PHYLOGENY OF THE GENUS *CHRISSIA* (OSTRACODA: CYPRIDIDAE) WITH DESCRIPTION OF A NEW SPECIES FROM CHINA

Qiang Kong [1], Ivana Karanovic [2,3], and Na Yu [4,*]

[1] School of Life Sciences, East China Normal University, Shanghai 200062, P.R. China
[2] Department of Life Science, College of Natural Science, Hanyang University, Seoul 133-791, South Korea
[3] Institute for Marine and Antarctic Studies, University of Tasmania, Private Bag 49, 7001, Hobart, Tasmania, Australia
[4] School of Life Sciences, East China Normal University, Shanghai 200062, P.R. China

ABSTRACT

A new species of the genus *Chrissia* Hartmann, 1957 is described from Dongqian Lake, East China. *Chrissia dongqianhuensis* n. sp. is characterized by short swimming setae on the second antenna. This character state is very rare in the genus, and besides the new species the feature is found only in *Chrissia ceylonica* (Daday, 1898). The two species can be distinguished from each other based on the shell shape: *C. ceylonica* has distinctly protruding posterior end with sinusoid postero-dorsal margin, while in *C. dongqianhuensis* the postero-dorsal margin is evenly rounded and the posterior end is not protruding. In addition, *C. dongqianhuensis* has only 5 swimming setae. *Chrissia* presently contains 35 living species. In order to assess the position of the new species within the genus and the position of *Chrissia* within Cyprididae, we analyzed morphological data and nucleotide sequences for 18S rDNA. A total of 18 morphological characters for 32 species were used. Molecular data include sequences from 29 species (18 from the GenBank, 11 newly obtained) belonging to several lineages of Cyprididae and Ilyocyprididae. Analysis of the morphological characters resulted in 16 equally parsimonious trees with several well-supported lineages, such as the African-Indian lineage of *Chrissia* with pointed extensions on their shells, and a predominantly Asian lineage with low shells with rounded margins. The molecular phylogeny suggests that Herpetocypridinae is polyphyletic and in need of revision, and also indicates that some sequences from the GenBank are probably misidentified. High bootstrap values support Cypridopsinae, Cypricercinae, and Dolerocypridinae, while Cyprinotinae seems to be polyphyletic.

KEY WORDS: 18S rDNA, cladistics, Herpetocypridinae, molecular phylogeny, taxonomy

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INTRODUCTION

The freshwater ostracode family Cyprididae contains 23 subfamilies (Martens and Savatenalinton, 2011). Karanovic (2012) pointed out that the positions of many species within genera and many genera within subfamilies are not clear, and should undergo a taxonomic revision. Most recently, Martens et al. (2012) contributed to the systematic revision of Cyprididae by erecting Bennelongiinae to include an Australian genus *Bennelongia* De Deckker and McKenzie, 1981, which was previously considered a member of Cypridinae (see Martens and Savatenallinton, 2011) or left without any subfamily assignment (see Karanovic, 2012). The majority of Cyprididae live in open freshwater bodies and relatively few taxa are obligate stygobionts. Despite the fact that Cyprididae lay dry resistant eggs and that invertebrates in open freshwater bodies tend to be more widespread compared to the subterranean species, the family has a very high rate of endemicity; only approximately 7% of about 1000 species described so far can be found in more than one zoo-geographical province (Martens et al., 2008).

Herpetocypridinae comprises approximately 140 Recent species, and is one of the most specious cyprid linages. In the taxonomic revision of this subfamily Martens (2001) divided it into three tribes: Herpetocypridini (with genera

Candonocypris Sars, 1896, *Herpetocypris* Brady and Norman, 1889 and *Ilyodromus* Sars, 1894); Stenocypridini (with genera *Acocypris* Vávra, 1895, *Ampullacypris* De Deckker, 1981, *Chrissia* Hartmann, 1957, *Stenocypria* Müller, 1901 and *Stenocypris* Sars, 1889); Psychrodromini (with genera *Psychrodromus* Danielopol and McKenzie, 1977, *Humphcypris* Martens, 1997, and *Somalcypris* Martens, 1997). Higuti et al. (2009) added the newly described genus *Paranacypris* Higuti, Meisch, and Martens, 2009 to the last tribe and changed its diagnosis, because all genera of Psychrodromini, except *Paranacypris*, have a rectangular terminal segment of the maxillular palp. In *Paranacypris*, the palp is spatula-shaped (see Higuti et al., 2009). In the discussion part of the paper Higuti et al. (2009) indicated that Herpetocypridinae is awash with homoplastic characters, which is also mirrored in the key to genera they provide. Four herpetocypridine genera (*Ampullacypris*, *Stenocypria*, *Paranacypris* and *Somalcypris*) are monospecific, and the two largest genera (*Chrissia* and *Stenocypris*) have 34 and 36 species, respectively. The other six genera have between 8 and 19 species. The subfamily has a global distribution (although so far not recorded from Antarctica), but the center of biodiversity is in the Southern Hemisphere, especially in Africa (see maps of distribution in Karanovic, 2012).

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* Corresponding author; e-mail: nyu@bio.ecnu.edu.cn

Hartmann (1957) described two genera from Lake Chrissie in South Africa: *Chrissia* Hartmann, 1957 and *Gesa* Hartmann, 1957. However, Martens (2001) showed that the only species of the latter, *Gesa dubia* Hartmann, 1957, is in fact just an early developmental stage of *Chrissia levetzovi* Hartmann, 1957, the type species of the former genus. In fact, Martens (2001) indicated that *C. levetzovi* was also described based on juvenile specimens, so that several characters (morphology of the marginal zone of the carapace and the spinal structure of the posterior margin of the uropodal ramus) should be excluded from the diagnosis of the genus as they are only present in juveniles. In his revision of Herpetocypridinae, Martens (2001) synonymized *Gesa* and *Parastenocypris* Hartmann, 1964 with *Chrissia*. *Chrissia* differs from its most closely related genus, *Stenocypris*, in the absence of radial bands of septa along the free shell margins. The two genera share many characters, such as a rectangular terminal segment of the maxillular palp, and a conspicuously asymmetrical uropodal ramus (Victor and Fernando, 1981; Martens, 2001; Karanovic, 2012). *Chrissia* has the highest diversity in temporary freshwater ecosystems in Africa and South-East Asia.

During the investigation of the Chinese ostracode fauna we collected a new species of *Chrissia*, which we describe in this paper. This new species has very short swimming setae, which is a character found only in one other *Chrissia* (*C. ceylonica*). On the other hand, short swimming setae are often encounterd among *Stenocypris*. In order to compare the new species with other *Chrissia*, we have performed a cladisic analysis based on 18 morphological characters for 31 species of *Chrissia*, and one out-group, *Stenocypris major* (Baird, 1859).

Molecular phylogenetic analysis of ostracodes above the genus level are rare, and only few studies involved genes such as 18S and 28S rDNA that have proved to be suitable for this kind of study (Hillis and Dixon, 1991; Jarman et al., 2000). The 18S rDNA sequence was used to infer phylogenetic relationship of the class Ostracoda (Yamaguchi and Endo, 2003), suborder Cytherocopina (Yamaguchi, 2003), and suborder Cypridocopina (Yu et al., 2005), while both 18S and 28S rDNA were used for a study of the evolution of vision in myodocopid ostracodes (Oakley and Cunningham, 2002; Oakley, 2005; Syme and Oakley, 2012). In order to study the phylogenetic position of the genus *Chrissia* within the subfamily Herpetocypridinae and the family Cyprididae, we sequencesd 18S rDNA from the new species and several other species belonging to other cyprdid genera (total of 10 species), and added 18 sequences from GenBank.

MATERIALS AND METHODS

Collecting and Taxonomic Methods

Samples were collected by passing sediment through a 58 μm sieve. The resulting residue was transported back to the laboratory in Ziplock bags. Living specimens were picked under a Motic B1-220A Binocular Microscope and preserved in 75% ethanol. Appendages were dissected in glycerin and sealed on a glass slide. Valves were stored dry on micropalaeontological slides. Scanning Electron Microscop (JSM-5610LV SEM) was used for detailed observations of the shell structure. All specimens of the new species are kept in the collection of the Biological History Museum of East China Normal University. Other material is kept at the Department of Life Science, Hanyang University (Seoul, South Korea).

Systematics follows the indexes of Martens and Savatenalinton (2011) and Karanovic (2012). Chetotaxy for all appendages follow Broodbakker and Danielopol (1982). Herein we follow the view of Meisch (2007) regarding the terminology and homology of the most posterior appendage on the ostracod body ("furca"). Setal classification system follows Garm (2004).

Abbreviations used in text and figures: A1, antennula; A2, antenna; Md, mandibula; Mxl, maxillula; L5, fifth limb; L6, sixth limb; L7, seventh limb; UR, uropodal ramus; LV, left valve; RV, right valve; L, length; H, height; W, width.

Cladistic Methods

Maximum parsimony (MP) cladistic analysis of the genus *Chrissia* was performed based on 18 morphological characters (see Table A1 in the online version of this journal, which can be accessed via http://booksandjournals. brillonline.com/content/journals/1937240x). Thirty one species were included in the analysis, forming the in-group, while *Stenocypris major* was chosen as an out-group taxon. All the characters in the cladistic analysis were equally weighted. The matrix (Table A1 in the online version of this journal, which can be accessed via http://booksandjournals.brillonline. com/content/journals/1937240x) was created using WinClada, version 1.00.08 (Nixon, 2002), and then analyzed using NONA, version 2 (Goloboff, 1999), with the Heuristic Search with default parameters.

Morphological characters used in the MP cladistic analysis.—

0. Marginal septae: present (0), absent (1);
1. Shape of the shell: anterior and posterior margins of RV and LV almost symmetrical (0), only RV with pointed part and valves asymmetrical (1);
2. H/L ratio: less than 40% (0), between 40 and 50% (1), more than 50% (2);
3. Greatest H: situated medially (0), situated anteriorly (1), situated posteriorly (2);
4. Postero-ventral margin: rounded, without pointed extension (0), pointed, with conical extension (1);
5. Anterior margin: rounded (0), pointed (1);
6. Pointed end on anterior margin: absent (0), situated medially (1), situated close to the ventral margin (2);
7. Dorsal margin: almost flat (0), arched (1);
8. Posterior margin: rounded (0), inclined and sometimes even straight (1);
9. Anterior and posterior margins: more or less equally wide (0), anterior margin wider than posterior one (1);
10. Swimming setae on A2: 5 setae or some of them reaching or exceeding tip of terminal claw (0), shorter (1);
11. Swimming setae on A2: reaching or exceeding middle of terminal claws (0), much reduced (1);
12. Right UR: slender (0), stout (1);
13. Serration on the posterior margin of the right UR: step-like arrangement (0), group-like arrangement (1);
14. The longest spine on the posterior margin of UR: shorter than the width of the ramus close to the base (0), equal or longer (1);
15. Anterior seta on the UR: reaching or exceeding 1/2 length of the anterior claw (0), not reaching 1/2 length of the anterior claw (1);
16. Posterior claw on the UR: more than 1/2 length of anterior one (0), around 1/2 length of anterior one (1);
17. Serration on the claws of UR: strong (0), weak (1).

PCR Amplification Methods.—Specimens for molecular analysis were examined with only shell removed under a compound microscope (objective 63× dry) in propylene glycol for identification to morpho-species. The whole soft body was used for the DNA extraction. List of species used in the molecular analysis, collecting localities and GenBank accession numbers are listed in the Table 1. DNA was extracted using LaboPass Tissue Mini extraction kit, following the manufacturer protocol. Fragments of nuclear 18S rDNA (on average 1800 bp) were amplified using the primer pair 18S-F1 (5′-TACCTGGTTGATCCTGCCAG-3′) and 18S-R9 (5′-GATCCTTCCGCAGGTTCACCTAC-3′) (see Yamaguchi and Endo, 2003). The PCR reaction was done in a TaKaRa PCR Thermal Cycler Dice in a 25 μl volume containing 5 μl of the DNA template, 2.5 μl, 10× ExTaq Buffer, 0.25 μl of TaKaRa Ex Taq (5 units/μl), 2 μl of dNDTP mixture (2.5 mM each), 1 μl each primer and 13.25 μl distilled H₂O.

The PCR protocol consisted of 5 min initial denaturation at 94°C, followed by 35 cycles consisting of denaturation at 95°C for 30 s, annealing

Table 1.   List of species used for PCR amplification, locality data and accession numbers.

Species name as shown on the ML tree	Locality	GPS coordinates	Accession number
Bradleycypris vittata (Sars, 1903)	South Korea, Choenggye Mountain, freshwater stream	37°25′25″N, 127°3′55.2″E	KM403072
Chrissia dongqianhuensis sp. nov.	Type locality (see below)	29°47′N, 121°38′E	KM403073
Genus_Mexico	Mexico, Chetumal, brackish pool	18°31′41″N, 88°15′57″W	KM403075
Heterocypris DY_China	P.R. China, small pond	46°1′13″N, 117°45′41″E	KM403076
Heterocypris sp. 3_China	P.R. China, rice field	31°27′15″N, 121°21′46″E	KM403077
Heterocypris sp_Korea	South Korea, Busan, pond	35°7′27″N, 129°7′25″E	KM403079
Heterocypris sp_Korea 1	South Korea, Busan, pond	35°7′27″N, 129°7′25″E	KM403078
Cyprinotus sp_Korea	South Korea, freshwater pool		KM403074
Stenocypris sp_Australia	Western Australia, Christmas Island, Spring	10°27′12″S, 105°42′09″E	KM403080
Tanycypris sp_Japan	Japan, Lake Biwa, littoral	35°4′33″N, 135°56′E	KM403081

at 48°C for 30 s and extension at 72°C for 1 min. A final extension at 72°C lasted for 5 min. The PCR products were electrophoresed on the 1% agarose gels, and, if DNA was present, the products were purified for sequencing reactions, using the LaboPass PCR Purification Kit following the guidelines provided with the kit. DNA was sequenced on ABI automatic capillary sequencer (Macrogene, Seoul, South Korea) using the same set of primers.

Methods for Molecular Phylogeny Reconstruction

All obtained sequences were visualized using Finch TV version 1.4.0. BLAST (Altschul et al., 1990) analyses of GenBank revealed that the obtained sequences are ostracod in origin and not contaminants. Each sequence is checked for quality and sites with possible law resolution, and corrected by comparing forward and reverse strands. Sequences were imported in MEGA 5.2.2 (Tamura et al., 2011) and all further analyses, including the addition of highly homologous sequences from the GenBank, were done using this software. We have chosen three species of *Ilyocypris* Brady and Norman, 1889 (Ilyocyprididae) as out-group for our analyses. Sequences for all three out-group species and another 15 highly similar sequences, all belonging to Cyprididae, were downloaded from GenBank. Sequences used in the analysis varied in length from 1871 (*Cypridopsis adusta* Sars, 1903) to 702 base pairs (*Heterocypris* sp. from Korea). They were aligned with ClustalW (Thompson et al., 1994) with MEGA default parameters. The K2 + G was calculated to be the best nucleotide substitution model for this data set, based on the Bayesian Information Criterion. Distance between sequences was estimated using this model and the gaps (missing data) were treated with pairwise deletion. The distance matrix is presented in Table A2 in the online version of this journal, which can be accessed via http://booksandjournals.brillonline.com/content/journals/1937240x. A Maximum Likelihood (ML) analysis was conducted using MEGA, with the best fit model, partial deletion (95%), Nearest-Neighbour-Interchange (NNI) as the heuristic search method, and the initial tree was created automatically (Defauls-NJ/BioNJ). The boostrap values (Felsenstein, 1985) were calculated with 500 replicants. We have also used the GBLOCKS SERVER v. 0.91b (Castresana, 2000) for detecting the ambiguous regions in the alignments.

SYSTEMATICS

Class Ostracoda Latreille, 1802
Subclass Podocopa G. W. Müller, 1894
Order Podocopida Sars, 1866
Suborder Cypridocopina Baird, 1845
Superfamily Cypridoidea Baird, 1845
Cyprididae Baird, 1845
Herpetocypridinae Kaufmann, 1900
Chrissia Hartmann, 1957
Chrissia dongqianhuensis n. sp.
(Figs. 1-4)

Type Locality.—The southern shore of Dongqian Lake, Ningbo, Zhejiang, P.R. China; 29°47′N 121°38′E. Collector Na Yu, 26 June 2012. Water depth approximately 2.0 m, pH 7.9, water temperature 25.5°C.

Other Locality.—A clear stream at the foot of Tiantong Mountain, Ningbo, Zhejiang, P. R. China; 29°48′N, 121°47′E. Collector Qiang Kong, 26 June 2012.

Material Examined.—Holotype female (soft parts dissected on one glass slide, shell kept on one micropalaontological slide ECNU201206/DQH5-1); 5 paratype females (1 female soft parts dissected on one glass slide, shell kept on one micropalaontological slide ECNU201206/DQH5-2; 4 females in ethyl alcohol kept for future DNA studies), all from the type locality. Eight females were collected from the other locality.

Etymology.—The species is named after its type locality.

Diagnosis.—Carapace in lateral view elongated and reniform, greatest height situated at mid-length, anterior margin slightly more rounded than posterior margin. Marginal septae absent, fused zone wide, pore canals straight, calcified inner lamella well developed anteriorly and posteriorly. LV overlaps RV ventrally. A1 with Rome organ. A2 with 5 reduced swimming setae, almost equal in length. Alpha seta of Md smooth. Mxl palp terminal segment elongate, *Zahnborsten* of third endite with fine serration. Basal segment of L6 with two short setae (*d1*- and *d2*-). UR asymmetrical, both without proximal seta, right ramus stout and slightly curved with rows of distal spines, upside 1/4 part with a short row of fine serration.

Description.—Valves L ranging from 1.1 to 1.3 mm, H ranging from 0.46 to 0.48 mm. Carapace elongated and reniform in lateral view, ventral margin slightly concave, anterior margin slightly more rounded than posterior margin, H:L = 2/5, the greatest height at mid-length, anterior and posterior margins with hair-like setae. Calcified inner lamella wide both anteriorly and posteriorly, anteriorly calcified inner lamella equaling 17% of total L (Fig. 1, arrow indicates anterior direction), fused zone wide on both valves, without septa along free margins. Marginal pore canals short and simple. LV overlaps RV ventrally. Surface of valves with puncta. Color of living specimen transparently whitish.

A1 (Fig. 2C) 7-segmented. First segment large and wide, with one dorsal and two long ventral setae. Second segment with short dorsal-apical seta, and short Rome organ. Third segment elongate with one relatively long dorsal and one short ventral setae. Fourth and fifth segments each with
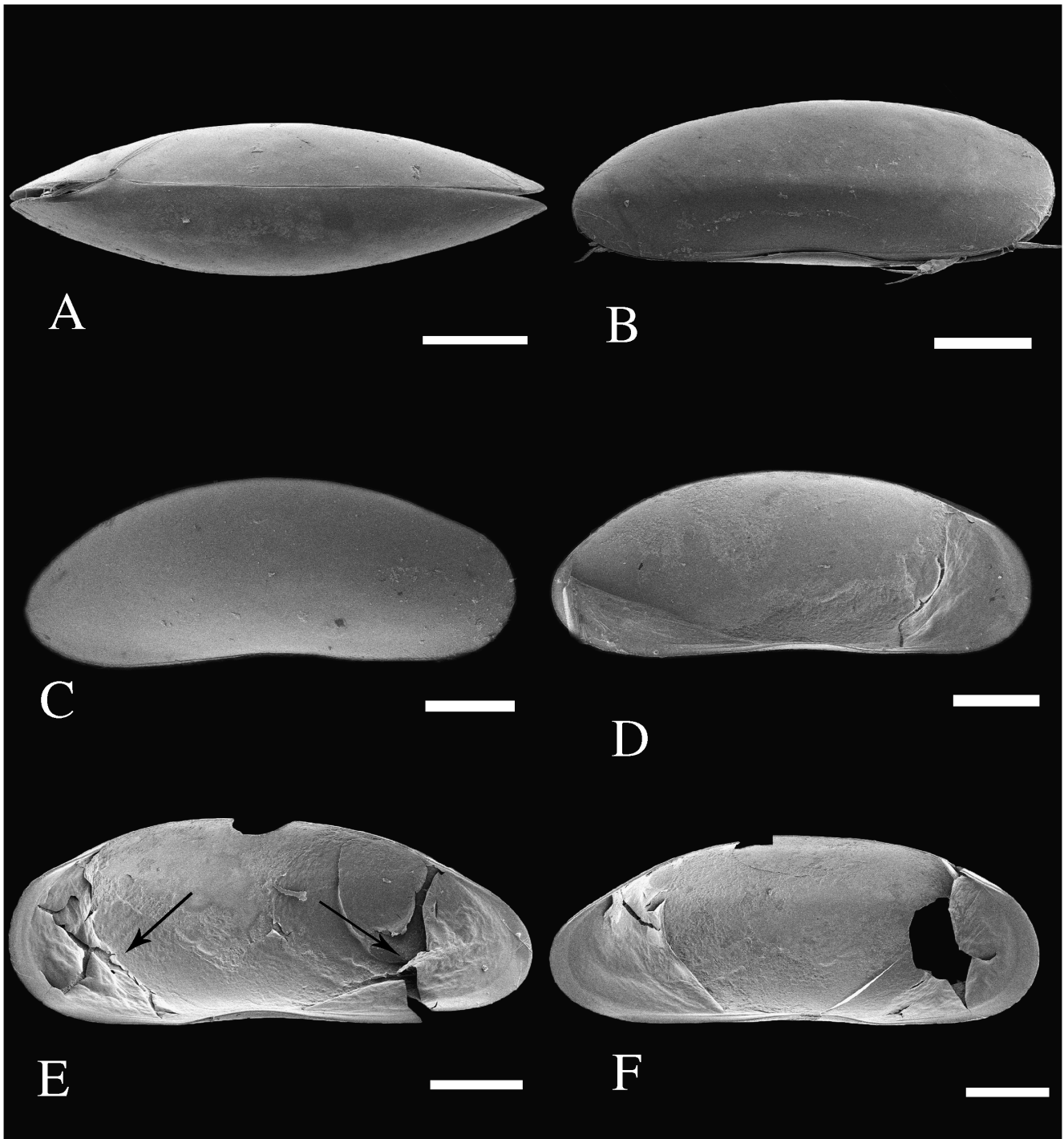
Fig. 1.   *Chrissia dongqianhuensis* n. sp., female. A, dorsal view of whole carapace; B, right lateral view of whole carapace; C, RV, outside view; D, F, LV, inside view; E, RV, inside view. Scale bar: 200 μm.

two dorsal setae (one short, one long) and two long ventral setae. Penultimate segment dorsally with two long and one short setae, ventrally with two setae (one short, one long). Terminal segment with one claw-like, two long setae and an aesthetasc *ya* (Wouters organ not examined).

A2 (Fig. 2D) 5-segmented. *Y* aesthetasc long and slender, 2-segmented, with five reduced natatory setae, which are subequal in length. Penultimate segment with three claws and three long z1-z3; claw *G2* short, about half-length of

G1, *G1* slightly longer than *G3*. Terminal segment short with long *GM*, claw *Gm* just 1/3 length of *GM*, aesthetasc *y3* about two times length of *Gm*.

Md-palp (Fig. 2E) 4-segmented. First segment with two hirsute setae, one slender seta and one smooth short α- seta. Second segment with short and hirsute β- seta; and three long and one shorter setae. Third segment with stout and hirsute γ- seta. Coxa endite well developed with three setae
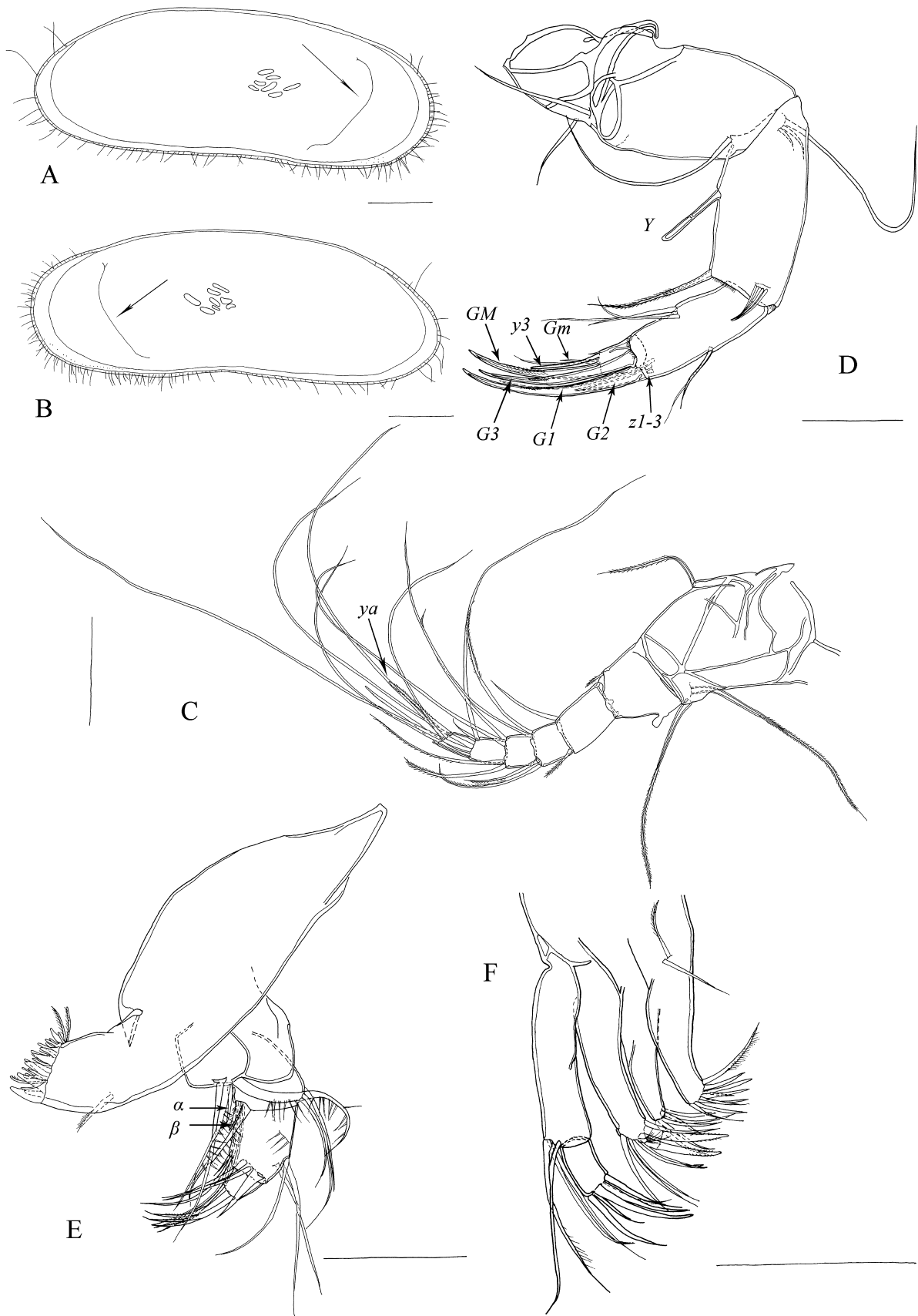
Fig. 2. *Chrissia dongqianhuensis* n. sp., female. A, LV, internal view; B, RV, internal view; C, A1; D, A2; E, Md; F, Mxl. Scale bars: 100 μm.

(two long and one short) on inner edge and one slender seta on outer edge.

Mxl (Fig. 2F) palp 2-segmented, endites elongated. First segment palp with four apical setae on outer edge; palp's terminal segment elongate with length 1.5 times that of distal width, with six apical setae. *Zahnborsten* of third endite with fine serration.

L5 (Fig. 3A) protopod with two unequal length *a*- setae and one *b*- seta, and *d*- seta on inner edge; distally with 14 apical setae of different length. Endopodite palp with one long and two short apical setae.

L6 (Fig. 3B) elongate, 5-segmented. Basal segment with two short setae (*d1*- and *d2*-). Seta *e*- on second segment as long as seta *f*- on third one. Penultimate segment with two *g*- setae, longer one about 1.5 times length of fifth segment, other very short. Terminal segment with claw *h2*-, seta *h3*- 2 times length of *h1*.

L7 (Fig. 3C) 4-segments. First segment with three long setae (*d1*-, *d2*- and *dp*-). Second segment with *e*- seta. Third segment undivided, with medial *f*- seta. Terminal segment with pincer (*h2*-) and long *h3*- seta.

UR (Fig. 3D, E) asymmetrical, right ramus stout, slightly curved, with distal spines, both without proximal seta. The denticulations of right UR on posterior margin in step-like arrangement, upside part with a short row of fine serration.

Attachment of UR (Fig. 3F) with well-developed triangular process at base, bifurcating distally, ventral branch straight and dorsal branch curved towards posterior at 1/3 of its length.

Genital field (Fig. 4A) rounded and without any extensions.

Males.—Not known.

Discussion

Affinities

Chrissia dongqianhuensis is a typical representative of the genus, having asymmetrical caudal rami, rectangular terminal segment of Mx1-palp, and missing septae on the marginal zone of valve. However, the new species can be easily distinguished from other representatives of the genus by extremely reduced swimming setae on the A2, i.e., they do not reach the middle of the penultimate segment. The only congener that has reduced swimming setae is *Chrissia ceylonica* (Daday, 1898) described from Sri Lanka. However, in *C. ceylonica*, the posterior end protrudes and the postero-dorsal margin is sinusoid, while in the new Chinese species the postero-dorsal margin is rounded and the posterior end is not protruding. Also, the claws on the UR of the new species seem to be more heavily serrated. The new species was collected in the shallow parts of the lake, probably crawling on the bottom, which can explain the reduction of the swimming setae, similarly *C. ceylonica* was found in swampy areas. Although short swimming setae are rarely found in *Chrissia*, at least seven species of *Stenocypris* have these setae reduced (see Smith and Kamiya, 2006). In the genera, *Humphcypris* Martens, 1997 and *Somalcypris* Martens, 1997, all species have reduced swimming setae on A2 (see Martens, 1997). In these two crenobiont genera, however, the UR is not so asymmetrical as in *Chrissia*, and

the basal setae on the T2 are much longer. Also, marginal zone consists of much more prominent and denser canals.

Neotenic Characters

All examined adult females lack eggs, which may raise a suspicion that they are actually in the A-1 developmental stage, the last one before the final molt. Beside the lack of eggs, there are other characteristics of the new species that may indicate that animals are not adults, which is based on the study of ontogenetic development of cyprids (Smith and Martens, 2000). First of all, it is very peculiar that the new species has only five swimming setae, a number characteristic for the A-1 instar. The final, most external, sixth seta is added only after the last molt. Nevertheless, some species have already been described with the reduced number of the swimming setae, such as, for example *Austromesocypris bluffensis* Karanovic, Eberhard, and Perina, 2012, a cyprid recently described from a Tasmanian cave (Karanovic et al., 2012). This species has only two swimming setae left, probably due to the adaptation to specific environmental conditions. The remaining two swimming setae, probably do not serve their function any more. It is likely that the loss of function leads to reduction of organs. The new species indeed has very short swimming setae, because it probably uses crawling as the mode of locomotion. In our opinion, it is very unlikely that the new species has reduced swimming setae because of the adaptations to the subterranean way of life, since it does not have any other morphological adaptations to this mode of life, and it also has a well-developed eye.

The second neotenic character found in the new species is the number of setae externally on the penultimate segment of the Md palp. Namely this species has only three setae, a characteristic of the A-1 instar (cyprid adults have four setae). While all candonines (Candonidae) always have three setae, this is a rare, but possible character state in adult Cyprididae. Chang et al. (2012) recently described *Tanycypris centa* Chang, Lee and Smith, 2012, a cyprid with three setae externally on the penultimate segment of Md palp.

The final, possibly neotenic feature, is a slightly swollen "h1" seta on the cleaning leg, but we have checked ovigerous females of *Stenocypris major* (see Fig. 4C) where this seta has a similar appearance.

The Chinese species has clearly developed genital field with chitinous structures and oviducts (see Fig. 4B), very similar to the state found in ovigerous *S. major* (see Fig. 4A). Therefore, we feel comfortable to describe *C. dongqianhuensis* and strongly believe that our examined specimens are adults.

Morphological Phylogeny

Our analysis of the morphological data set (see Table A1 in the online version of this journal, which can be accessed via http://booksandjournals.brillonline.com/content/journals/1937240x) resulted in 16 equally parsimonious trees, 64 steps long, with a consistency index of 0.32 and a retention index of 0.69. The majority rule of the 16 trees (Fig. 5) was two steps longer, with a consistency index of 0.31 and a retention index of 0.67. The new species clusters together with *C. halyi* (Ferguson, 1969), *C. spinosa*
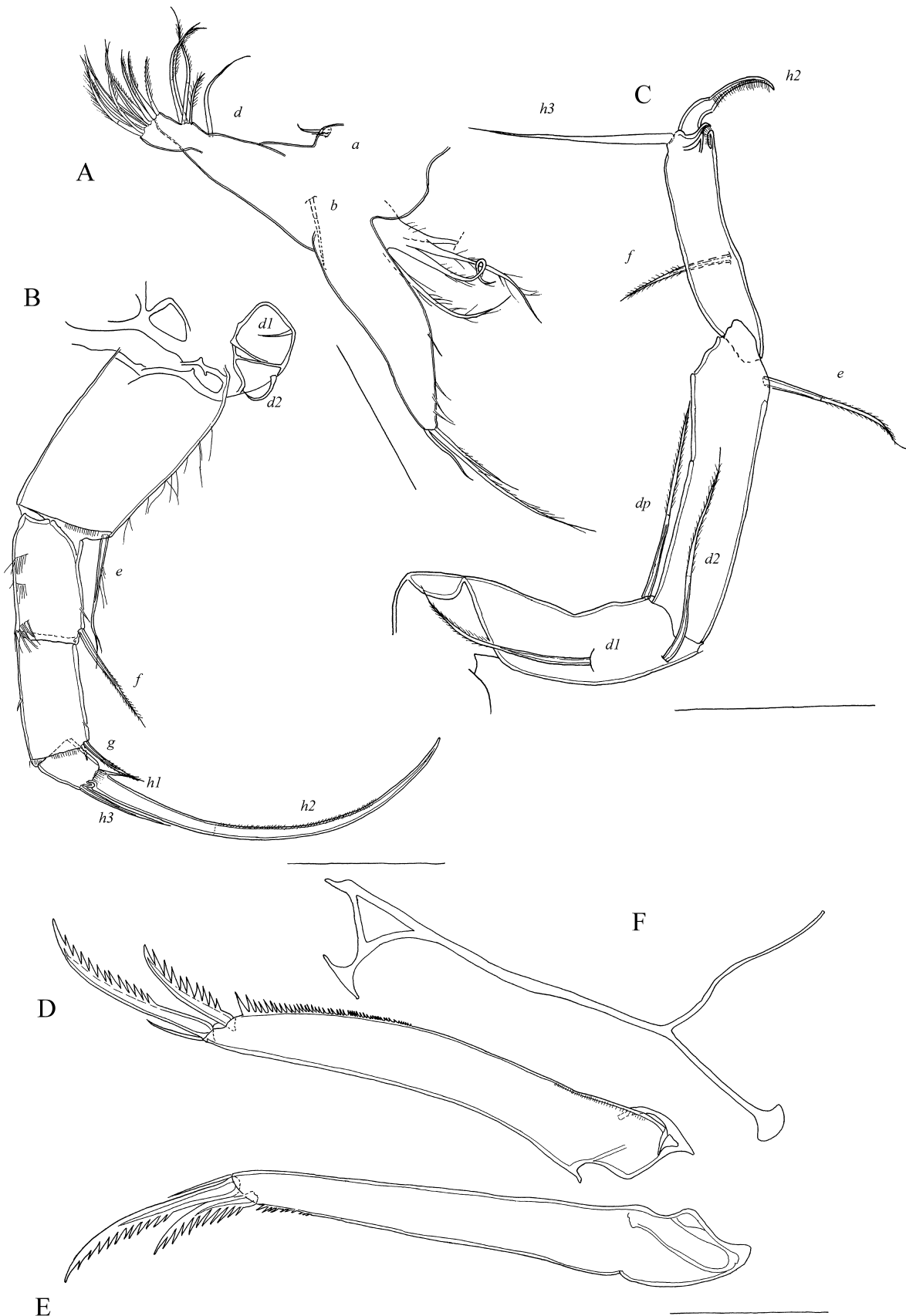
Fig. 3.   *Chrissia dongqianhuensis* n. sp., female. A, L5; B, L6; C, L7; D, Right UR; E, Left UR; F, Attachment of UR. Scale bars: 100 μm.
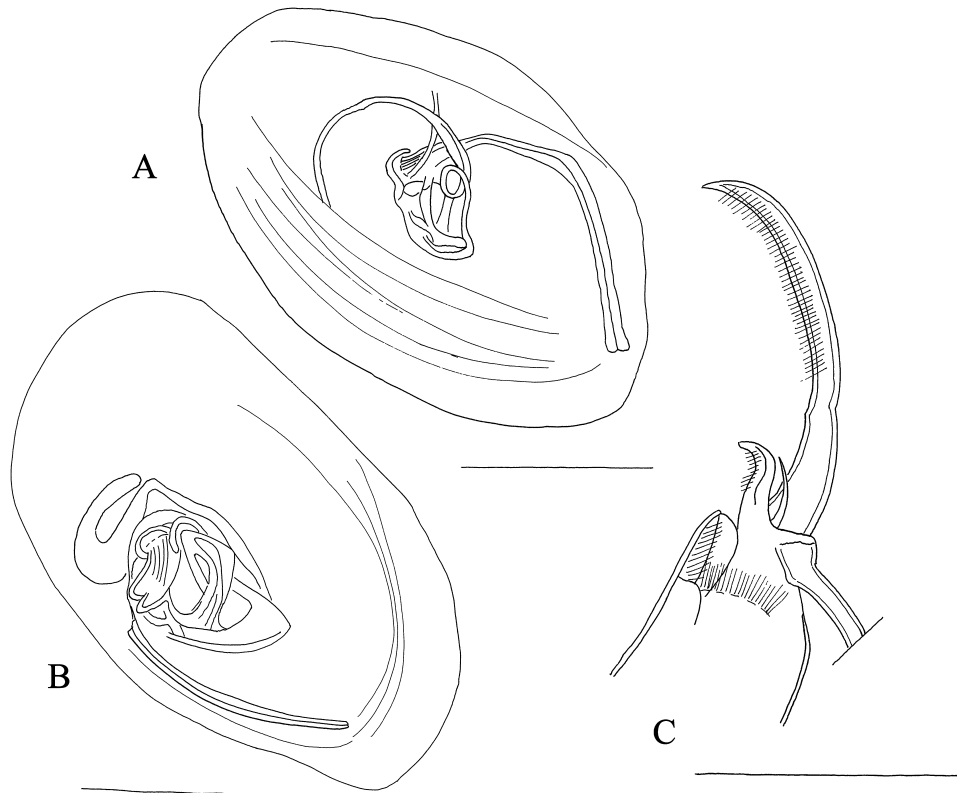
Fig. 4.   *Chrissia dongqianhuensis* n. sp., female. A, genital field, *Stenocypris major*, female; B, genital field; C, L7. Scale bars: 100 μm.

(Tressler, 1937), *C. ousmana* Ghetti, 1972, and *C. ceylonica*. They all have a very low carapace, with greatest high situated around the middle, and an almost flat dorsal margin. They are represented as the clade "III" in Fig. 5. This clade, along with the clades "I" and "II," consists mostly of species distributed in Asia, North Africa, and Central India. Only the type species, *C. levetzovi* is known from South Africa. *Chrissia levetzovi* is very closely related to *C. formosa* (Klie, 1938), a species which has been repeatedly reported throughout South-East Asia (Klie, 1938; Okubo, 1974, 2004; Victor and Fernando, 1981; Smith et al., 2011), and the differences noted between the two species and coded as such in the matrix may be the results of the fact that *C. levetzovi* was described after juveniles (see Martens, 2001). Another species that is very similar to *C. formosa* is *C. vittata* Okubo, 1974, described from Japan (Okubo, 1974). Nevertheless, three basal clades (I, II, III) represent a more primitive lineage of the genus *Chrissia* that is characterized by low to medium high carapaces, rounded margins, and the serration on the posterior margin of the UR not so prominent (character number 14). The clade "IV" on the tree is composed of species distributed in Africa below the Equator and two Indian species, *C. achandii* (George et al., 1993) and *C. goddeerisi* (George et al., 1993), both described from South-West India. The two Indian species cluster together with *C. cultrata* (Müller, 1900) from Kenya and three South African species *C. ametra* (Müller, 1908), *C. ametra minor* (Müller, 1908), and *C. bispinosa* (Müller, 1914). These six species represent the clade "VI" and are characterized by valves that have a pointed antero-ventral and/or postero-

ventral margins. Here we want to point out that *C. ametra* and *C. ametra minor* should be considered as two separate species because the latter subspecies is much higher and has arched dorsal margin. These two characters are not seen as variable in *Chrissia* and are, in fact, important for species identification. The clade "VII" contains only South African species with inclined posterior margin (sometimes even straight), and of a medium high. These species are very similar to the clade "V," with the exception that the species of the latter clade have very high carapaces and highly arched dorsal margins, but are all known from Africa and Madagascar. The present analysis included most of the species presently assigned to *Chrissia*, and the tree is mostly well resolved with well supported branches. There are several exceptions, but it has to be noted that most species of *Chrissia* have been described long time ago, and their descriptions are quite limited. Even though the carapace in ostracods is considered to be awash with homoplasy (Tinn and Oakley, 2008; Oakley et al., 2012) in this analysis most of the characters are of the shell, and they have been proven here to support (to some extent) groups within commonly recognized zoogeographic regions. Although the soft parts morphology is very conservative in this genus, it is also rarely described in many details, which left us with little choice for the analysis. This analysis pointed out the existence of at least three lineages in the genus: 1) which is considered to be more primitive and with low to medium sized ostracods distributed mostly in Asia, 2) with African-Indian species with pointed antero-ventral and/or postero-ventral margin, and 3) with much
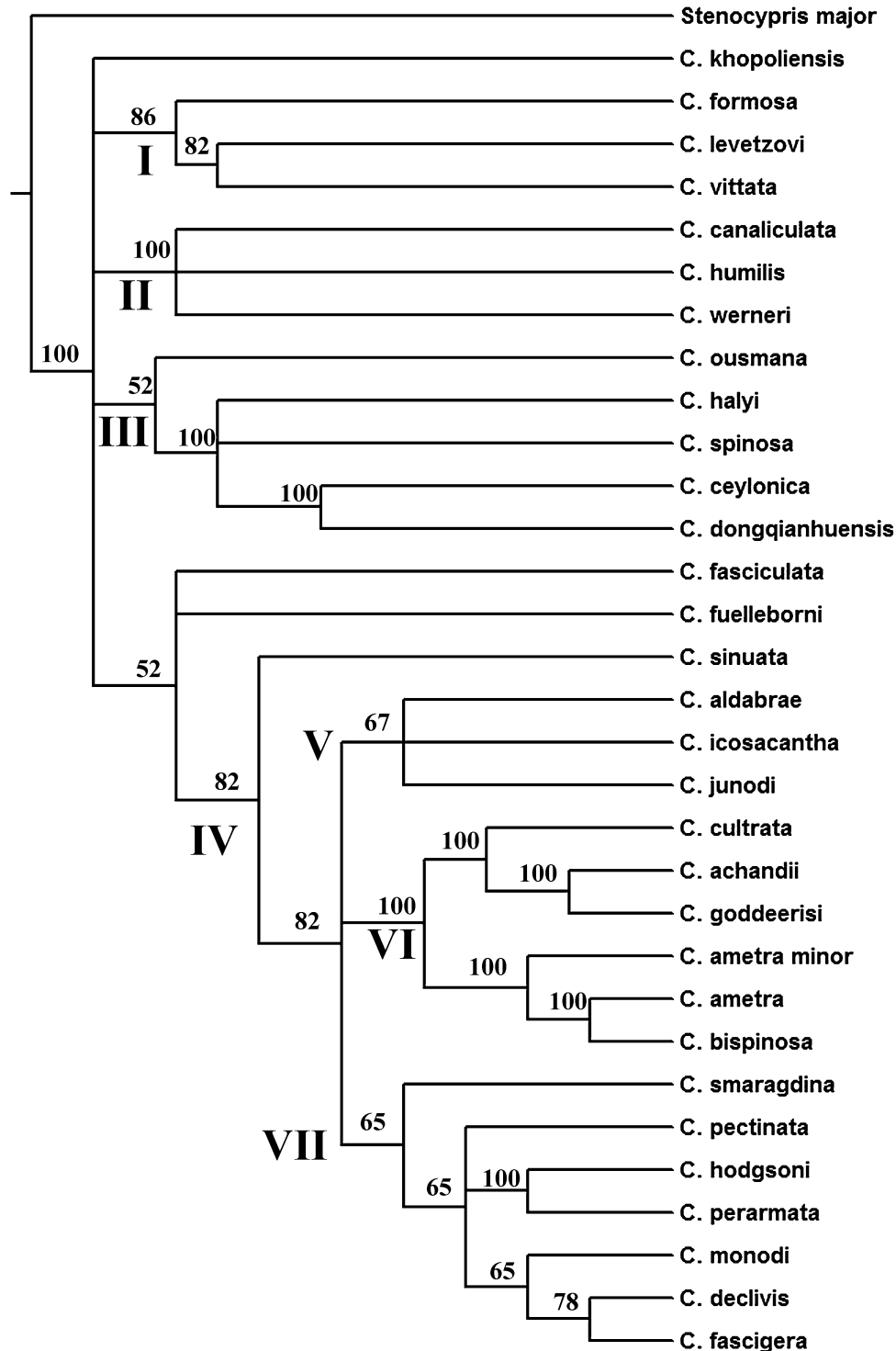
Fig. 5.   Maximum parsimony (MP) cladistic analysis of the genus *Chrissia* based on 18 morphological characters.

higher species, arched dorsal and (mostly) inclined posterior margins, of predominantly African species.

Molecular Phylogeny

The ML tree (Fig. 6) was rooted with *Ilyocypris* and it is characterized by a very law support of basal nodes but a high support of terminal nodes. Based on this tree it ap-

pears that the *Stenocypris major*, sequence Z22850.1, is a sister taxon to all other in-group taxa of Cyprididae. This sequence was downloaded from the GenBank (after the BLAST search of highly similar sequences) and it is possible that the authors who submitted it (still an unpublished paper) misidentified the species. This is supported by the fact that *Stenocypris* sp., sequence AY622195.1, another Gen-
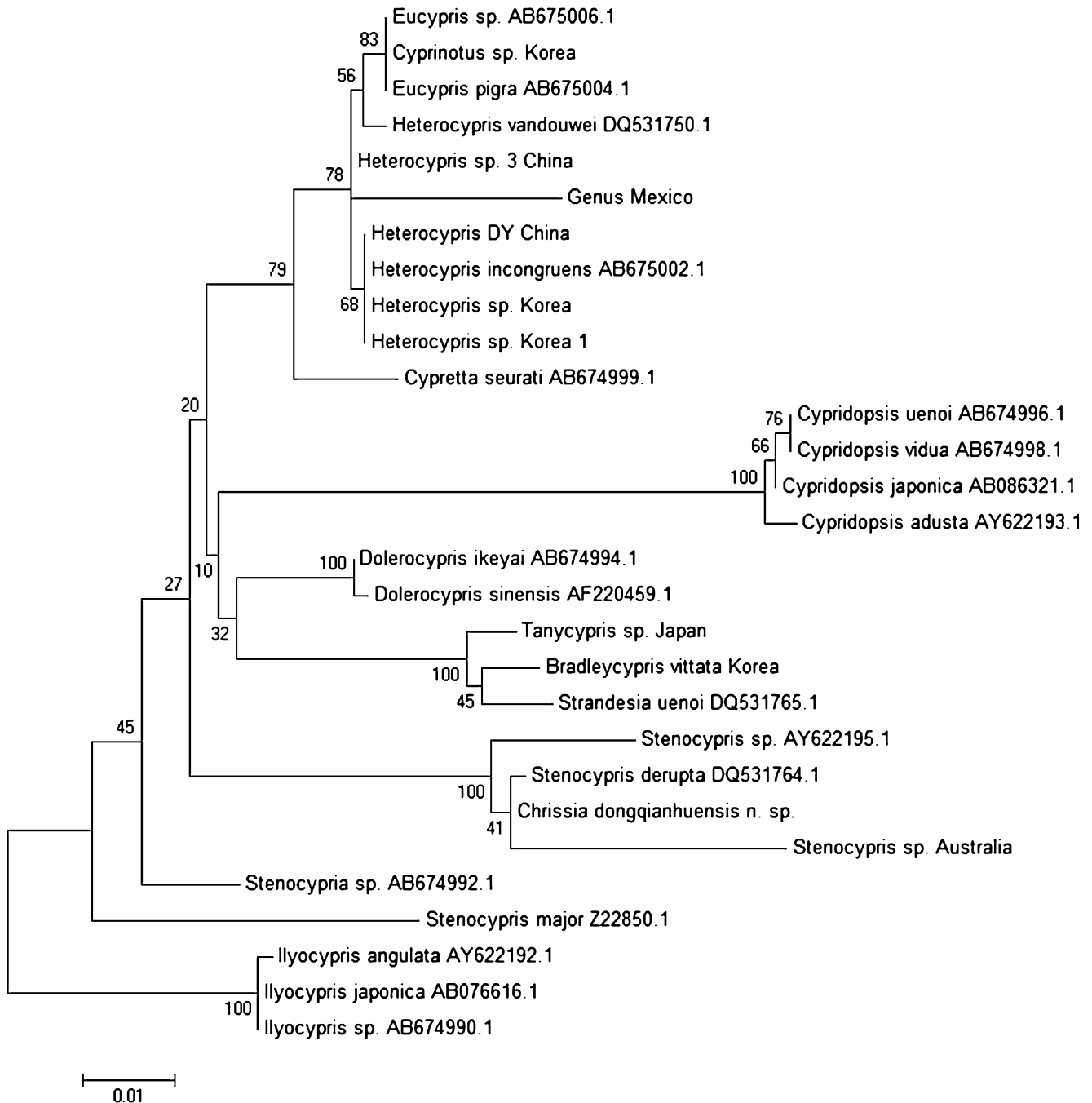
Fig. 6. Phylogenetic relationships of 29 species based on 18S rDNA sequences: maximum likelihood (ML) tree with 500 replicates, numbers on branches indicate bootstrap values.

Bank sequence, was submitted to the databank by one of us (Na Yu) and was subsequently identified as *S. major*. The name change in GenBank will follow the publication of the present paper. The average distance between *Stenocypris major*, sequence Z22850.1, and sequences of other *Stenocypris/Chrissia* is also very high, ranging from 6 to 9%. Unfortunately, we had only the new species as a representative of *Chrissia*, and on the ML tree it clusters together with three other *Stenocypris*: *Stenocypris* sp._AY622195.1, *S. derupta*_DQ531764.1, and *Stenocypris* sp. from Aus-

tralia. The last is a new species that will be described in a separate paper. The ML branch support for the *Stenocypris-Chrissia* is 100, the greatest distance (5%, see Table A2 in the online version of this journal, which can be accessed via http://booksandjournals.brillonline.com/content/journals/1937240x) is between *Stenocypris* sp. from Australia and *Stenocypris* sp._AY622195.1, while the lowest between distance (1%) is between *C. dongqianhuensis* and *S. derupta*. Beside *Stenocypris* and *Chrissia*, we had only one more representative of Herpetocypridinae, *Stenocypria*

sp._AB674992.1, a sequence also downloaded from the GenBank, but still not published. The genus *Stenocypria* Müller, 1901 is monospecific with a Palearctic distribution (Karanovic, 2012). According to Martens (2001) this genus "sits uncomfortably in the Stenocyprini, as it is the only species with symmetrical furcae with posterior seta." The distribution of the species and genera of herpetocypridines on our tree indicated that this subfamily needs a revision because of the position of *Stenocypria* and the fact that *Chrissia* shows less molecular differences from some *Stenocypris* than they show between each other. Indeed, looking at the taxonomic descriptions of the taxa of *Stenocypris*, it becomes clear that for many species the marginal zone does not carry the so called "septae," which is the most important (actually, the only) difference between *Stenocypris* and *Chrissia*. Some examples are: *S. acuta* (Vavrá, 1895), *S. bimucronata* Vavrá, 1906, *S. damasi* (Kiss, 1959), *S. dybowskii* Grochmalicki, 1913 and *S. exsiccate* Vavrá, 1897 (Vavrá, 1895, 1897, 1906; Grochmalicki, 1913; Kiss, 1959). Our phylogeny based on morphology alone also indicates several clear lineages in *Chrissia* (see above).

Another conspicuous branch on the ML tree is the one that consists of species of Cyprinotinae (*Heterocypris* Claus, 1892 and *Cyprinotus* Brady, 1886) and Eucypridinae (*Eucypris* Vávra, 1891). Average distances between species of this clade are between 0% and 2%. Although we have only provisionally identified species *Heterocypris* DY from China, *Heterocypris* sp. 3 from China, *H.* sp. from Korea, and *H.* sp. from Korea 1 as *Heterocypris incongruens* (Ramdohr, 1808) a cosmopolitan species (see Meisch, 2000), we still must compare their COI sequences to make sure they belong to the same species, despite some variability in their morphology. Interestingly, *Cyprinotus* sp. from Korea is more closely related to the two species of *Eucypris* than to *Heterocypris* sp. However, *Eucypris pigra* (Fischer, 1851) is an outstanding member of the genus (Meisch, 2000). Therefore, drawing any conclusion on the systematics of Eucypridinae based on two sequences, both (most probably) belonging to *E. virens*, is premature. Sequences from true *Eucypris* would help to resolve this issue. The sequence we have identified as "Genus Mexico" belongs to an as yet undescribed taxon from Yucatan, whose morphology does not conform neither to Eucypridinae, nor Cyprinotinae. Description of this genus is in preparation together with the revision of the *Cyprinotus-Heterocypris-Eucypris* complex. All other internal branches are well supported and clearly include only members of one subfamily.

Although we have rooted the tree with Ilyocyprididae as the apparently most closely related family to Cyprididae, it is clear that some distances within Cyprididae are greater than between the out-group and some in-group taxa. While the average distance between Ilyocyprididae and any ingroup subfamily does not exceed 7%, distances between many subfamilies are higher. For example, the distance between Cypridopsinae (*Cypridopsis* branch) and *Stenocypris*/*Chrissia* is between 7 and 10%. Yamaguchi et al. (2003) constructed a molecular phylogeny of cytherocopines based on the 18S rDNA sequence, and found that the average distances between families vary between 2 and 9%, and between in-group and out-group (bairdioideans and cytherel-

loideans) between 6.8 and 11.8%. Branches of individual Cytherocopinae for the most part had relatively high bootstrap values, but the phylogenetic relationships between taxa is mostly not resolved and have low bootstrap support. Better taxon sampling should eventually improve branch supports, but the number of sequences (31) used in Yamaguchi et al. (2003) was quite similar to the present work (29). Very high divergence rates we have found between subfamilies and even within one subfamily (Herpetocypridinae) could indicate that the level of subfamily is not adequate to mirror the antiquity and divergence time between cypridid lineages. In addition, Cypridoidea (Cyprididae and Candonidae) is about 400 million years old (Martens et al., 1998), and it is possible that 18S rDNA sequence cannot resolve relationships among such old lineages. After deleting the ambiguous parts of our alignment using the GBLOCK server, the newly proposed alignments did not change either the resulting ML tree, or the average distances between the sequences.

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APPENDIX

Table A1. Data matrix for the species of genus *Chriss* and the outgoup. Characters correspond to Fig. 4.

Taxon	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Stenocypris major	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chrissia achandii	1	0	0	2	1	0	0	1	1	1	1	0	0	1	1	1	0	1
C. aldabrae	1	0	2	1	0	0	0	1	1	1	0	0	0	0	1	0	1	0
C. ametra	1	1	1	0	1	1	1	0	1	1	0	0	0	0	1	1	0	1
C. ametra minor	1	1	2	0	1	1	1	1	0	1	0	0	0	0	1	1	0	1
C. bispinosa	1	1	0	0	1	1	1	1	1	1	?	?	?	?	?	?	?	?
C. canaliculata	1	0	1	2	0	0	0	0	1	0	0	0	0	1	0	0	0	0
C. ceylonica	1	0	0	0	0	0	0	0	0	1	1	1	1	0	0	1	0	0
C. cultrata	1	0	2	0	1	0	0	1	1	1	0	0	0	1	1	?	1	1
C. declivis	1	0	1	0	0	0	0	0	1	1	?	?	0	0	0	1	1	0
C. dongqianhuensis	1	0	0	0	0	0	0	0	0	1	1	1	1	0	0	1	0	0
C. fasciculata	1	0	2	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0
C. fascigera	1	0	1	0	0	0	0	0	1	1	?	?	0	1	1	1	0	0
C. formosa	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0
C. fuelleborni	1	0	2	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0
C. goddeerisi	1	0	2	2	1	1	2	1	1	1	1	0	0	1	1	1	1	1
C. halyi	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0
C. hodgsoni	1	0	1	0	0	0	0	0	1	0	1	0	0	0	1	0	0	1
C. humilis	1	0	1	2	0	0	0	1	0	1	0	0	0	0	0	0	0	0
C. icosacantha	1	0	2	0	0	0	0	1	1	1	0	0	0	0	1	0	0	0
C. junodi	1	0	2	0	0	0	0	1	1	1	1	0	0	0	1	0	0	0
C. khopoliensis	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C. levetzovi	1	0	0	0	0	0	0	1	0	1	0	0	1	1	0	0	1	0
C. monodi	1	0	1	0	0	0	0	0	1	1	0	0	0	0	1	0	0	0
C. ousmana	1	0	1	0	0	0	0	0	0	0	1	0	?	0	0	0	0	0
C. pectinata	1	0	1	0	0	0	0	0	1	1	?	?	0	0	1	0	0	1
C. perarmata	1	0	0	0	0	0	0	0	1	0	0	0	?	0	1	0	0	1
C. sinuata	1	0	2	0	0	0	0	1	0	0	0	0	0	0	1	0	0	1
C. smaragdina	1	0	1	0	0	0	0	1	1	1	?	?	0	0	1	0	0	1
C. spinosa	1	0	0	0	0	0	0	0	0	0	1	0	?	0	0	1	1	0
C. vittata	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0
C. werneri	1	0	0	2	0	0	0	0	0	1	0	0	1	0	0	0	0	1

Table A2.   Distance matrix of 18S rDNA sequences from 29 ostracod species.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1. *Bradleycypris_vittata_Korea*	0.06																											
2. *Chrissia_dongqianhuensis_*n._sp.	0.05	0.07																										
3. *Cyprelta_seurati_*AB674999.1	0.07	0.09	0.05																									
4. *Cypridopsis_adusta_*AY622193.1	0.07	0.09	0.05	0																								
5. *Cypridopsis_japonica_*AB086321.1	0.07	0.09	0.05	0	0																							
6. *Cypridopsis_uenoi_*AB674996.1	0.07	0.1	0.06	0.01	0.01	0																						
7. *Cypridopsis_vidua_*AB674998.1	0.04	0.06	0.04	0.06	0.06	0.06	0.07																					
8. *Dolerocwris_ikeyai_*AB674994.1	0.04	0.07	0.04	0.06	0.06	0.06	0.07	0																				
9. *Dolerocypris_sinensis_*AF220459.1	0.05	0.06	0.03	0.07	0.07	0.07	0.07	0.04	0.04																			
10. *Eucypris_pigra_*AB675004.1	0.05	0.06	0.03	0.07	0.07	0.07	0.07	0.04	0.04	0																		
11. *Eucypris_sp._*AB675006.1	0.06	0.07	0.05	0.07	0.07	0.07	0.08	0.05	0.05	0.02	0.02																	
12. Genus_Mexico	0.06	0.07	0.04	0.07	0.07	0.07	0.08	0.05	0.05	0.01	0.01	0.02																
13. *Heterocypris_*DY_China	0.06	0.07	0.03	0.06	0.06	0.06	0.07	0.04	0.04	0.01	0.01	0.02	0															
14. *Heterocypris incongruens_*AB675002.1	0.06	0.07	0.03	0.07	0.07	0.07	0.07	0.04	0.04	0.01	0	0.02	0	0														
15. *Heterocypris_sp._3_China*	0.06	0.07	0.03	0.07	0.06	0.06	0.07	0.04	0.04	0	0	0.02	0	0	0													
16. *Heterocypris_sp._Korea*	0.05	0.05	0.02	0.06	0.06	0.06	0.06	0.03	0.03	0.01	0.01	0.02	0	0	0	0												
17. *Heterocypris_sp._Korea_1*	0.05	0.05	0.02	0.07	0.06	0.06	0.06	0.03	0.03	0.01	0.01	0.02	0.01	0	0	0	0.01											
18. *Cyprinotus_sp._Korea*	0.04	0.05	0.02	0.07	0.06	0.06	0.07	0.03	0.03	0	0	0.02	0.01	0	0	0	0.01	0.01										
19. *Heterocypris vandouwei_*DQ5 31750.1	0.05	0.06	0.04	0.07	0.07	0.07	0.07	0.04	0.05	0.01	0.01	0.02	0.01	0.01	0	0	0.01	0.01	0.01									
20. *Ilyocypris angulata_*AY622192.I	0.06	0.07	0.05	0.06	0.06	0.06	0.07	0.05	0.05	0.04	0.04	0.05	0.05	0.04	0.04	0.04	0.05	0.05	0.05	0.05								
21. *Ilyocypris japonica_*AB076616.1	0.06	0.07	0.05	0.06	0.06	0.06	0.07	0.05	0.05	0.04	0.04	0.06	0.05	0.04	0.05	0.04	0.05	0.05	0.05	0.05	0							
22. *Ilyocypris_sp._*AB674990.I	0.06	0.07	0.05	0.06	0.06	0.06	0.07	0.05	0.05	0.04	0.04	0.06	0.05	0.04	0.05	0.04	0.05	0.05	0.05	0.05	0	0						
23. *Stenocypria_sp._*AB674992.1	0.05	0.06	0.04	0.07	0.07	0.07	0.08	0.04	0.04	0.04	0.04	0.06	0.05	0.04	0.05	0.05	0.03	0.03	0.05	0.05	0.05	0.05						
24. *Stenocypris derupta_*DQ531764.1	0.06	0.01	0.07	0.1	0.1	0.1	0.1	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.06	0.06	0.07	0.07	0.08	0.08	0.07					
25. *Stenocypris_major_*Z22850.1	0.06	0.07	0.04	0.07	0.07	0.07	0.08	0.05	0.05	0.04	0.04	0.06	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.08	0.08	0.04	0.07				
26. *Stenocypris_sp._Australia*	0.08	0.03	0.07	0.1	0.1	0.1	0.1	0.07	0.07	0.07	0.07	0.08	0.07	0.07	0.07	0.07	0.07	0.06	0.07	0.09	0.08	0.08	0.06	0.03	0.09			
27. *Stenocypris_sp._*AY622195.1	0.08	0.04	0.08	0.1	0.1	0.1	0.11	0.07	0.07	0.07	0.07	0.08	0.08	0.08	0.07	0.07	0.06	0.06	0.07	0.08	0.08	0.08	0.07	0.04	0.06	0.05		
28. *Strandesia uenoi_*DQ5 31765.1	0.01	0.07	0.07	0.09	0.09	0.09	0.09	0.07	0.07	0.08	0.08	0.06	0.06	0.08	0.06	0.05	0.11	0.11	0.08	0.08	0.09	0.09	0.08	0.09	0.1	0.08	0.1	
29. *Tanycypris* sp._Japan	0.01	0.07	0.05	0.07	0.07	0.07	0.08	0.05	0.05	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.05	0.06	0.06	0.07	0.07	0.06	0.07	0.07	0.08	0.09	0.01