

## THE TAXONOMY AND PHYLOGENY OF *CANDIDIA* (TELEOSTEI: CYPRINIDAE) FROM TAIWAN, WITH DESCRIPTION OF A NEW SPECIES AND COMMENTS ON A NEW GENUS

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**ABSTRACT.** – The cyprinid genus, *Candidia* Jordan & Richardson, 1909, is revised and the taxonomic status and the phylogenetic position of related cyprinids from Taiwanese waters are clarified. The molecular phylogenetic analyses inferred from mtDNA complete D-loop sequences for related cyprinid genera and species from adjacent waters have revealed that three species including so-called “*Zacco*” *temminckii* (Temminck & Schlegel, 1846), “*Zacco*” *sieboldii* (Temminck & Schlegel, 1846) and “*Zacco*” *koreanus* Kim, Oh & Hosoya, 2005, belong to a monophyletic clade with *Candidia* and *Parazacco*, and are not related to *Zacco*. A new genus, *Nipponocypris* is described and for which *Leuciscus temminckii* Temminck & Schlegel, 1846, is designated as the type species with three species considered valid: two described from Japan viz. “*Z.*” *temminckii*, “*Z.*” *sieboldii*, and one from Korea, “*Z.*” *koreanus* Kim et al., 2005. A new cyprinid, *Candidia pingtungensis*, new species, was collected from southern Taiwan. This new species can be distinguished from *Candidia barbatus* (Regan, 1908) by its meristic features and specific colouration. The taxonomy of the genus *Candidia* is revised and a diagnostic key to all valid members of *Candidia* is provided.

**KEY WORDS.** – new species, *Candidia*, molecular phylogeny, Cyprinidae, fish taxonomy, Taiwan.

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### INTRODUCTION

Cyprinid fish form the largest group of primary freshwater fish in Taiwan (Shen & Tzeng in Shen, 1993; Chen & Fang, 1999). Among this insular freshwater fish fauna are species which exhibit a high degree of endemism (Chen & Chang, 2005). The so-called barbel’s stripe minnow, “*Opsariichthys*” *barbatus* Regan, 1908, was first described from Central Taiwan and assigned into the cyprinid genus *Candidia* by Jordan & Richardson (1909). More recently *Candidia* has been treated as a valid genus endemic to Taiwan (Tzeng, 1986; Wang et al., 1997; Chen, 1998; Chen & Fang, 1999; Chen & Chang, 2005; Wu et al., 2007; Chen et al., 2008) except some ichthyologists has ever grouped it as *Zacco* (Shen & Tzeng, in Shen, 1993).

Wang et al. (1997) firstly provided genetic evidence for

clarifying the generic status of the genera *Zacco* and *Candidia*. On the basis of the results obtained from their isozymatic analysis, they disagreed with the generic placement of “*Zacco*” *barbatus* as proposed in Shen (1993). Wu et al. (2007) have provided molecular evidence for the existence of two discrete clades of the *Candidia barbatus* complex based on mtDNA D-loop sequences among the different populations from all the major river basins in Taiwan. After our intensive morphological study of these two clades, we have concluded that the southern clade belong to an undescribed species, endemic to a very limited geographical range in southern Taiwan. More recently, another research team, Wang et al. (2007), in their phylogenetic approach using mtDNA CytB gene sequences, have independently reported the close relationship between “*Zacco*” *temminckii* species complex and *Candidia barbatus*.

Our main aim here has been to use a rather sensitive genetic marker: complete D-loop sequence, to allow us to clarify the phylogenetic relationships among related species of the *Candidia* group and similar species, namely the “*Zacco*” *temminckii* species complex which shares a very similar overall colouration pattern (Kawanabe et al., 2002; Hosoya et al., 2003; Kim et al., 2005). Our other aims have been to describe the new taxon of *Candidia* from recent discoveries of cyprinids for Taiwan attributed by the first author’s team (Chen & Chang, 2005; Wu et al., 2007) as well as to establish a new genus from the Japanese and Korean fresh waters which is closely related to genus *Candidia* that is based on both morphological and molecular approaches.

## MATERIALS AND METHODS

**Sample collection.** – Specimens were collected by either using a casting net or electro-fishing. Whole specimens and partial fin tissues used for molecular analysis were directly preserved in 95% ethanol. All fish samples used for molecular biological analyses are listed in Table 1. Specimens used for morphological studies were preserved in 10% formalin solution before being transferred into 70% ethanol for long-term storage.

The cyprinid specimens used in this current research have been deposited in the Pisces collections of the National Museum of Marine Biology/Aquarium, Pingtung (NMMB), National Museum of Natural Science, Taichung (NMNS) and the Institute of Marine Biology, National Taiwan Ocean University, Keelung (NTOU).

**Molecular phylogenetic analysis.** – All DNA extraction of the samples was carried out according to the general protocols of the Phenol-chloroform method (Sambrook et al., 1989; Chen et al., 1998, 2002). The DNA fragment of about 1200 bp, including the full length of the D-loop region, were amplified by polymerase chain reaction (PCR) using primers based on the flanking region (CYP-THRA: 5’–AAAGCATCGGTCTTGTAATCCGAAG–3’; CYP-12SB: 5’–CATGCGGAGTTTCTTAGGTC–3’) that were designed from the conserved sequences of tRNA-PHE and 12S rRNA (Chen et al., 1998, 2002; Chen & Chang, 2007; Wu et al., 2007). PCR was done in a MODEL 2700 or 9700 thermal cycler (Perkin-Elmer) and 30–40 cycles were completed. The 25 µL reaction volume contained 14.4 µL of sterile distilled water, 2.5 µL of 10× PCR buffer (Takara), 2.0 µL of dNTP (2.5 mM each), 2.5 µL of each primer, 0.1 µL of 0.5 unit Ex Taq (Takara) and 10 µL of template. The thermal cycler profile was as follows: denaturation at 94°C for 15 seconds, annealing at 50 °C for 15 seconds and extension at 72°C for 60 seconds. A negative control without template was carried out for each run of PCR. The PCR products were run on a 1.0% L 03 agarose gel (Takara) and stained with ethidium bromide for band characterization under ultraviolet transillumination.

Double-stranded PCR products were purified using a kit (Roche, High Pure Product Purification kit), before

undergoing direct cycle sequencing with dye-labeled terminators (ABI Big-Dye kit). The sequencing primers used were either same as those for PCR or following two primers: CYP-DLMF1: 5’–CATGCGGAGTTTCTTGTC–3’ and CYP-DLMR2: 5’–GCTCGGCATGTTGGGTAA–3’. All sequencing reactions were performed according to the manufacturer’s instructions. Labeled fragments were analyzed using as ABI PRISM Model 377-64 DNA Automated sequencer (ABI).

Nucleotide sequence alignment was verified manually after running the software of CLUSTAL W (Thompson et al., 1994) and BIOEDIT version 5.9 (Hall, 2001). The analysis of aligned mutation sites were conducted using Molecular Evolutionary Genetics Analysis (MEGA) version 3 (Kumar et al., 2004) for aligned mutation sites analysis.

The parsimony (MP) analysis was carried out using PAUP\* version 4.0B10 (Swofford, 2003) using heuristic search. Branch support was established via bootstrap analysis (2000 replications). For the Bayesian (BI) analysis, the best-fitting model for sequence evolution was determined for mtDNA D-loop sequences using MrMODELTEST version 2.2 (Nylander, 2005). The BI analyses were performed using MrBayes 3.0 (Ronquist & Huelsenbeck, 2003). The posterior probabilities of each node were computed from remaining 75% of all sampled trees.

**Morphological studies.** – The meristic counts and morphometric measurements generally follow that of Fang (2000) and Hosoya et al. (2003). Meristic abbreviations are as follows: A, anal fin rays; CPS, surrounding scales of caudal peduncle; D, dorsal fin rays; LL, lateral-line scales; LLa, transverse scale rows above lateral-line scales; LLb, transverse scale rows below lateral-line scales; P1, pectoral fin rays; P2, pelvic fin rays; Ph, pharyngeal teeth; PreD, predorsal scales; and VC, vertebral count.

## RESULTS

**Molecular phylogenetic analysis.** – The complete D-loop sequences have been amplified and sequenced for both *Opsariichthys* and *Candidia* genus groups. The total lengths of complete mtDNA D-loop sequence of cyprinid fishes are from 925 bp to 936 bp. The shorter sequences, 925 bp, were obtained from *Opsariichthys uncirostris* (Temminck & Schlegel, 1846), *Opsariichthys bidens* Günther, 1873 and *Opsariichthys pachycephalus* Günther, 1868; the longer sequence, 936 bp, was obtained from *Nicholsicypris normalis* (Nichols & Pope, 1927). The sequence lengths of all members of *Candidia* genus group are from 926 to 928 bp.

The result of MP analysis by heuristic search yielded only one tree with minimal tree length 562; the consistency index being 0.601 and homoplasy index 0.399. The BI analysis yielded the tree topology (Fig. 1) which is almost the same with MP analysis and the result clearly supports two distinct paraphyletic clades with the assigned outgroup based on cyprinid species with distinct ventral keel: both *Aphyocypris*

Table 1. The OTU codes of cyprinid species of *Opsariichthys* and *Candidia* genus groups used for mitogenetic sequence analysis.

Code	Species name	Locality
CABAT1	<i>Candidia barbatus</i>	Tanshuei River, Taipei, Taiwan
CAPIT1	<i>Candidia pingtungensis</i>	Fongkang River, Pingtung, Taiwan
NPKOK1	<i>Nipponocypris koreanus</i>	Gwangcheon River, Kyong Sanbuk-Do, Korea
NPSIJ1	<i>Nipponocypris sieboldii</i>	Biwa Lake, Otsu, Honshu, Japan
NPTEJ1-2	<i>Nipponocypris temminckii</i>	Biwa Lake, Otsu, Honshu, Japan
PASPC1	<i>Parazacco spilurus</i>	Hongkong, China
OPUNJ1-2	<i>Opsariichthys uncirostris</i>	Biwa Lake, Otsu, Honshu, Japan
OPBIC1-2	<i>Opsariichthys bidens</i>	Changtangjiang, Zhejiang, China
OPEVC1	<i>Opsariichthys evolans</i>	Changtangjiang, Zhejiang, China
OPEVC2	<i>Opsariichthys evolans</i>	Julongjiang, Fujian, China
OPEVC3	<i>Opsariichthys evolans</i>	Mulan River, Fujian, China
OPEVT4	<i>Opsariichthys evolans</i>	Tanshuei River, Taipei, Taiwan
OPPAT1	<i>Opsariichthys pachycephalus</i>	Genfang River, Taipei, Taiwan
OPPAT2	<i>Opsariichthys pachycephalus</i>	Shihu River, Taipei, Taiwan
ZAPLJ1	<i>Zacco platypus</i>	Kyoto, Honshu, Japan
ZAPLJ2	<i>Zacco platypus</i>	Kagoshima, Japan
APKIT1	<i>Aphyocypris kikuchii</i>	Hualian River, Hualian, Taiwan
NINOC1	<i>Nicholsicypris normalis</i>	Hongkong, China

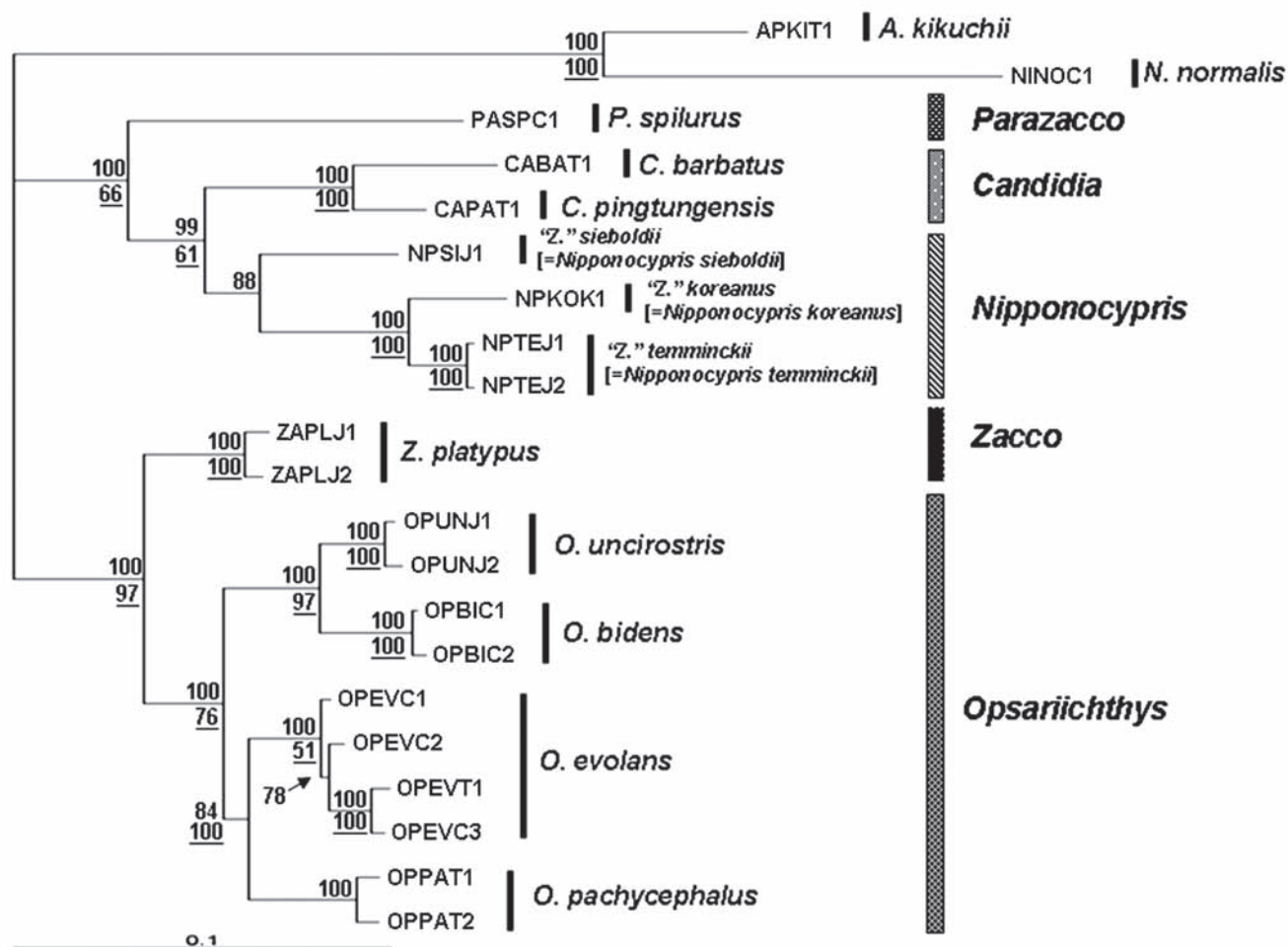


Fig. 1. Phylogenetic tree reconstructed by Bayesian Inference method based on complete mtDNA D-loop sequences of *Opsariichthys* and *Candidia* genus groups from Eastern Asiatic waters. The posterior probability listed above the branch. The consensus tree of MP analysis showing the similar topology as above listed with the values of bootstrap support (> 50) of 1,000 replications shown below the branch with underline.

*kikuchii* (Oshima, 1919) and *Nicholsicypris normalis* (Nichols & Pope, 1927). The clade I is *Opsariichthys-Zacco* group, the basal group is *Zacco platypus*. The clade II is *Parazacco-Candidia* group, the basal group is *Parazacco spilurus* (Günther, 1868) (Fig. 2). Both clades are supported with high bootstrap support and posterior probability. Under the molecular support, the two Japanese minnows as both so-called “*Zacco*” *temminckii* and “*Zacco*” *sieboldii* and one Korean minnow “*Zacco*” *koreanus* have never been grouped into clade I (with the type species of *Zacco*: *Z. platypus*) by any of clustering methods. In contrast, the group including “*Zacco*” *koreanus*, “*Zacco*” *temminckii*, and “*Zacco*” *sieboldii*, in both methods of phylogenetic analysis, group with the sister group which includes two supposedly valid species of *Candidia* from Taiwan. Based on the molecular evidence, these five different stripe minnows (all sharing the longitudinal stripes) should form a monophyletic group within the plesiomorphic unit: *Parazacco spilurus* with complete ventral keel to “*Zacco*” *temminckii* complex with no distinct ventral keel’. Therefore, the generic position of the so-called “*Zacco*” *temminckii* complex is reassigned in the following systematic section.

**SYSTEMATICS**

**Key to 3 genera of stripe minnows from China, Taiwan, Korea and Japan:**

- 1. Body very strongly laterally compressed in adult, ventral keel sharply pointed and complete after pelvic fin; anal fin iv, 11–12 ..... *Parazacco* (southern China)
- Body slightly laterally compressed in adult, ventral keel absent on anterior half region from pelvic fin base to anus; anal fin iii, 8–10 ..... 2
- 2. Maxillary barbels present, ventral keel present on posterior half of region from pelvic fin to anus ..... *Candidia* (Taiwan)
- Maxillary barbels absent, ventral keel absent or indistinct ....  
..... *Nipponocypris* new genus (Japan and Korea)

***Candidia* Jordan & Richardson, 1909**

**Key to 2 endemic species of *Candidia* from Taiwan**

- 1. Lateral-line scales usually 53–57 (modally 55); predorsal scales 23–25 (modally 23); scales above lateral line 11–13; scales below lateral-line modally 5; one pair of barbels present; body with almost uniform grayish above a middle longitudinal bluish-black stripe which is irregular and interrupted on anterior half region ..... *Candidia barbatus* (Regan, 1908)



Fig. 2. The specimen photograph of *Parazacco spilurus*, male, NTOUP-2008-10-422, 79.6 mm SL, Dongchung, Daryushan, Hongkong, People's Republic of China.

- Lateral-line scales usually 47–49 (modally 48); predorsal scales 19–21 (modally 20); scales above lateral line 9–10; scales below lateral-line modally 3; more than one pair of barbels present; body with a broad silvery white stripe on dorsal half of lateral above a middle longitudinal deep black stripe ..... *Candidia pingtungensis*, new species

**Diagnosis.** – This genus, *Candidia*, can be distinguished by the following combination of features: dorsal fin rays iii, 7–8; anal fin rays iii, 8–10; lateral-line scales 46–60; scales above lateral-line 9–13; predorsal scales 19–26; pharyngeal teeth 1–2, 3–4, 4–5 to 4–5, 3–4, 1–2; maxillary barbels present with a total of 2–4 barbels on both side of mouth corner; weak and shallow ventral keel present on the posterior half region of ventral mid-line from pelvic fin base to anus; body with a conspicuous longitudinal dark stripe from anterior trunk to caudal fin base.

***Candidia barbatus* (Regan, 1908)  
(Figs. 3a & 4)**

*Opsariichthys barbatus* Regan, 1908: 359 (type locality: Lake Candidius, Nantou County, Taiwan).

*Candidia barbatus* - Jordan & Richardson, 1909: 169.

*Zacco barbatus* - Banarescu, 1968: 309; Tzeng in Shen, 1993: 141.

*Candidia barbatus* - Zhu, 1995: 25; Tzeng, 1986: 69; Wang et al., 1997: 171; Chen & Fang, 1999: 64; Sado & Kimura, 2002: 350; Chen & Chang, 2005: 38.

**Material examined.** – All specimens from Taiwan: NTOUP-2007-07-019, 5 ex., 77.7–138.1 mm SL, Shuanshi River, Taipei County, Coll. J. H. Wu, 1 Jul.2003; NTOUP-2007-07-027, 2 ex., 77.5–109.5 mm SL, Shuanshi River, Taipei County, Coll. J. H. Wu, 31 Mar.2003; NTOUP-2007-07-029, 3 ex., 80.7–88.8 mm

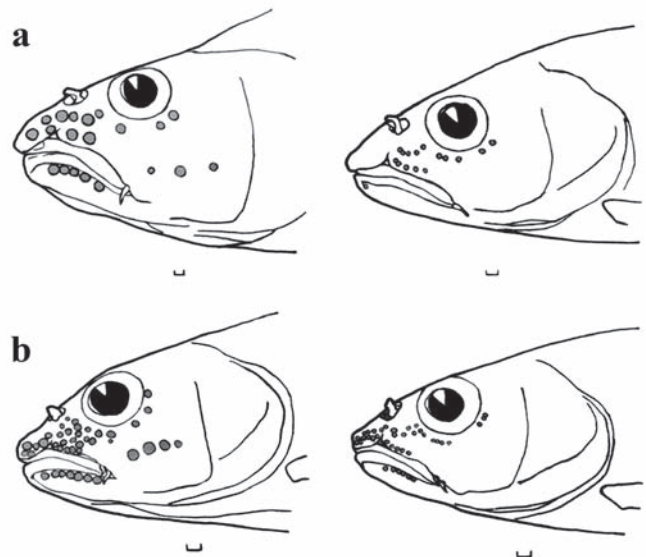


Fig. 3. Head illustrations of two *Candidia* species in Taiwan: a, *Candidia barbatus*: male (left), NTOUP-2007-07-009, 116.0 mm SL, female (right), NTOUP-2007-07-009, 106.4 mm SL, Joshui River, Natou County, Taiwan; b, *Candidia pingtungensis*, new species: male (left), NTOUP-2007-07-010, 77.1 mm SL; female (right), NTOU-2007-07-010, 72.1 mm SL, Szuchung River, Pingtung County, Taiwan. Scale bars = 1 mm.

SL, Shuanshi River, Taipei County, Coll. J. H. Wu, 10 May 2004; NTOUP-2007-07-020, 8 ex., 55.0–85.2 mm SL, Kingsawan River, Taipei County, Coll. Y. M. Ju, 14 Sep.2003; NTOUP-2007-07-030, 2 ex., 71.0–78.4 mm SL, Kingsawan River, Taipei County, Coll. Y. M. Ju, 8 May 2003; NTOUP-2007-07-031, 10 ex., 50.1–87.1 mm SL, Kulinkun River, Taipei County, Coll. J. H. Wu, 7 Aug.2003; NTOUP-2007-07-011, 11 ex., 64.3–91.2 mm SL, Beiszukun River, Taipei County, Coll. J. H. Wu, 11 Aug.2003; NTOUP-2007-07-014, 10 ex., 65.0–96.5 mm SL, Shinghuaten River, Taipei County, Coll. J. H. Wu, 10 Aug.2003; NTOUP-2007-07-032, 2 ex., 76.3–86.1 mm SL, Tanshuei River, Taipei County, Coll. J. H. Wu, 9 Aug.2003; NTOUP-2007-07-033, 5 ex., 81.0–92.3 mm SL, Tanshuei River, Taipei County, Coll. Y. M. Ju, 15 Aug.2003; NTOUP-2007-07-034, 2 ex., 71.1–78.0 mm SL, Tanshuei River, Taipei County, Coll. C. W. Wang, 17 Nov.2004; NTOUP-2007-07-035, 1 ex., 65.6 mm SL, Tanshuei River, Taipei County, Coll. J. H. Wu, 9 Aug.2003; NTOUP-2007-07-015, 10 ex., 75.8–126.9 mm SL, Fongshan River, Hsinchu County, Coll. J. H. Wu, 12 Aug.2003; NTOUP-2007-07-017, 10 ex., 98.0–148.8 mm SL, Chonkang River, Miaoli County, Coll. J. H. Wu, 12 Aug.2003; NTOUP-2007-07-022, 7 ex., 68.5–98.0 mm SL, Holong River, Miaoli County, Coll. C. W. Wang, 27 Dec.2003; NTOUP-2007-07-036, 1 ex., 66.1 mm SL, Holong River, Miaoli County, Coll. J. H. Wu, 18 Nov.2004; NTOUP-2007-07-037, 2 ex., 66.3–73.4 mm SL, Holong River, Miaoli County, Coll. J. H. Wu, 19 Sep.2003; NTOUP-2007-07-013, 10 ex., 71.7–118.7 mm SL, Taan River, Miaoli County, Coll. J.H. Wu, 19 Sep.2003; NTOUP-2007-07-018, 7 ex., 84.9–121.4 mm SL, Tajar River, Taichung County, Coll. J. H. Wu, 18 Sep.2003; NTOUP-2007-07-025, 3 ex., 73.4–118.3 mm SL, Tajar River, Taichung County, Coll. J. H. Wu, 15 Mar.2003; NTOUP-2007-07-023, 4 ex., 87.0–134.4 mm SL, Wu River, Taichung County, Coll. J. H. Wu, 15 Aug.2003; NTOUP-2007-07-038, 1 ex., 85.5 mm SL, Wu River, Taichung County, Coll. Y. M. Ju, 13 Feb.2003; NTOUP-2007-07-039, 3 ex., 70.5–89.6 mm SL, Wu River, Taichung County, Coll. C. W. Wang, 18 Sep.2003; NTOUP-2007-07-012, 2 ex., 70.5–71.6 mm SL, Wu River, Taichung County, Coll. J. H. Wu, 16 Oct.2003; NTOUP-2007-07-009, 8 ex., 74.2–104.6 mm SL, Joshui River, Nantou County, Coll. J. H. Wu, 18 Sep.2003; NTOUP-2007-07-040, 2 ex., 66.8–76.6 mm SL, Joshui River, Nantou County, Coll. C. W. Wang, 14 Feb.2003; NTOUP-2007-07-028, 1 ex., 128.3 mm SL, Parchan River, Chiayi County, Coll. Y.M. Ju, 15 Sept.2001; NTOUP-2007-07-041, 2 ex., 62.2–89.6 mm SL, Parchan River, Chiayi County, Coll. J. H. Wu, 15 Feb.2003;



Fig. 4. The specimen photographs of *Candidia barbatus*: a, male, NTOUP-2008-10-420, 108.6 mm SL, Yurui Stream, Keelung River, Keelung City, Taiwan; b, female, NTOUP-2008-10-420, 103.7 mm SL, the locality same as above.

NTOUP-2007-07-042, 2 ex., 51.3–86.1 mm SL, Parchan River, Chiayi County, Coll. J. H. Wu, 27 Mar.2003; NTOUP-2007-07-016, 10 ex., 77.4–109.2 mm SL, Huwei River, Yunlin County, Coll. J. H. Wu, 1 Apr.2003; NTOUP-2007-07-026, 3 ex., 86.1–113.3 mm SL, Tzengwen River, Tainan County, Coll. J.H. Wu, 28 Mar.2003; NTOUP-2007-07-043, 1 ex., 85.6 mm SL, Tzengwen River, Tainan County, Coll. C. W. Wang, 10 Apr.2003; NTOUP-2007-07-044, 1 ex., 67.9 mm SL, Tzengwen River, Tainan County, Coll. C. W. Wang, 15 Feb.2003; NTOUP-2007-07-045, 5 ex., 56.7–60.9 mm SL, Tzengwen River, Tainan County, Coll. J. H. Wu, 1 Apr.2003; NTOUP-2007-07-046, 5 ex., 83.9–103.9 mm SL, Kaoping River, Pingtung County, Coll. J.H. Wu, 13 Nov.2003; NTOUP-2007-07-047, 3 ex., 70.5–96.3 mm SL, Kaoping River, Pingtung County, Coll. Y. M. Ju, 18 Dec.2003; NTOUP-2007-07-048, 2 ex., 70.0–77.4 mm SL, Kaoping River, Pingtung County, Coll. C. W. Wang, 13 Nov.2003; NTOUP-2008-10-420, 2 ex., 103.7–108.6 mm SL, Yu-Rui Stream, Keelung River, Tanshuei River basin, Keelung City, Coll. J. H. Huang, 20 Oct.2008.

**Diagnosis.** – *Candidia barbatus* can be distinguished from another congeneric species by unique combination of following features: dorsal fin rays iii, 7; anal fin rays iii,

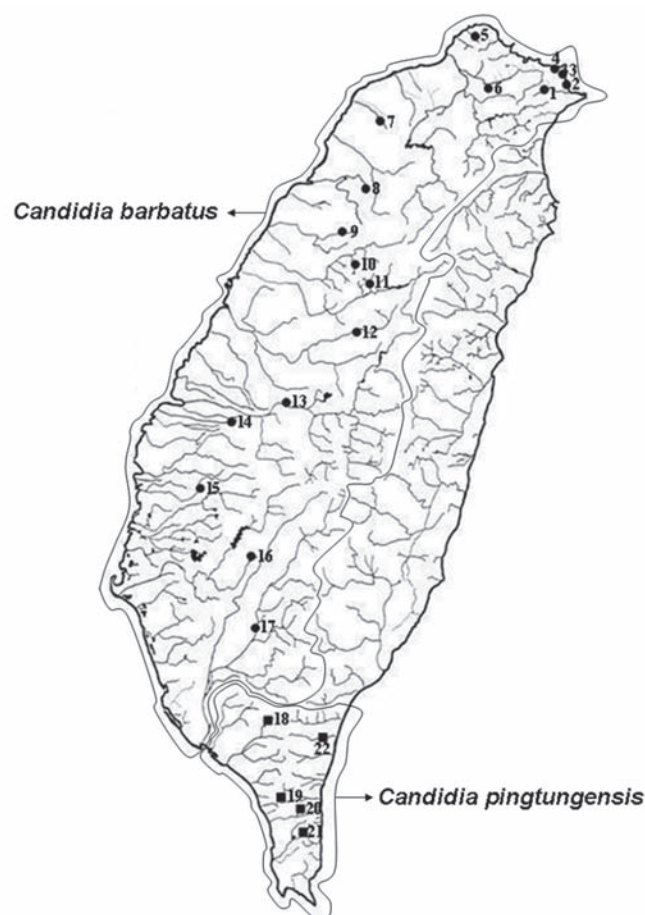


Fig. 5. The sampling localities of two species of *Candidia* in Taiwan. The upper circle indicates the distribution range of *Candidia barbatus*, and the lower square mark indicates the distribution range of *Candidia pingtungensis*, new species. The numbers represent the names of localities as follows: 1, Shuanshi R.; 2, Kingsawan R.; 3, Beiszukun R.; 4, Kulinkun R.; 5, Shinghuaten R.; 6, Tanshuei R.; 7, Fongshan R.; 8, Chonkang R.; 9, Holong R.; 10, Taan R.; 11, Tajar R.; 12, Wu R.; 13, Joshui R.; 14, Huwei R.; 15, Parchan R.; 16, Tzengwen R.; 17, Kaoping R.; 18, Linbian R.; 19, Fansan R.; 20, Fongkan R.; 21, Szuchung R. and 22, Jinlun R.

9–10; pectoral fin rays I, 13–15 (modally 14); lateral-line scales 51–60 (usually 53–57 and modally 55); scales above lateral-line 11–13 (modally 12); scales below lateral-line 4–6 (modally 5); predorsal scales 22–26 (modally 23); gill rakers 2–3 + 6–7 (total 9–10); pharyngeal teeth usually 1, 4, 5 – 4, 4, 1; shallow ventral keel on the posterior half region between pelvic fin and anus; a series of 4–6 nuptial tubercles on cheek and 3–5 nuptial tubercles on lower jaw in adult male; eye small, cheek depth about 1.5–2.0 times of eye diameter in adult male; body with a broad, longitudinal bluish-black stripe from upper opercle to caudal fin base which is irregular and interrupted on anterior half region and forming a large round black mark on caudal fin base; dorsal half of lateral usually uniform dark grayish with very narrow grayish region above bluish-black stripe; ventral half with some blackish brown small spots; and eye with semicircular red-black region on the dorsal one-third to half of eye iris.

**Distribution.** – This species occurs in most northern river drainages of Taiwan, along basins of western slope from the Central Mountain Ridge and southwards to the Kaoping River (Fig. 5).

***Candidia pingtungensis*, new species  
(Figs. 3b & 6)**

*Candidia* sp. Chen & Chang, 2005: 44 (southern Taiwan).

**Material examined.** – All specimens from Taiwan: **Holotype.** NTOUP-2007-07-21-1, 79.8 mm SL, Fongkang River, Pingtung County, Coll. J. H. Wu, 19 Jul.2003. **Paratypes.** NTOUP-2007-07-021-2, 11 ex., 48.7–65.4 mm SL, NTOUP-2007-07-024, 11 ex., 49.5–94.9 mm SL, other data same as holotype; NTOUP-2007-07-010, 26 ex., 50.6–114.3 mm SL, Szuchung River, Pingtung County, Coll. J. H. Wu, 19 Jul.2003; NTOUP-2007-07-049, 5 ex., 46.5–64.8 mm SL, Linbien River, Pingtung County, Coll. J. H. Wu, 5 Jul.2003; NTOUP-2007-07-050, 10 ex., 47.0–64.3 mm SL, Fansang River, Pingtung County, Coll. J. H. Wu, 25 Nov.2003; NTOUP-2007-07-051, 1 ex., 67.7 mm SL, Fongkang River, Pingtung County, Coll. J. H. Wu, 5 Apr.2003; NTOUP-2007-07-052, 3 ex.,

55.2–79.1 mm SL, Kinlun River, Taitung County, Coll. Y. M. Ju, 21 May 2003.

**Diagnosis.** – *Candidia pingtungensis* can be distinguished from another congeneric species by the following unique combination characters: dorsal fin rays iii, 7; anal fin rays iii, 9–10 (modally 9); pectoral fin rays I, 14; lateral-line scales 46–50 (modally 48); scales above lateral-line 9–10 (modally 10); scales below lateral-line 3–4 (modally 3); predorsal scales 19–21 (modally 20); gill rakers 2 + 6 (total 8); pharyngeal teeth always 1, 3, 5 – 4, 4, 1; shallow ventral keel on the posterior half of region between pelvic fin and anus; a series of 3–5 nuptial tubercles on cheek and 5–7 nuptial tubercles on lower jaw in adult male; eye large, eye diameter about equal to cheek depth in adult male; body with an uniform broad, longitudinal black stripe from upper opercle, through mid-lateral to caudal fin base; dorsal side dark grayish, a broad silvery white stripe just above the longitudinal black stripe; ventral side silvery-white; dorsal fin pinkish-red with distal transverse black bars in male; and eye with red-black region on dorsal two-third of eye iris.

**Description.** – Dorsal fin rays iii, 7; anal fin rays iii, 9–10 (modally 9); pectoral fin rays I, 14; pelvic fin rays I, 8–9 (modally 8) (frequency distribution listed in Table 2). Lateral-line scales 46–50 (modally 48); scales above lateral-line 9–10 (modally 10); scales below lateral-line 3–4 (modally 3); predorsal scales 19–21 (modally 20); scales surrounding caudal peduncle 7–8 (modally 8); vertebral count 4 + 40–42 (modally 41) (frequency distribution listed in Table 3); gill-rakers 2 + 6; and pharyngeal teeth 1, 3–4 (modally 3), 3–5 (modally 5) – 4–5 (modally 4), 3–4 (modally 4), 1.

Body elongate and compressed laterally, belly rounded, from pelvic to anal fin with shallow ventral keel located on the posterior half of region in front of anus. Mouth slightly rounded. Maxillary oblique and extended to vertical of anterior margin of pupil. A dermal notch between nostrils and upper jaw which is more distinct in males. Upper jaw bent upwards especially in male. Maxillary barbels 3–4 which usually as 2 + 2 (left + right side) in adult with some of smaller young ones even lacking one barbel as either 1 + 2 or 2 + 1. Eye moderately large, dorso-lateral in position. Eye diameter about equal to cheek depth in adult male. Inter-orbital region larger than snout length. Body with moderately small cycloid scales which are larger posteriorly. Lateral line complete and running downward abruptly above pectoral fin and along the ventral profile into middle of caudal fin base. Origin of dorsal fin inserted around middle. Pelvic fin rounded, mature male with large triangular-shaped anal fin which extends beyond vertical of caudal fin base; but female with small anal fin never extending to vertical of caudal fin base. Caudal fin forked, lower lobe slightly longer than upper lobe. A series of conspicuous, sharply conical tubercles along opercle, pre-opercle, snout and present on both upper and lower jaws and with 6–7 large tubercles below lower jaw in adult male; while smaller tubercles are present in adult female.

**Colouration in life.** – Body with a broad, longitudinal black



Fig. 6. The specimen photographs of *Candidia pingtungensis*, new species: a, male, NTOUP-2007-07-021-1, holotype, 79.8 mm SL, Fongkang River, Pingtung County, Taiwan; b, female, NTOUP-2007-07-010, paratype, 94.8 mm SL, the locality same as above.

Table 2. Frequency distribution of fin-ray counts of two *Candidia* species in Taiwan.

Species	D iii			A iii				P1 i				P2 i		
	7	8	M	8	9	10	M	13	14	15	M	8	9	M
<i>Candidia barbatus</i>	170	1	7.0	12	152	7	9.0	17	173	48	14.1	147	88	8.4
<i>Candidia pingtungensis</i>	68	–	7.0	–	64	4	9.1	–	41	5	14.1	33	12	8.3

Table 3. Frequency distribution of scale and vertebral counts of two *Candidia* species in Taiwan.

Species name	LL															
	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	M
<i>Candidia barbatus</i>	–	–	–	–	–	3	20	30	33	38	33	26	15	13	7	55.1
<i>Candidia pingtungensis</i>	6	10	12	9	3	–	–	–	–	–	–	–	–	–	–	48.4

Species name	LLa						LLb				
	9	10	11	12	13	M	3	4	5	6	M
<i>Candidia barbatus</i>	–	–	46	56	15	11.6	–	49	54	4	4.3
<i>Candidia pingtungensis</i>	6	18	–	–	–	9.8	14	9	–	–	3.3

Species name	PreD										CPS				
	19	20	21	22	23	24	25	26	M	7	8	9	10	11	M
<i>Candidia barbatus</i>	–	–	–	2	37	30	20	10	24.0	–	–	10	59	47	10.3
<i>Candidia pingtungensis</i>	4	13	6	–	–	–	–	–	20.1	2	16	–	–	–	7.9

Species name	VC			
	40	41	42	M
<i>Candidia barbatus</i>	34	98	35	41.0
<i>Candidia pingtungensis</i>	10	47	9	41.0

M = Average

stripe which starts from postorbital region, extending through upper opercle, through mid-lateral to caudal fin base. Dorsum dark grayish, a rather broad silvery white band just above the longitudinal black stripe. Ventral side silvery white. Postorbital region light yellow in dorsal half and reddish orange in ventral half. Bright red seen on ventral side of both pectoral, pelvic fins in male, and similar colouration pattern in female but of a lighter red, pelvic fin white. Dorsal fin membrane pinkish-red with distal transverse black bars in male. Pectoral fin orange. Anal and caudal fins pale white to light yellow. Eye with red-black region on dorsal two-third of iris.

**Colouration in preserved specimens.** – Dorsal region grayish brown, belly light yellow. A longitudinal pale white stripe above the black stripe. Opercle pale white. Dorsal fin with only black transverse bars remaining. All orange, red or silvery-white colouration decolourised.

**Distribution.** – The new species occurs from the Tongkang River southwards to all western drainages of Hengchuen

Peninsula and also to the eastern drainage of the Kongkou River and most of Pingtung County (Fig. 5).

**Habitat preference.** – This new species is found in anterior region of pool and edge-water habitats up to 2 m depth with the benthic substratum of small pebbles to large boulders under slow to moderate water flow, in small and very clear tributaries and headwaters with better shaded vegetation along the stream bank and the pH value of water is 7.5–8.8.

**Etymology.** – This species is named after the “County of Pingtung”, in southern Taiwan.

**Remarks.** – *Candidia pingtungensis* (Figs. 3b & 6) can be distinguished from its closest congener, *Candidia barbatus* (Figs. 3a & 4) in Taiwan by the following features: (1) a broad silvery white stripe on dorso-lateral side (vs. absence of such a broad stripe on dorso-lateral body); (2) less lateral-line scales 46–50 which modally 48 (vs. 51–60 which modally 55); (3) less predorsal scales modally 20 (vs. 23); (4) less scales below lateral-line modally 3 (vs. 5); and (5) 3–4 barbels in adult (vs. 2).

Table 4. Morphometry of two *Candidia* species in Taiwan.

Species	<i>Candidia barbatus</i>						<i>Candidia pingtungensis</i> , new species						
	male			female			holotype	male			female		
Sex	45			76			1	8			16		
N													
Standard length (mm)	69.8~148.9			64.3~138.1			79.8	67.7~106.6			64.3~94.9		
% in SL	min	max	mean	min	max	mean		min	max	mean	min	max	mean
Head length	26.3	33.0	29.5	26.8	33.5	30.0	30.8	27.1	30.8	29.2	27.9	32.3	29.6
Body depth	24.0	30.2	26.4	23.6	30.4	26.5	29.1	27.3	29.1	28.2	25.1	29.8	26.9
Body width in dorsal origin	12.0	19.2	15.9	12.4	19.6	15.6	17.8	15.6	18.8	17.1	13.0	24.6	16.8
Body width in anal origin	8.6	14.9	11.8	8.3	13.7	11.1	13.6	11.4	14.7	13.0	8.6	13.2	11.1
Caudal peduncle depth	10.1	12.8	11.3	10.2	13.5	11.7	11.7	10.5	12.0	11.5	10.6	12.3	11.2
Caudal peduncle length	13.3	22.2	19.3	15.4	21.3	19.2	20.8	19.0	20.9	20.3	18.2	21.0	19.3
Predorsal length	49.8	54.6	52.6	50.5	56.2	53.5	52.3	51.0	52.9	51.8	51.1	54.7	53.1
Preanal fin length	65.7	72.4	68.7	67.6	74.9	70.5	68.9	66.9	68.9	67.8	67.9	71.4	70.1
Distance of snout to anus	64.4	71.3	67.8	65.5	72.6	69.5	68.5	66.0	68.5	67.1	67.0	70.6	69.3
Prepelvic length	47.3	53.4	50.2	47.9	53.5	51.0	50.6	48.6	50.8	49.7	48.5	52.2	50.5
Pelvic fin ray length	14.4	18.8	15.9	13.2	17.6	15.0	16.4	15.5	18.9	16.9	14.1	16.7	15.3
Pectoral fin ray length	17.6	21.8	20.2	17.9	21.9	19.6	21.8	19.7	22.3	21.3	19.1	22.5	20.8
Upper lobe of caudal fin	18.0	22.2	20.2	17.9	22.9	20.4	16.7	16.7	21.3	19.6	20.2	24.8	21.7
Anal fin ray length	19.7	29.9	25.1	17.6	29.2	23.0	24.7	20.0	30.1	25.9	19.6	27.4	22.8
Dorsal fin base length	11.0	14.2	12.5	9.7	13.0	11.4	11.8	11.8	14.3	12.8	9.9	12.3	11.6
Anal fin base length	11.6	16.8	13.7	9.7	14.6	12.3	13.6	13.5	15.5	14.5	11.5	14.0	12.6
% in HL													
Snout length	28.3	35.0	31.4	26.7	37.6	30.7	30.5	29.6	33.2	31.5	27.4	32.9	30.7
Interorbital width	32.4	40.8	36.9	24.5	39.2	36.1	37.6	37.0	40.4	38.9	34.8	40.0	38.0
Eye diameter*	16.2	25.5	20.7	17.1	29.0	22.7	20.4	20.4	25.3	22.2	22.1	28.1	24.7
Snout to rear skull	65.1	77.8	69.4	62.4	76.2	69.1	68.9	68.9	74.7	71.5	65.4	75.2	71.5
Head width	47.6	61.4	55.0	47.7	60.6	54.6	58.3	55.4	58.6	57.2	48.3	60.3	56.1
Head width in nasal section	31.1	44.0	36.1	31.0	41.9	35.2	35.4	33.9	39.1	36.5	32.5	40.6	36.2
Head depth	63.3	76.0	67.7	59.5	74.0	67.0	68.0	65.6	71.6	68.4	37.8	69.2	64.8
Upper jaw length	37.2	42.0	39.1	35.6	42.9	39.6	38.3	35.9	40.3	38.8	36.8	42.6	40.3
Snout to rear preopercle	16.1	20.7	18.2	14.1	20.6	18.0	18.3	16.7	19.3	17.9	16.2	19.9	17.7
Cheek depth	19.1	28.1	23.5	17.2	30.7	22.5	23.3	19.7	23.3	21.7	19.0	23.2	21.6

***Nipponocypris*, new genus**

(Fig. 7)

**Type species.** – *Leuciscus temminckii* Temminck & Schlegel, 1846.

**Materials examined.** – *Nipponocypris temminckii* (Temminck & Schlegel, 1846): NMMBP- 06230, 2 ex., 86.3–103.2 mm SL, the small rivers of the Biwa Lake, Japan, Coll. I-S. Chen, 17 May 2002; NMNS01388, 2 ex., 96.2–112.8 mm SL, Hino, Shiga, Japan, Coll. K. Watanabe, D. Takahashi, T. Komiya and N. H. Jang-Liaw, 16 Oct.2005; NTOUP-2008-10-423, 3 ex., 38.4–39.3 mm SL, a



Fig. 7. The specimen photograph of *Nipponocypris temminckii*, female, NMNS01388, 112.8 mm SL, Hino, Shiga, Japan. Photograph by Nian-Hong Jang-Liaw.

small tributary of South to Kohung, Choluham-Do, Korea, Coll. S. P. Huang, 26 Oct.2008; NTOUP-2008-10-424, 21 ex., 40.8–60.5 mm SL, main river in Kohung, Choluham-Do, Korea, Coll. S. P. Huang, 27 Oct.2008. ***Nipponocypris sieboldii* (Temminck & Schlegel, 1846):** NMNS01393, 2 ex., 92.4–99.4 mm SL, Kasatsu, Shiga, Japan, Coll. K. Watanabe, D. Takahashi, T. Komiya & N. H. Jang-Liaw, 16 Oct.2005; NMNS01402, 1 ex., 67.9 mm SL, Yogo river, Shiga, Japan, Coll. K. Watanabe, T. Komiya & N. H. Jang-Liaw, 15 Oct 2005; NMNS01450, 13 ex., 38.8–92.3 mm SL, Hino, Shiga, Japan, Coll. K. Watanabe, D. Takahashi, T. Komiya & N. H. Jang-Liaw, 16 Oct.2005. ***Nipponocypris koreanus* (Kim, Oh & Hosoya, 2005):** NTOUP-2008-10-425, 4 ex., 16.7–76.2 mm SL, small tributary near Pogyonga Temple, Gwancheon River, Kyong Sangbuk-Do, Korea, Coll. S. P. Huang, 25 Oct.2008.

**Diagnosis.** – The new genus can be distinguished from other Cyprinid genera by the following unique combination of characters: dorsal fin rays iii, 7–9; anal fin rays iii, 8–10; lateral-line scales 43–60; scales above lateral-line 9–13; predorsal scales 20–26; pharyngeal teeth 1–2, 3–4, 5-5, 2–4, 1–2; absence of maxillary barbels; ventral keel completely absent; and body with a distinct longitudinal bluish to grayish black stripe.

**Etymology.** – The new generic name, *Nipponocypris*, is



referred to the type species described from freshwaters of Japan (Nippon).

**Distribution.** – The three nominal species of *Nipponocypris* are all endemic to the main islands of Japan and Korean peninsula.

#### Key to three species of *Nipponocypris* from Japan and Korea:

1. Anal fin rays iii, 10; lateral line scales not less than 54; scales above lateral line not less than 13 .....  
..... *Nipponocypris sieboldii* (Temminck & Schlegel, 1846)
- Anal fin rays iii, 9; lateral line scales not more than 53; scales above lateral line not less than 12 ..... 2
2. Scales above lateral-line 11–12; conspicuous red marking on upper margin of the eyes; antero-lateral part of body red and anterior margin of pectoral fin yellow in male .....  
.... *Nipponocypris temminckii* (Temminck & Schlegel, 1846)
- Scales above lateral line 9–10; absence of red on upper margin of the eyes; antero-lateral part of body yellow and anterior margin of pectoral fin red in male .....  
..... *Nipponocypris koreanus* (Kim, Oh & Hosoya, 2005)

#### DISCUSSION

The generic status for the genus *Zacco* assigned from the type species — *Zacco platypus* (Temminck & Schlegel, 1846) originally by Jordan & Evermann, 1902 has been included and maintained for another three members of so-called “*Zacco*” *temminckii*, “*Zacco*” *sieboldii*, and even “*Zacco*” *koreanus* and some Chinese species by Japanese and Chinese ichthyologists (Chen, 1998; Hosoya et al., 2003; Kawanabe et al., 2003; Kim et al., 2005). It is plausible to re-assign into the member of *Candidia* genus group rather than traditional classification to “*Zacco*” based on current phylogenetic evidence of mtDNA D-loop sequences and previous work from mtDNA Cyt B gene sequences (Wang et al., 2007). It is improper to clump “*Zacco*” *temminckii* complex simply under the ‘typical *Zacco*’. In our molecular survey and that of Wang et al. (2007), all molecular evidence provided by different mitogenetic markers seems to indicate the stable and close relationship between both “*Zacco*” *temminckii* complex and its sister group — Taiwanese *Candidia* species. However, we consider the generic position for *Parazacco* to differ in comparison with the Cyt B gene phylogeny of Wang et al. (2007). Wang et al. (2007) did not show the two very conspicuous paraphyletic clades: i.e. clade I: the *Opsariichthys-Zacco* group and clade II: the *Parazacco-Candidia* group. The current phylogenetic insight allowed by the D-loop sequences seems to be more congruent with our conclusions for monophyly of *Candidia* genus group (*Candidia*, *Parazacco* & *Nipponocypris*) based on sharing their common plesiomorphic feature as representing the longitudinal deep black to bluish black stripe on lateral body with a series of varied degree of ventral keel reduction for different genera. While the more-informative sites of highly divergent D-loop sequences can be used for detailed analysis of the current group of OTUs, further molecular surveys

using different genetic markers are desirable. They may provide better phylogenetic resolution for very complicated evolutionary histories such as the case of *Opsariichthys* genus complex in East Asian river basins.

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