Zoological Studies **51**(2): 222-231 (2012)

Zoological Studies

Systematic Study of the *Simocephalus* Sensu Stricto Species Group (Cladocera: Daphniidae) from Taiwan by Morphometric and Molecular Analyses

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(Accepted September 9, 2011)

Shuh-Sen Young, Mei-Hui Ni, and Ming-Yun Liu (2012) Systematic study of the *Simocephalus* sensu stricto species group (Cladocera: Daphniidae) from Taiwan by morphometric and molecular analyses. *Zoological Studies* **51**(2): 222-231. There is some controversy regarding the traditional taxonomy of the *Simocephalus* sensu stricto species group. We conducted molecular and morphometric analyses to differentiate the 3 species from this group found in Taiwan: *S. vetulus* (O.F. Müller, 1776), *S. vetuloides* Sars, 1898, and *S. mixtus* Sars, 1903. The landmark method was employed, followed by a transfer into 24 characteristic values for a principal component analysis (PCA), the results of which indicated morphometric overlap among these species. The dorsal angle, brood size, and body length were smallest in *S. vetulus*, medium in *S. vetuloides*, and largest in *S. mixtus*. In the *Simocephalus* sensu stricto group from Taiwan, the dorsal angle and body length were significantly correlated with brood size in a quadratic manner. In the molecular analysis, 98 specimens of *Simocephalus* were used, and the 641-bp mitochondrial DNA cytochrome oxidase subunit 1 sequence was employed as a marker to analyze the genetics of *S. vetulus*, *S. vetuloides*, *S. mixtus*, *S. serrulatus* (Koch, 1841), and *S. heilongjiangensis* Shi and Shi, 1994. *Simocephalus vetulus*, *S. vetuloides*, and *S. mixtus* shared several haplotypes, and the interspecific genetic distance was merely 0.00671-0.00785, which is within the range of intraspecific differences. We concluded that *S. vetulus*, *S. vetuloides*, and *S. mixtus* in Taiwan belong to the same species and should be treated as *S.* cf. *vetulus*. The number of species of *Simocephalus* in Taiwan is thus reduced to 3: *S.* cf. *vetulus*, *S. serrulatus*, and *S. heilongjiangensis*.
http://zoolstud.sinica.edu.tw/Journals/51.2/222.pdf

Key words: Systematics, Biodiversity, *Simocephalus*, Freshwater zooplankton.

The general morphologies of *Simocephalus vetulus* (O.F. Müller, 1776), *S. vetuloides* Sars 1898, and *S. mixtus* Sars 1903 are very similar. Sars (1916) first discriminated *S. vetulus* and *S. vetuloides* based on the dorsoposterior valve angle. After that, many authors defined *S. vetuloides* by a more-protruding dorsal valve margin and more-numerous and larger denticles on the posterior dorsal valve margin compared to *S. vetulus* (Uéno 1966, Chiang and Du 1979, Yoon and Kim 1987 2000, Shi and Shi 1996, Kim 1998, Orlova-Bienkowskaja 2001, Tuo 2002). Other authors treated *S. vetuloides* as a local form (Johnson 1953) or as a synonym of *S. vetulus* (Fryer 1957, Harding 1961, Sharma 1978, Negrea 1983, Michael and Sharma 1988). Sars (1903) described *S. mixtus* as having a more-protruding (to the rear) dorsal valve margin and larger denticles on the posterior dorsal valve margin compared to *S. vetulus* and *S. vetuloides*. Flössner (1972) and Negrea (1983) treated *S. mixtus* as a synonym of *S. vetulus*. After that, Orlova-Bienkowskaja (1998)

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made a more-detailed revision and treated *S. mixtus* as a valid species.

Orlova-Bienkowskaja (2001) proposed a different method of discriminating *S. vetulus*, *S. vetuloides*, and *S. mixtus.* She drew an inner circle along the shell posterior, the diameter of which and the prominence of the dorsal valve being key features for identification. The shell posterior of *S. vetulus* ends without an extending shell spine, the inner circle is larger than in *S. vetuloides* and *S. mixtus*, while *S. mixtus* has more-protruding dorsal valves than *S. vetuloides*. The diameter of the inner circle of *S. mixtus* is larger than the prominence portion, and *S. vetuloides* differs from *S. mixtus* in that the diameter of the inner circle of *S. vetuloides* is smaller than the prominence portion.

In the past, many authors proposed *S. vetulus* to be a cosmopolitan species first des-cribed from the Old World, as it was found in many areas, with the exception of New Zealand and Australia (Werestschagin 1923, Uéno 1927, Rylov 1930, Hemsen 1952, Harding 1961, Manuilova 1964, Uéno 1966, Chiang and Du 1979, Rajapaksa and Fernando 1982, Boonsom 1984, Yoon and Kim 1987, Kim 1998, Mizuno and Takahashi 1991, Du 1993, Hann 1995, Shi and Shi 1996, Michael and Sharm 1998, Tuo 2002). Orlova-Bienkowskaja (2001) indicated that the distribution of *S. vetulus* was limited to northern Africa and Europe, while *S. vetuloides* had a limited distribution in eastern Siberia. Outside of Africa, Europe, and eastern Siberia, *Simocephalus* sensu stricto comprises *S. punctatus* Orlova-Bienkowskaja, 1998, *S. elizabethae* (King, 1853), and *S. mixtus*. *Simocephalus mixtus* is a cosmopolitan species distributed in Asia, Eastern Europe, North Africa, and North America. *Simocephalus* (*Coronocephalus*) *serrulatus* (Koch, 1841) is also regarded as a cosmopolitan species, as it is distributed in Asia, Europe, Africa, North America, South America, and Australia (Orlova-Bienkowskaja 2001).

Based on the description by Orlova-Bienkowskaja (2001) and other morphological comparisons, Tuo (2002) described 3 species of *Simocephalus* from Taiwan, *S. serrulatus*, *S. vetulus*, and *S. vetuloides*. Since then, this extensive collection has increased to include *S. heilongjiangensis* Shi and Shi, 1994 and *S. mixtus* Sars from southern Taiwan (Ni 2005). At some collection sites, *S. vetulus* and *S. vetuloides* were found simultaneously as were *S. vetuloides* and *S. mixtus* (Ni 2005). Morphological similarities among *S. vetulus*, *S. vetuloides*, and *S. mixtus* are large, with the exception of the shape of the dorsal valve. However, the shape of the dorsal valve of cladocerans may be affected by the brooding status, with growing embryos pushing the valve more prominently outwards, than in individuals without eggs.

The species level is recognized as the basic unit of biodiversity (Mayer and Ashlock 1991). Nowadays, alpha taxonomy is still based mainly on morphology. Morphometry is one of several possible methods to determine species and analyze morphological differences between closely related species and populations (Chen et al. 2010). With the advent of molecular technology for DNA sequencing, morphologically cryptic species have been increasingly revealed, and the use of DNA markers as a new tool to overcome morphological impediments was suggested (Tautz et al. 2003). The ideal DNA-based identification system (DNA barcodes) would employ a single gene, and be suitable for any organism in the taxonomic hierarchy. Folmer et al. (1994) designed a universal primer for the mitochondrial cytochrome oxidase subunit I (COI) gene, which subsequently became a popular marker to study invertebrates. Hebert et al. (2003), Tautz et al. (2003), Blaxter (2004), Lefébure et al. (2006), and Costa et al. (2007) suggested that the COI gene appears to be an appropriate molecular marker (as a DNA barcode) on several taxonomic scales, but particularly at the species level. We attempted to clarify the taxonomic status of *S. vetulus*, *S. vetuloides*, and *S. mixtus* in Taiwan by morphometric comparisons and used the mitochondrial (mt)DNA COI gene marker as a new character.

This paper is our 1st step dealing with *vetulus*-like populations of *Simocephalus* in Taiwan, which are currently regarded as conspecific to the Palaearctic cosmopolitan species. We thus attempted to improve the taxonomy of the genus *Simocephalus* by solving a small piece of the puzzle from the overall picture.

MATERIALS AND METHODS

Samples were taken from many temporary freshwater bodies throughout Taiwan using a plankton net. Each sample was fixed in 70% ethanol (EtOH), later preserved in 95% EtOH and stored at a low temperature (< -20°C). Within 72 h, each raw sample was sorted and identified under a stereomicroscope. In total, 187 individuals (170

with eggs) were collected in 2003 and 2004 and used for the morphometric analysis: 45 individuals of *S. vetulus* from 8 sites, 72 individuals of *S. vetuloides* from 11 sites, and 70 individuals of *S. mixtus* from 10 sites. From this set of 187 individuals, 72 individuals, including 22 individuals of *S. vetulus* from 8 sites, 28 individuals of *S. vetuloides* from 11 sites, and 22 individuals of *S. mixtus* from 10 sites, were selected for the DNA analysis. Additionally, 7 individuals of *S. serrulatus* (Fig. 1) from 3 sites and 19 individuals of *S. heilongjiangensis* (Fig. 1) from 5 sites were also included in the DNA analysis. *Daphnia similoides* Hudec, 1991 (Daphniidae) and *Diaphanosoma dubium* Manuilova, 1964 (Sididae) from Taiwan were analyzed in order to obtain outgroup sequences.

Morphometric analysis

Lateral-view images of *S. vetulus*, *S. vetuloides*, and *S. mixtus* were taken using a digital camera under a stereomicroscope for the morphometric study. Morphometric characters were extracted from the photographic images, and 8 morphometric data points were used to construct

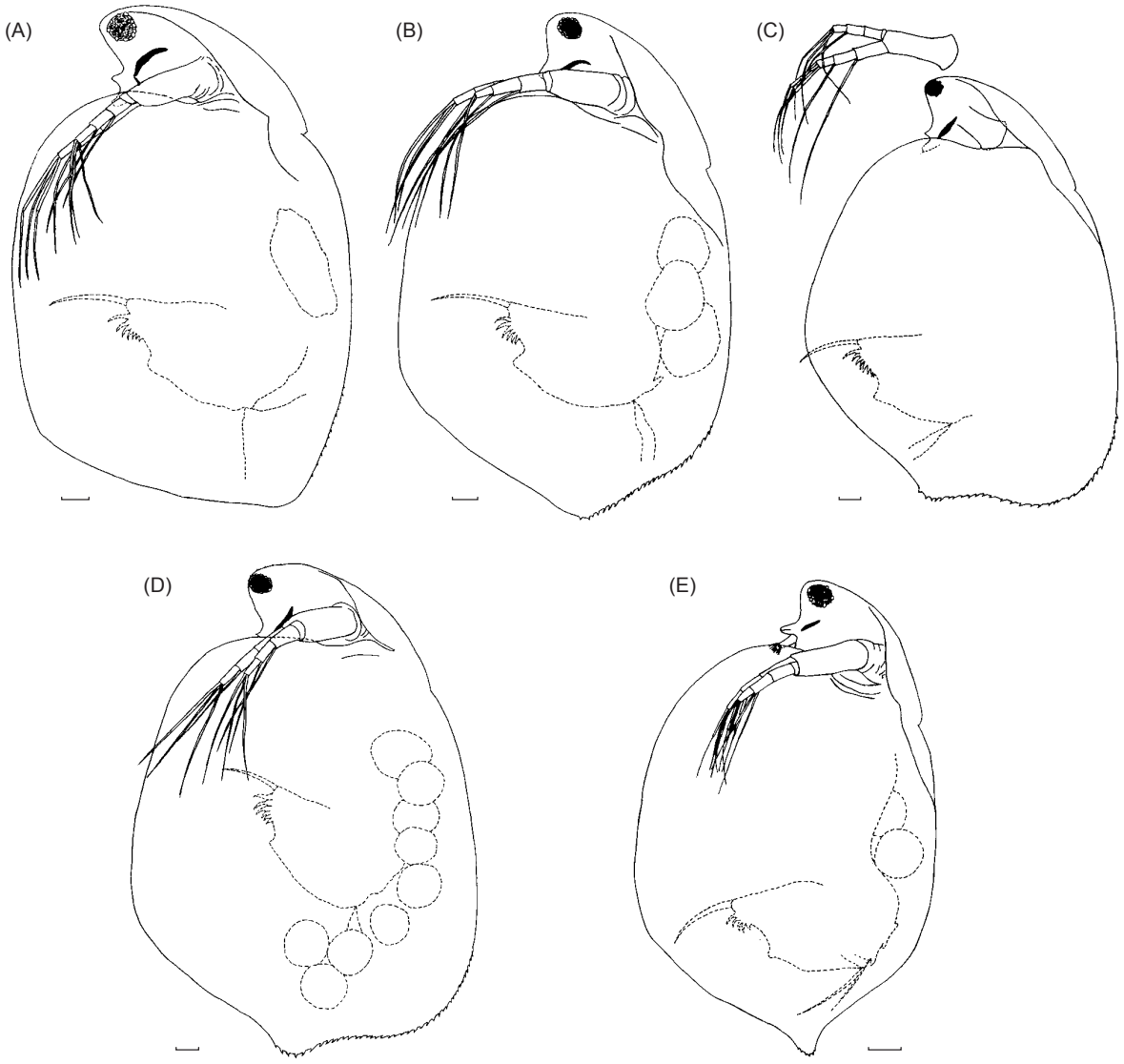

Fig. 1. General morphology of female *Simocephalus* with summer eggs found in Taiwan (all drawings are original). (A) *S. vetulus*; (B) *S. vetuloides*; (C) *S. mixtus*; (D) *S. serrulatus*; (E) *S. heilongjiangensis*. The valve shape is the major difference among *S. vetulus*, *S. vetuloides*, and *S. mixtus*; *S. serrulatus* has teeth on the top of its head, and *S. heilongjiangensis* has a different posterior end of the valve. Scale bars = 0.1 mm.

24 length measurements, each of which was divided by body length to obtain size-free ratios (Fig. 2). The body length and dorsal valve angle (Fig. 2) were also measured on the photographic images, and the clutch size of each individual was assessed under a microscope. SPSS vers. 10.0.1 (Chicago, IL, USA) was used to analyze the numerical data. The data matrix was tested using the Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy and by the Bartlett X^2 test prior to the principle component analysis (PCA). For individuals with eggs, Pearson's correlation analyses and non-linear regressions among the dorsal angle, body length, and clutch size were carried out.

DNA extraction, amplification, and sequencing

Total genomic DNA was extracted using Chelex (InstaGene Matrix BIO-RAD 7326030, Bio-Rad Laboratories, Hercules USA) from single animals. Each animal was taken from 95% EtOH and placed into pure water for 1 h for cleaning. After that, each animal was placed at the bottom of a 0.5-ml centrifuge tube for 30 min to dry in a speed vacuum-drying system. Dried samples were then ground up by needles, and 50 μl of a 5% Chelex solution was used to extract the DNA by incubation at 56°C for 2-3 h, followed by incubation at 90°C for 8 min. For each polymerase chain reaction (PCR), 5 μl of upper cleaning was used as the DNA template after centrifugation at 10^4 rpm (9168g) for 3 min.

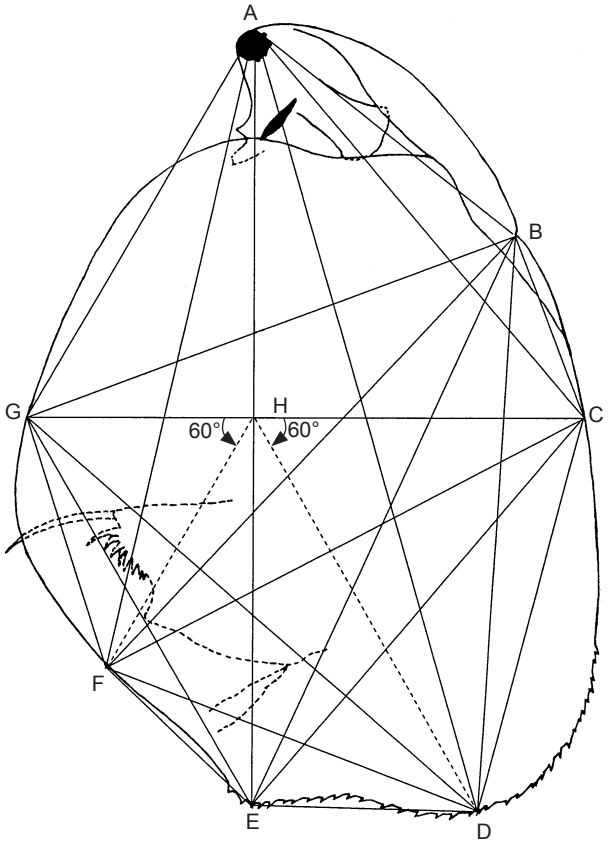

Fig. 2. Morphometry of each specimen extracted from 8 data points (A-H), from which we constructed 24 length measurements; each length measurement was then divided by body length (AE) to obtain size-free ratios. The angle between lines AE and ED was taken as the dorsal valve angle.

We employed the universal primers, LCO 1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2918 (5'-TAAACTTCAGGGTGACCAA AAAATCA-3') (Folmer et al. 1994), to amplify the mitochondrial COI gene by a PCR. Each PCR sample had a total volume of 50 μl and consisted of pH 9.2 buffer solution (50 mM Tris-HCl, 16 mM ammonium sulfate, 2.5 mM $MgCl_2$, and 0.1% Tween 20), 5 pM of each primer, 50 μM of dNTPs, 2 units of *Taq* DNA polymerase (super Therm DNA polymerase, *Bio-Taq*, BioKit Biotechnology, Miaoli Taiwan), and 10-50 ng of genomic DNA. The PCRs were performed in an Eppendorf Mastercycler gradient 384 machine (Eppendorf, Hamburg, Germany). Thermocycling began with 5 min of preheating and continued with 35 cycles at 94°C for 30 s, primer annealing at 51°C for 45 s, and extension at 72°C for 45 s; followed by incubation at 72°C for 10 min for full extension of the DNA and ended with 4°C holding. PCR products were electrophoresed in 2% agarose gels, after which the gels were stained with ethidium bromide (EtBr) and photographed under an ultraviolet light box. DNA fragments were excised from the gel and extracted using a 1-4-3 DNA extraction kit (Gene-Spin, Protech, Taipei, Taiwan) to obtain purified DNA. Sequences of DNA fragments were resolved on an ABI3730 automated sequencer (Applied Biosystems, Carlsbad, California USA) using 20-50 ng of template with 5 pM of the LCO1490 primer.

Alignment, genetic diversity, and phylogeny

After a search of GenBank, all COI sequences of *Simocephalus* were downloaded and aligned with our sequences. The download sequences included *S. vetulus* from the UK (accession no., DQ889172: Costa et al. 2007), *S.* cf. *punctatus* from Mexico and Guatemala (EU702310 and EU702282, Elias-Gutierrez et al. 2008), *S.* cf. *exspinosus* from Mexico and Guatemala

(EU702296 and EU702279, Elias-Gutierrez et al. 2008), *S.* cf. *mixtus* from Mexico and Guatemala (EU702305 and EU702281, Elias-Gutierrez et al. 2008), and *S. serrulatus* from Mexico and Guatemala (EU702312, Elias-Gutierrez et al. 2008). COI gene sequences were aligned by eye using the BioEdit program vers. 7.0.2 (Hall 1999). We calculated the haplotype diversity (Hd, Nei 1987), nucleotide diversity (π, Nei 1987), genetic distance (D_{xy}, Nei 1987), and average genetic distances between each pair of species using MEGA 3 vers. 3.0 (Kumar et al. 2004). *Daphnia similoides* and *Diaphanosoma dubium* were used as outgroups, and the phylogenetic tree was derived using all sequences by the Neighbor-joining (NJ) and maximum-parsimony (MP) methods (Saitou and Nei 1987) based on Kimura 2-parameter (K2P) distances with 1000 bootstraps using MEGA 3.

RESULTS

Morphometric comparisons of *Simocephalus vetulus*, *S. vetuloides*, and *S. mixtus*

The KMO value for the morphometric data matrix was 0.81, and Bartlett's X^2 was 2583.96 (*d.f.* = 276; *p* = 0.000), demonstrating the suitability of the PCA. After the PCA, 91% of the variance was explained by the 1st, 2nd, and 3rd components combined. On the 1st and 2nd component plots, *S. vetulus* and *S. mixtus* were separated from each other, but *S. vetuloides* was mixed with both groups; thus, they did not separate very well into 3 different species (Fig. 3).

Simocephalus vetulus individuals with eggs (*n* = 170) (clutch sizes ranged 1-4, dorsal valve angle ranged 39.5°-74.8°) had fewer eggs than the 2 other species; *S. vetuloides* (clutch sizes ranged 1-12, dorsal valve angle ranged 41.5°-69.5°) was intermediate; and *S. mixtus* (clutch sizes ranged 1-30; dorsal valve angle ranged 63.4°-97.5°) had the most eggs. In a pooled analysis of these 3 species, Pearson's correlation between the dorsal valve angle and clutch size was $r = 0.725$ ($p = 0.000$), and between body length and clutch size was $r = 0.70$ ($p = 0.000$). The relationship between clutch size (Y) and dorsal valve angle (X) fit a quadratic function $Y = 0.0088X^2 - 0.9091X + 25.3361$ ($r^2 = 0.53$), and the one between clutch size (Y) and body length (X) also fit a quadratic function $Y = 9.81X^2 - 20.48X + 12.00$ ($r^2 = 0.49$). Hence, irrespective of the species, clutch size was positively correlated with the dorsal valve angle and body length. The valve shape was not a species-specific character, but rather it depended on the clutch size.

Molecular analysis of COI sequences

We used 110 COI sequences from *S. vetulus* (*n* = 22), *S. vetuloides* (*n* = 28), *S. mixtus* (*n* = 10), *S. serrulatus* (*n* = 7), *S. heilongjiangensis* (*n* = 19), *Daphnia similoides* (*n* = 5), and *Diaphanosoma dubium* (*n* = 7) for the phylogenetic analysis. Each sequence was 641 bp long. Twelve haplotypes were detected for the 5 species of *Simocephalus* with 151 segregation sites; the genetic diversity, Hd, was 0.891, and the nucleotide diversity, π, was 0.07049. *Simocephalus vetulus* had 4 haplotypes from 8 sites (Hd = 0.576), *S. vetuloides* had 6 haplotypes from 11 sites (Hd = 0.802), *S. mixtus* had 4 haplotypes from 9 sites (Hd = 0.636), *S. serrulatus* had 2 haplotypes from 3 sites (Hd = 0.571), and *S. heilongjiangensis* had 3 haplotypes from 6 sites (Hd = 0.374) (Table 1).

Genetic distances (D_{xy}) between each pair of species based on the COI gene ranged 0.00671-0.1604 (Table 2). Genetic distances among *S. vetulus*, *S. vetuloides*, and *S. mixtus* were all < 0.01, while those between *S. serrulatus* and the other species were > 0.15, and those between

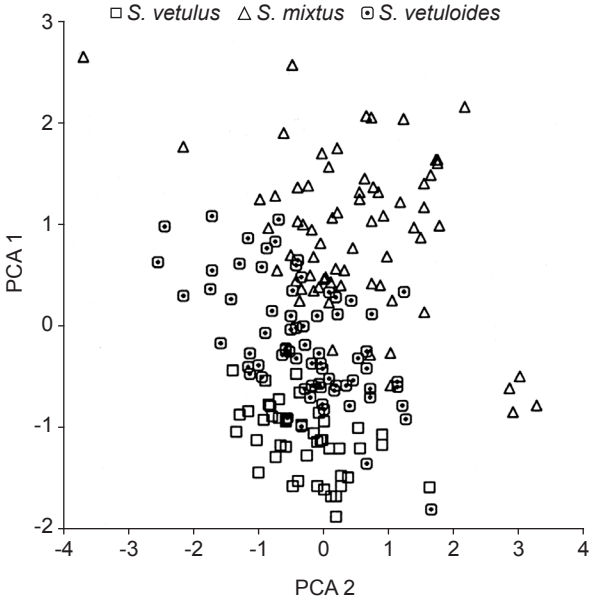

Fig. 3. Results of the principal component analysis of the morphometric dataset: 1st and 2nd principle component plot. *Simocephalus vetulus* and *S. mixtus* were well separated with a distribution gap, while *S. vetuloides* filled the gap and mixed with those 2 species.

S. heilongjiangensis and the other species were > 0.14.

In the phylogenetic NJ tree (Fig. 4), *S. vetulus*, *S. vetuloides*, and *S. mixtus* (hap *a-g*) were mixed together as a well-supported group with a bootstrap value of 99%. *Simocephalus serrulatus* (hap *k-l*) and *S. heilongjiangensis* (hap *h-j*) were well separated, with each group being supported by a 99% bootstrap value. The dorsal valve shape variation was not associated with genetic differences based on the COI gene. The most protruding valve shape (*S. mixtus*) was common in haplotypes *a* and *b*. Valve shapes of *S. vetulus* and *S. vetuloides* were also common

Table 1. Haplotypes (Hap) of each species of *Simocephalus* and their collection sites

Haplotype	*n*	Collection sites
S. vetulus	22	8 collection sites; HD = 0.576; π = 0.00806
Hap *a*	14	scA (3), scB (3), scC (2), zb (6)
Hap *e*	2	dgA (2)
Hap *f*	3	dy (3)
Hap *g*	3	scE (2), hsB (1)
S. vetuloides	28	11 collection sites; HD = 0.802; π = 0.00777
Hap *a*	10	hsA (3), scD (3), sf (1), xse (3)
Hap *b*	5	dd (1), khC (1), mn (3)
Hap *c*	3	lj (3)
Hap *d*	6	bs (6)
Hap *e*	2	khB (2)
Hap *g*	2	gA (2)
S. mixtus	22	10 collection sites; HD = 0.636; π = 0.00535
Hap *a*	12	gA (2), dh (3), dy (3), hsA (1), scF (3)
Hap *b*	6	dd (2), dy (1), tt (3)
Hap *e*	3	dgB (3)
Hap *g*	1	gs (1)
S. serrulatus	7	3 collection sites; HD = 0.571; π = 0.00357
Hap *k*	4	mf (4)
Hap *l*	3	gs (1), sf (2)
S. heilongjiangensis	19	6 collection sites; HD = 0.374; π = 0.00140
Hap *h*	15	pjA (3), pjB (4), pjC (4), pjD (4)
Hap *i*	2	khA (2)
Hap *j*	2	khA (2)

bs: Baoshan (Hsinchu County); dd: Dadu (Taichung County); dgA-B: Dongang A-B (Pingtung County); dh: Dahu (Miaoli County); dy: Dayuan (Taoyuan County); gA: Green Grass Lake (Hsinchu City); gs: Guanxi (Hsinchu County); hsA-B: Hengshan A-B (Hsinchu County); khA-C: Kaohsiung City A-C; lj: Longjing (Taichung County); mf: Minfu (Hsinchu city); mn: Meinong (Kaohsiung County); pjA-D: Pingzhen A-D (Taoyuan County); scA-E: Hsinchu City A-E; sf: Shinfeng (Hsinchu County); tt: Taitung city; xse: Xiangshan (Hsinchu City); zb: Zhubei (Hsinchu County).

Table 2. Genetic distances (D_{xy}) among *Simocephalus* species from Taiwan based on mitochondrial DNA cytochrome oxidase subunit I sequences

	S. vetuloides	*S. mixtus*	*S. vetulus*	*S. serrulatus*
S. vetuloides	-	-	-	-
S. mixtus	0.00671			
S. vetulus	0.00785	0.00698		
S. serrulatus	0.15550	0.15572	0.15473	
S. heilongjiangensis	0.16017	0.16046	0.15945	0.14391

in haplotype *b*. Haplotypes *e* and *g* were shared by all 3 morphospecies (Fig. 5, Table 1). We reconstructed the phylogenetic trees by including both our sequences and downloaded sequences, and obtained NJ and MP phylogenetic trees with similar tree structures (Fig. 6). Haplotypes *a-g* from Taiwan were all placed in the same group.

DISCUSSION

DNA barcoding can be helpful in species identification within cryptic species groups (Hebert et al. 2004, Belyaeva and Taylor 2009). In general, sequence divergences are much lower among individuals of a species than between closely related species. For example, congeneric species of moths exhibit an average sequence divergence of 6.5% in the mitochondrial COI gene, whereas divergences among conspecific individuals average only 0.25% (Moore 1995, Hebert et al. 2004). Similar values were obtained in birds, with intraspecific divergences of COI averaging 0.27%, whereas congener divergences averaged 7.93% (Hebert and Stoeckle et al. 2004). Among 1781 congeneric species pairs of crustaceans, only 1.3% had COI gene divergences of < 2%, 13.4% had COI gene divergences ranging 4%-8%, and 81.8% had COI gene divergences ranging 8%-32% (Hebert et al. 2003). In a study of the scale of intercontinental divergence for the cladoceran genus *Daphnia*, Adamowicz et al. (2009) observed a pairwise sequence divergence within the *D. obtusa* complex of up to a maximum of 16.9%, with divergences of up to 19% within the *D. longispina* complex. In our study, *S. serrulatus* and *S. heilongjiangensis* showed 14%-16% COI divergence from each other and from *Simocephalus* sensu stricto. These interspecific differences were similar to most crustaceans (Hebert et al. 2003).

Based on the morphological differences described by Orlova-Bienkowskaja (2001), 3 species - *S. vetulus*, *S. vetuloides*, and *S. mixtus* - were previously recorded in Taiwan. Indeed, our morphometric analysis of the valve shape revealed a significant difference between *S. vetulus* and *S. mixtus* from Taiwan, which appeared to support their taxonomic status as different species. However, when all 3 putative species were included in the analysis, the PCA did not separate *S. vetulus*, *S. vetuloides*, and *S. mixtus* from one another, as they formed a morphological continuum. This is consistent with a single morphologically variable species. Furthermore, differences in valve shape among *S. vetulus*, *S. vetuloides*, and *S. mixtus* collected in Taiwan were not associated with genetic variations. The genetic distances in COI among them were very small (0.6%-0.8%), a divergence level that corresponds

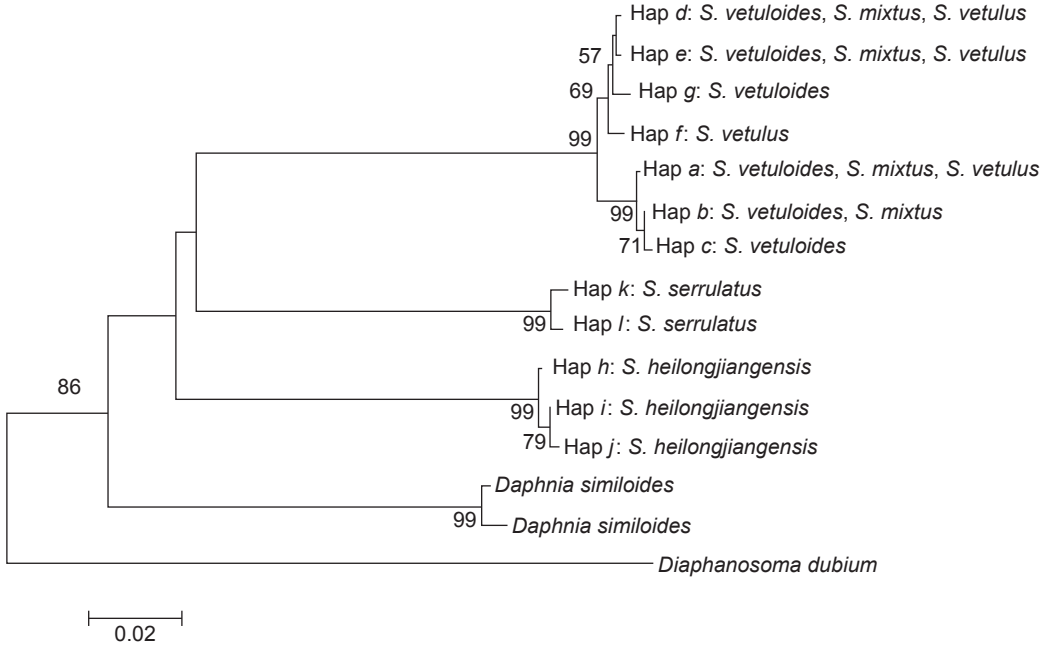

Fig. 4. Phylogenetic tree for *Simocephalus* species in Taiwan, derived using the Neighbor-joining (NJ) method based on mitochondrial (mt)DNA cytochrome oxidase subunit I (COI) sequences. The numbers indicate support values for 1000 bootstrap calculations.

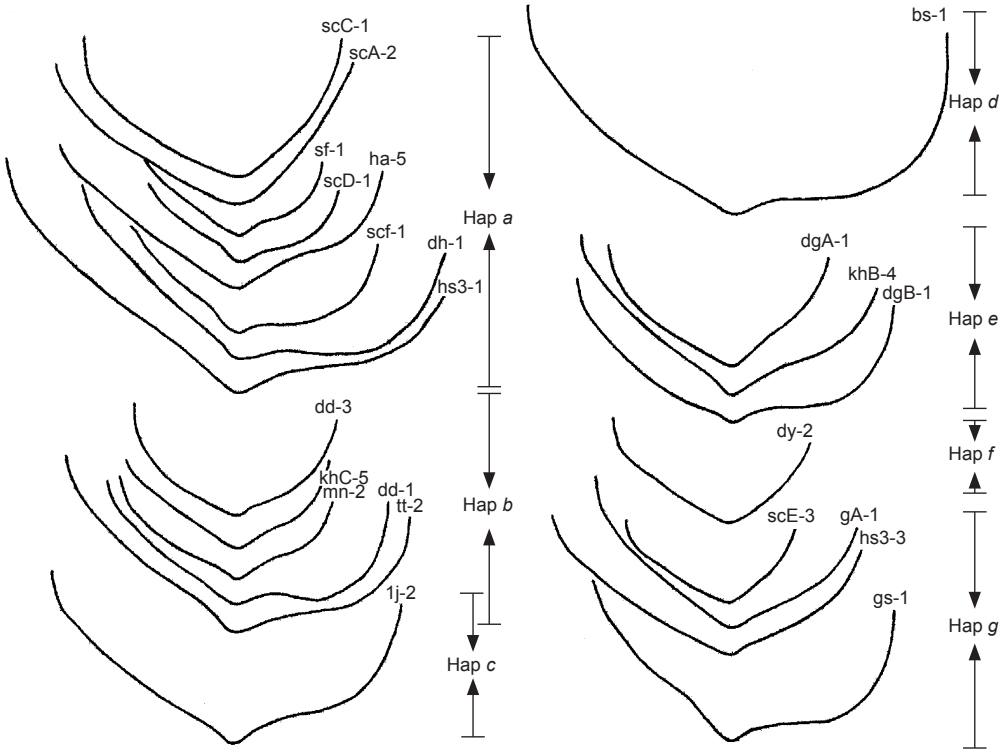

Fig. 5. Dorsal valve shapes of different haplotypes belonging to *Simocephalus vetulus*, *S. vetuloides*, and *S. mixtus*. Haplotypes *a*, *b*, *e*, and *g* have different valve shapes with large-scale variations.

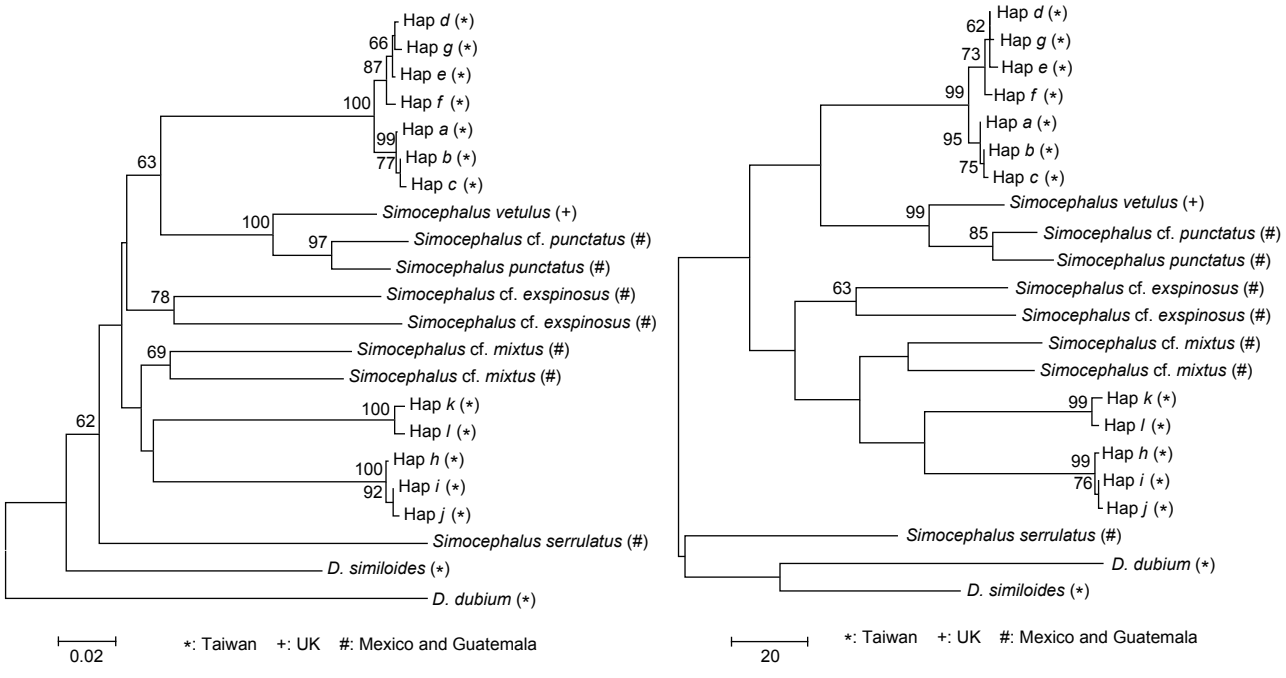

Fig. 6. Reconstructed phylogenetic trees of *Simocephalus*. Sequences from GenBank were included in this analysis: *S. vetulus* (accession no., DQ889172) from the UK, *S.* cf. *punctatus* (EU702310 and EU702282) from Mexico and Guatemala, *S.* cf. *exspinosus* (EU702296 and EU702279) from Mexico and Guatemala, *S.* cf. *mixtus* (EU702305 and EU702281) from Mexico and Guatemala, and *S. serrulatus* (EU702312) from Mexico and Guatemala. Both the Neighbor-joining (NJ) and maximum-parsimony (MP) trees shared similar branching structures. Haplotypes *a-f* from our study were all grouped together.

to intraspecific variations. Therefore, we prefer to treat all morphotypes of *Simocephalus* sensu stricto from Taiwan as a single species, *S.* cf. *vetulus*, as the publication time of *S. vetulus* was earlier than those of the other 2 species.

COI sequence comparison of *S.* cf. *vetulus* from Taiwan with the European *S. vetulus* showed that these were not conspecific (Fig. 6). As no sequences of *S. mixtus* or *S. vetuloides* from the areas of their primary distribution were available for comparison, it remains unclear whether the species found in Taiwan are conspecific with those species. It is possible that *Simocephalus* found in Taiwan is either *S. mixtus* or *S. vetuloides* or a new undescribed species. Future studies should compare sequences of *S. vetulus*, *S. mixtus*, and *S. vetuloides* collected from the type locations with sequences of *S.* cf. *vetulus* from Taiwan to verify its taxonomic status.

According to allozymic studies by Hann (1995), intraspecific differentiation within *S.* cf. *vetulus* in North America was very slight. North American and European populations were genetically distinct according to the allozyme data, but no morphological distinctiveness was identified. In the past, conspecific populations from different continents were believed to be widespread within the Cladocera based on morphological identifications. An intercontinental distribution of a species is generally presumed to be a result of passive transport by migratory birds or other dispersal mechanisms (Dumont and Negrea 2002, Adamowicz et al. 2009). The alternative hypothesis of geographical isolation assumes that gene flow among populations of cosmopolitan species on different continents is interrupted, and therefore the question is how large their genetic divergence is relative to the geographical dis-continuum scale. For example, Xu et al. (2009) explored the global phylogeography of the non-cosmopolitan freshwater cladoceran *Polyphemus pediculus* (Linnaeus, 1761) (Crustacea, Onychopoda) using 2 mitochondrial genes, COI and 16s ribosomal (r) RNA, and 1 nuclear marker, 18s rRNA. The *P. pediculus* complex represents an assemblage of at least 9 largely allopatric, cryptic species. The Far East harbors exceptionally high levels of genetic diversity at both the regional and local scales. In contrast, little genetic subdivision is apparent across the formerly glaciated regions of Europe and North America.

Similar to Xu et al. (2009) and many other previous studies on cosmopolitan cladoceran species (Ishida et al. 2006, Rowe et al. 2007, Belyaeva and Taylor 2009, Abreu et al. 2010), our results indicate that *S.* cf. *vetulus* from Taiwan is probably not the same species as *S. vetulus* from the UK, and *S. serrulatus* from Taiwan is not conspecific with *S.* cf. *serrulatus* from Mexico. *Simocephalus* cf. *vetulus* from Taiwan appears to be geographically isolated from populations on other continents. Future studies should collect barcodes of all morphospecies of *Simocephalus* from different locations around the world in order to reconstruct their systematic relationships.

Acknowledgments: We thank the National Science Council of Taiwan for their grant (NSC87-2311-B-134-001) to support part of this work. We are very grateful to the anonymous reviewers for their critical and constructive comments on our manuscript.

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