length (CPL); DFL—Dorsal Fin Length; ED—Eye Diameter; HL—Head Length (HL); IW—Interorbital Width; PL—Pectoral–Ventral Length; PAL—Preanal Length; PDL—Predorsal Length; PFL—Pectoral Fin Length; POL—Preorbital Length; PVL—Preventral Length; SL—Standard Length (SL); VFL—Ventral Fin Length.

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

	1	2	3	4	5	6	7
<i>N. brevipinna</i> Chen & Chen, sp. nov.							
<i>N. fimbriata</i> Chen & Chen, sp. nov.	4.2–4.5 (4.3)						
<i>N. laterimaculata</i>	2.2–2.5 (2.3)	3.0–3.6 (3.3)					
<i>N. longibarba</i>	2.7–3.3 (3.0)	4.2–4.5 (4.3)	1.6–1.9 (1.8)				
<i>N. nigrolinea</i> Chen & Chen, sp. nov.	5.0–6.2 (5.6)	3.0–3.9 (3.4)	4.4–5.3 (4.8)	5.0–6.2 (5.6)			
<i>N. qujiangensis</i> Chen & Chen, sp. nov.	2.5–3.9 (2.9)	3.0–4.4 (3.6)	1.6–3.0 (2.1)	1.6–3.3 (2.3)	4.1–5.6 (4.7)		
<i>N. xinjiangensis</i>	5.6–5.9 (5.7)	3.0–3.6 (3.3)	5.0	5.6–5.9 (5.7)	5.0–5.9 (5.4)	4.4–5.9 (5.1)	
<i>N. sp.</i>	5.0–5.3 (5.2)	2.5–3.0 (2.7)	3.9	5.0–5.3 (5.2)	4.4–4.7 (4.6)	4.4–5.3 (5.0)	3.9

4 Discussion

Seven species of *Niwaella* have been recorded from China: *N. brevipinna* Chen & Chen, **sp. nov.**, *N. fimbriata* Chen & Chen, **sp. nov.**, *N. laterimaculata*, *N. longibarba*, *N. nigrolinea* Chen & Chen, **sp. nov.**, *N. qujiangensis* Chen & Chen, **sp. nov.**, and *N. xinjiangensis* (Table 4).

Both morphological and molecular analysis revealed that *N. fimbriata* Chen & Chen, **sp. nov.** and *N. nigrolinea* Chen & Chen, **sp. nov.** are closely related (Figs 24–25). Their differences are mentioned in the diagnosis of *N. fimbriata* Chen & Chen, **sp. nov.** They can be distinguished from all other congeners from China by having a conspicuous longitudinal black strip and 19–22 small surface blotches on the dorsum (vs. an inconspicuous strip and 17–24 large surface blotches); the short

and dense vertical bars on the dorsolateral surface and a row of long, sparse, broad vertical stripes below the lateral midline posterior to the dorsal fin (Figs 2, 6, 8) (vs. short and dense vertical bars or broad, long stripes on the dorsolateral surface, and a row of long, sparse broad vertical stripes below the lateral midline or a longitudinal stripe along the lateral midline (Figs 12, 16, 18, 22, 26–30)).

Four species, *N. brevipinna* Chen & Chen, **sp. nov.**, *N. laterimaculata*, *N. longibarba*, and *N. qujiangensis* Chen & Chen, **sp. nov.**, are closely related (Figs 24–25). Their differences are partly mentioned in the diagnosis of *N. brevipinna* Chen & Chen, **sp. nov.** and *N. qujiangensis* Chen & Chen, **sp. nov.** In addition, *N. brevipinna* Chen & Chen, **sp. nov.** differs from *N. longibarba* and *N. qujiangensis* Chen & Chen, **sp. nov.** by having slender vertical bars discontinuous along dorsal upper blotches (vs. large vertical bars continuous along dorsal upper blotches); the lower lip wrapped the mandible, with developed mental lobes (vs. lower lip and mandible separated, with undeveloped mental lobes); 4 branched rays in anal fin (vs. 5); 6 branched rays in dorsal fin (vs. 7); 5 branched rays in ventral fin (vs. 6). *Niwaella qujiangensis* Chen & Chen, **sp.**

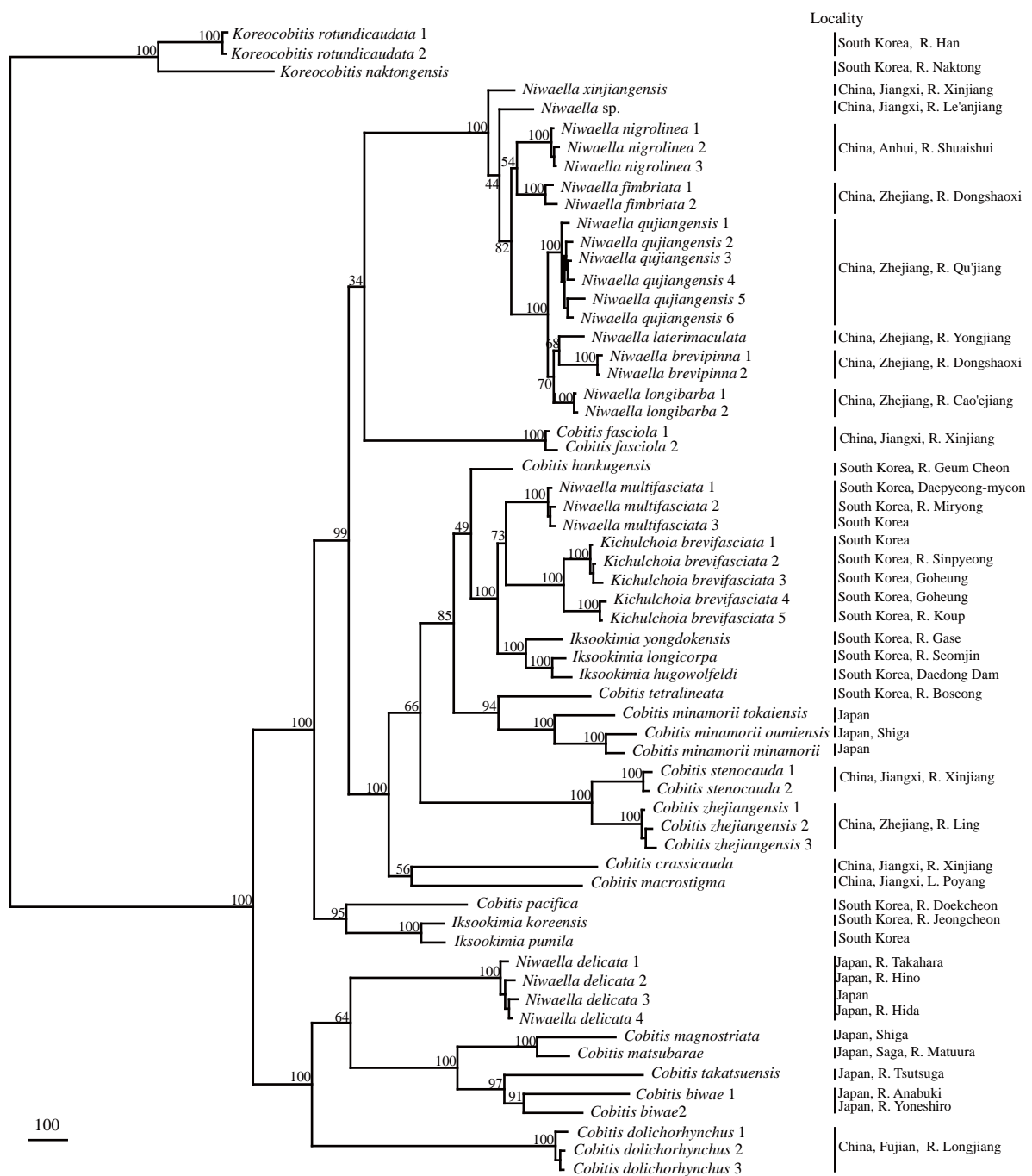


Figure 24. Bayesian tree of new species and its closely relatives in the East Asia recovered from mitochondrial cytochrome *b* based on GTR + G + I model. Bayesian posterior probabilities are shown in nodes.

nov. is distinguished from *N. laterimaculata* by having large vertical bars continuous along dorsal blotches (vs. slender vertical bars discontinuous along dorsal upper blotches); suborbital spine thick and straight (vs. suborbital spine slender and curved); caudal peduncle long, length of caudal peduncle 6.4–8.9 (mean 7.4) in SL (vs. caudal peduncle short, length of caudal peduncle 7.3–10.7 (mean 8.9) in SL). Moreover, these four new species are easily distinguished from *N. xinjiangensis*, *N. delicate* (from Japan), and *N. multifasciata* (from Korea) by having small, dense, slender vertical bars on the dorsolateral surface and a row of long, sparse, slender vertical stripes below the lateral midline (vs. large blotches on the dorsolateral surface and a longitudinal stripe or a row of small round blotches along the lateral midline in *N. xinjiangensis* and *N. delicate* (Nalbant, 1963: fig. 4; Sawada & Kim, 1977: fig. 2b; Kim & Lee, 1995: fig. 6b), broad and long vertical stripes on the flank

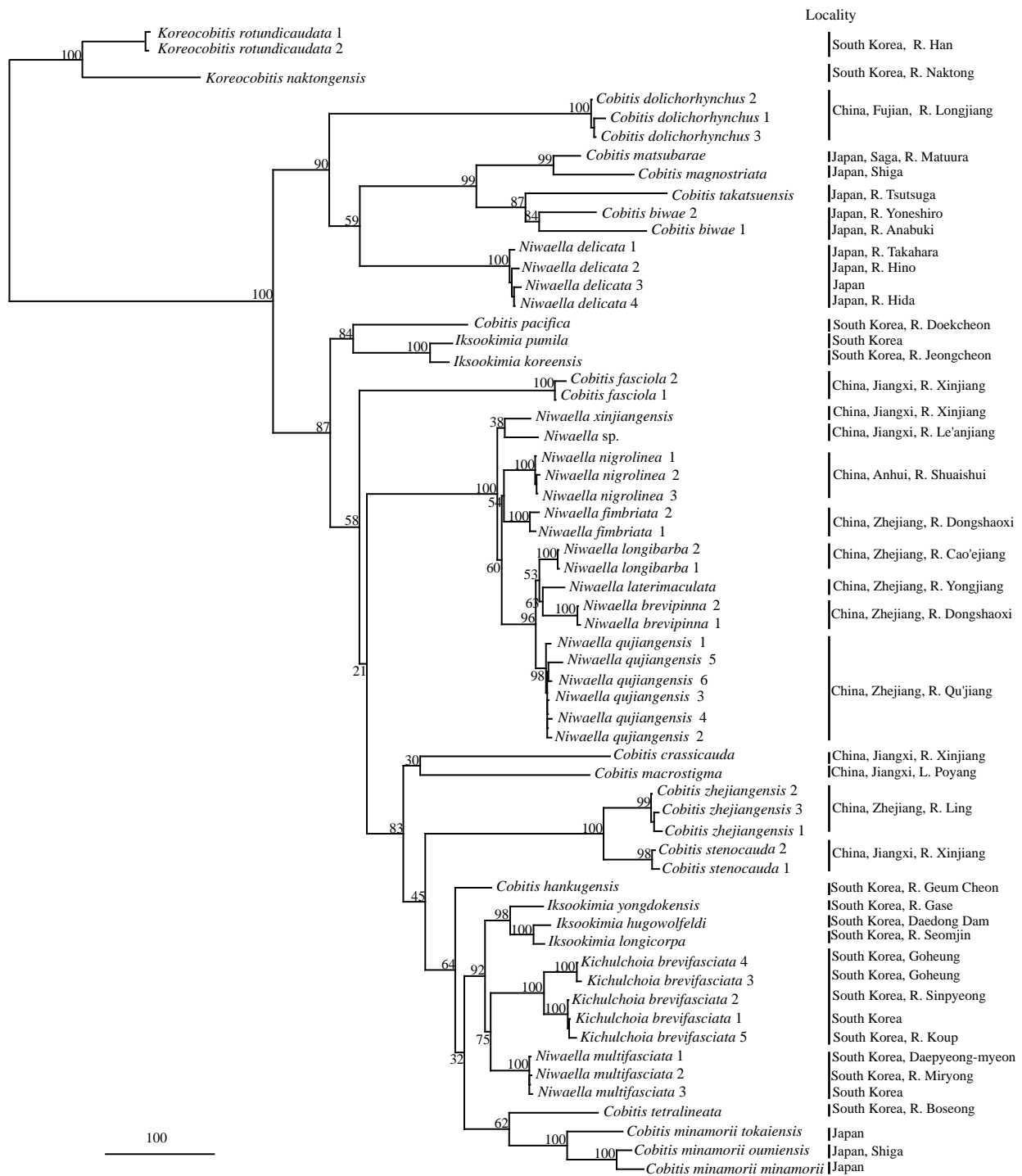
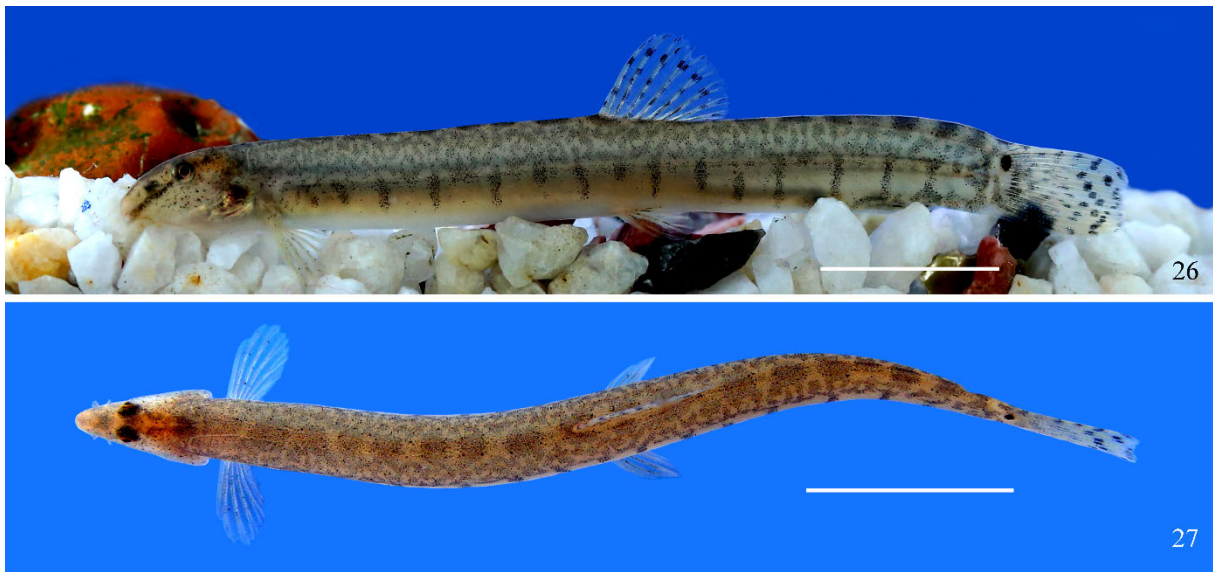


Figure 25. Maximum likelihood tree of new species and its closely relatives in the East Asia inferred from mitochondrial cytochrome *b* gene sequences based on GTRGAMMIX model. Clade credibility values are given for nodes with bootstrap support.



Figures 26–27. *Niwaella laterimaculata*, color in life, the Yongjiang River, Xikou, Zhejiang, China. 26. Lateral view. 27. Dorsal view. Scale bars = 1 cm.

in *N. multifasciata* (Kim & Lee, 1995: fig. 6c)).

Niwaella brevipinna Chen & Chen, **sp. nov.** is similar to Korean species *Kichulchoia brevifasciata* by having only 4 branched rays in anal fin and well developed mental lobes, but different by the short barbels, length of the maxillo-mandibular barbel 4.0–4.7 (mean 4.4) in HL (vs. long barbels, length of the maxillo-mandibular barbel 2.5–3.9 (mean 3.2) in HL (Kim & Lee, 1995)); mental lobes with bluntly-round tip (vs. mental lobes of the lower lip being pointed with a slightly filiform tip (Kim & Lee, 1995: fig. 3a)); ventral fins at the same level as the second branched dorsal-fin ray (vs. ventral fins at the same level as the origin of the dorsal fin (Kim & Lee, 1995: figs 1–2)). Both *Kichulchoia* and *Niwaella* are characterized by no sexual dimorphism and dorsal fin situated on second half of body, and different from *Cobitis* by those characters. Nalbant (1963) erected the genus *Niwaella* as the above reasons. While Kim *et al.* (1997) erected the genus *Kichulchoia* for separating it from *Niwaella* on the basis of presence of 4 branched rays in anal fin. Our molecular result showed that *Kichulchoia* is the sister lineage of the genus *Niwaella* (Figs 24–25). And there is a low level of sequence divergence between *K. brevifasciata* and *N. multifasciata* (9.9%). *Niwaella brevipinna* Chen & Chen, **sp. nov.** and *N.*



Figures 28–29. *Niwaella longibarba*, color in life, the Cao'ejiang River, Shengzhou, Zhejiang, China. 28. Lateral view. 29. Dorsal view. Scale bars = 1 cm.

laterimaculata are closely related with a low genetic distance (2.3%). 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). Based on the molecular data and morphology features, such as the sexual dimorphism that is used to define groups in the family Cobitidae, the validity of the *Kichulchoia* is in doubt. In this paper, we assigned *N. brevipinna* Chen & Chen, **sp. nov.** to the genus *Niwaella*.

Recent molecular works showed that the monophyly of the genera *Cobitis*, *Kichulchoia*, *Niwaella*, and *Iksookimia* are not supported (Šlechtová *et al.*, 2008; Chen *et al.*, 2013, 2015; Chen & Chen, 2013). Our result also agrees that. Therefore, several scholars proposed *Kichulchoia*, *Niwaella*, and *Iksookimia* as synonyms of *Cobitis* (Perdices *et al.*, 2016). Chen *et al.* (2013) reevaluated the diagnostic characters for genera *Cobitis* and *Iksookimia* and queried the validity of *Iksookimia*. In this paper, we queried the validity of the *Kichulchoia* and considered it as synonym of *Niwaella*. Although, the monophyly of the genus *Niwaella* is not supported by the molecular phylogeny, we considered *Niwaella* as valid. Based on the observations in the field, the representatives of genera *Cobitis* and *Niwaella* are observed together at the same sites. The type specimens of *Cobitis* are characterized by secondary sexual characters and sturdy body, and different from *Niwaella* by those characters.



Figures 30. *Niwaella xinjiangensis*, the Xinjiang River, Guangfeng, Jiangxi, China. Lateral view. Scale bars = 1 cm.

Key to the seven species of *Niwaella* in China.

1. Lower lip wrapped the mandible..... 2
Lower lip and mandible separated..... 3
2. Color pattern consisting of broad vertical bars continuous along dorsal upper blotches; a longitudinal stripe along the lateral midline ahead to the dorsal fin and then gradually below the lateral midline, or 16–18 vertical stripes; caudal peduncle long, length of caudal peduncle 6.4–8.0 (mean 7.2) in SL; suborbital spine slender, with long processus latero-caudalis, less than one-third of processus medio-caudalis (Xinjiang River)..... *N. xinjiangensis* Chen & Chen, 2005
Color pattern consisting of slender vertical bars discontinuous along dorsal upper blotches; a row of 15–18 vertical stripes below the lateral midline; caudal peduncle short, length of caudal peduncle 6.7–8.0 (mean 7.5) in SL; suborbital spine thick, with short processus latero-caudalis, less than one-fifth of processus medio-caudalis (Dongshaoxi River) *N. brevipinna* Chen & Chen, **sp. nov.**
3. Short and dense vertical bars on the dorsolateral surface, and a row of long and sparse vertical stripes below the lateral midline posterior to the dorsal fin 4
On the dorsolateral surface, short and dense vertical bars above the lateral midline, and a row of long and sparse vertical stripes below the lateral midline 5
4. 19–20 conspicuous blotches on the dorsum; suborbital spine straight, with short processus latero-caudalis, less than one-fifth of processus medio-caudalis; caudal peduncle short, length of caudal peduncle 6.5–7.5 (mean 7.0) in SL (Dongshaoxi River).....
..... *N. fimbriata* Chen & Chen, **sp. nov.**
19–22 inconspicuous blotches on the dorsum; suborbital spine curved, with long processus latero-caudalis, less than one-third of processus medio-caudalis; caudal peduncle long, length of caudal peduncle 5.8–6.8 (mean 6.2) in SL (Shuaishui River)
..... *N. nigrolinea* Chen & Chen, **sp. nov.**
5. Color pattern consisting of vertical bars discontinuous along dorsal upper blotches; barbels shorter than eye diameter; caudal peduncle short, length of caudal peduncle 7.3–10.7 (mean 8.9) in SL (Yongjiang River)..... *N. laterimaculata* (Yan & Zheng, 1984)
Color pattern consisting of vertical bars continuous along dorsal upper blotches; barbels longer than or equal to eye diameter; caudal peduncle long, length of caudal peduncle average less than 8.0 in SL..... 6
6. Suborbital spine thick and straight, with shorter processus latero-caudalis, more than one in 10 of processus medio-caudalis; caudal peduncle long, length of caudal peduncle 6.4–8.9 (mean 7.4) in SL (Qujiang River)..... *N. qujiangensis* Chen & Chen, **sp. nov.**
Suborbital spine slender and curved, with long processus latero-caudalis, less than one-third of processus medio-caudalis; caudal peduncle short, length of caudal peduncle 7.1–9.0 (mean 7.9) in SL (Cao'ejiang River) *N. longibarba* Chen & Chen, 2005

Table 4. Comparison of diagnostic characters of four Chinese species of *Niwaella*.

Species	Blotches on dorsum	Blotches on dorsolateral surface	Mandible and lower lip	Suborbital spine	Caudal peduncle
<i>N. brevipinna</i> Chen & Chen, sp. nov.	An inconspicuous stripe with 17–24 small, short surface blotches	Slender vertical bars discontinuous along dorsal blotches, and 15–18 vertical stripes below the lateral midline	Lower lip wrapped mandible	Thick and curved, short processus latero-caudalis, less than one-fifth of processus medio-caudalis	Short, length of caudal peduncle 6.7–8.0 (7.5) in SL
<i>N. fimbriata</i> Chen & Chen, sp. nov.	A conspicuous stripe with 19–20 small, short surface blotches	Short, dense vertical bars on the dorsolateral surface, and 8–10 long, sparse vertical stripes below the lateral midline	Separated	Thicker and straight, short processus latero-caudalis, less than one-fifth of processus medio-caudalis	Long, length of caudal peduncle 6.5–7.5 (7.0) in SL
<i>N. qujiangensis</i> Chen & Chen, sp. nov.	An inconspicuous stripe with 17–23 long surface blotches	Moderately large vertical bars continuous along dorsal blotches, and 15–19 vertical stripes below the lateral midline	Separated	Thick and straight, shorter processus latero-caudalis, more than one in 10 of processus medio-caudalis	Short, length of caudal peduncle 6.4–8.9 (7.4) in SL
<i>N. laterimaculata</i>	An inconspicuous stripe with 17–20 small, short surface blotches	Slender vertical bars discontinuous along dorsal blotches, and 16–20 vertical stripes below the lateral midline	Separated	Slenderer and curved, shorter processus latero-caudalis, less than one-seventh of processus medio-caudalis	Shorter, length of caudal peduncle 7.3–10.7 (8.9) in SL
<i>N. longibarba</i>	An inconspicuous stripe with 16–20 long surface blotches	Moderately large vertical bars continuous along dorsal blotches, and 16–19 vertical stripes below the lateral midline	Separated	Slenderer and curved, long processus latero-caudalis, less than one-third of processus medio-caudalis	Shorter, length of caudal peduncle 7.1–9.0 (7.9) in SL
<i>N. nigrolinea</i> Chen & Chen, sp. nov.	A conspicuous stripe with 19–22 inconspicuous small, short surface blotches	Short, dense vertical bars on the dorsolateral surface, and 8–11 long, sparse vertical stripes below the lateral midline	Separated	Thicker and curved, long processus latero-caudalis, less than one-third of processus medio-caudalis	Longer, length of caudal peduncle 5.8–6.8 (6.2) in SL
<i>N. xinjiangensis</i>	An inconspicuous stripe with 17–20 large, long surface blotches	Broad vertical stripes continuous along dorsal blotches, and a dark stripe along the lateral midline beyond the dorsal fin and then gradually below the lateral midline, or 16–18 vertical stripes below the lateral midline	Lower lip wrapped mandible	Slender and curved, longer processus latero-caudalis, less than one-third of processus medio-caudalis	Long, length of caudal peduncle 6.4–8.1 (7.2) in SL

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