



## Research paper

## A new freshwater ostracod genus from the northern Neotropical region and its phylogenetic position in the family Cyprididae (Podocopa)

Hyunsu Yoo<sup>a</sup>, Sergio Cohuo<sup>b,c</sup>, Laura Macario-Gonzalez<sup>b,c</sup>, Ivana Karanovic<sup>a,d,\*</sup><sup>a</sup> Hanyang University, Department of Life Science, College of Natural Sciences, 17 Haengdang-dong, Seongdonggu, Seoul 133-791, South Korea<sup>b</sup> Institut für Geosysteme und Bioindikation, Technische Universität Braunschweig, Langer Kamp 19c D-38106 Braunschweig, Germany<sup>c</sup> El Colegio de la Frontera Sur (ECOSUR), Unidad Chetumal, Av. Centenario km 5.5, Chetumal, 77014 Quintana Roo, Mexico<sup>d</sup> Institute of Marine and Antarctic Studies, University of Tasmania, Private Bag 49, 7001 Hobart, Tasmania, Australia

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## ABSTRACT

*Manuelcypris* n. gen., along with two species *M. chetumalensis* n. sp. and *M. tabascena* n. sp., are described from Southern Mexico. Another three species are transferred into the new genus: *M. cisternina* (Furtos, 1936) comb. nov., known from the Yucatan Peninsula, was originally described in the genus *Eucypris* Vávra, 1891, while *M. punctata* (Keyser, 1975) comb. nov., from Florida, and *M. antillensis* (Broodbakker, 1982) comb. nov., from the West Indies, were originally described in the genus *Heterocypris* Claus, 1892. The latter two new combinations are based only on the literature data, while for *M. cisternina* we examined its type material. The new genus is attributed to Cyprinotinae, but with an isolated position based on the absence of any seta on the basal segment of the walking leg and the reduced chaetotaxy of the fifth leg. The validity of this genus is confirmed with molecular markers, 18S rDNA and 28S rDNA which were used in the phylogenetic reconstruction along with several newly obtained sequences and others downloaded from the GenBank belonging to closely related taxa. Two methods, Maximum Parsimony and Maximum Likelihood, used in the analysis support the new genus with 100 bootstrap values. We also provide a taxonomic key for the five representatives of the new genus.

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## 1. Introduction

Ostracods of the family Cyprididae Baird, 1845 are the dominant group in open freshwater ecosystems, accounting for more than a half of the circa 2000 freshwater species described so far (Karanovic, 2012; Martens et al., 2008; Martens and Savatnalinton, 2011). The family comprises over 20 subfamilies most of which have a global distribution. Generally, endemism across zoogeographical provinces on the subfamily level is very rare in ostracods (Martens et al., 2008). In the family Cyprididae, a handful of families (i.e. Batucypridinae, Herpetocypridinae, Lio-cypridinae, Ngarawinae, Pelocypridinae) are each restricted to one

zoogeographical province. This may, however, be the result of the lack of research or unresolved phylogenetical relationships. During 250 years since the first Cyprididae ostracod, *Cypridopsis vidua* (Müller, 1776) was described, systematics of this family became more complex and in the recent years several taxonomic and phylogenetic studies attempted to revise a few subfamilies and genera. For example, Martens (1989) and Martens et al. (1992) revised Eucypridinae and proposed several important taxonomic characters (such as, for example the presence of “c” seta on L5 and the length ration between d1 and d2 setae on L6) for distinguishing between genera; Martens (1986), and Martens (1990, 1992) revised Megalocypridinae and Cypridinae; Savatnalinton and Martens (2009) revised Cypricerinae, while Martens (2001) revised Herpetocypridinae. All these revisions were based on the morphological characters (both of the carapace and soft parts) and still a thorough revision of any of the subfamilies based on molecular markers is lacking. However, several studies included representatives of various Cyprididae subfamilies in the attempt to resolve phylogenetic relationships within ostracods in general or the positions of various genera within their respective subfamilies.

**Abbreviations:** A1, antennula; A2, antenna; UR, uropodal ramus; H, height; L, length; LV, left valve; Md, mandible; Mdp, mandibular palp; Mx, maxillula; RV, right valve; L5, L6, L7, first, second and third thoracopods respectively; W, width.

\* Corresponding author at: Hanyang University, Department of Life Science, College of Natural Sciences, 17 Haengdang-dong, Seongdonggu, Seoul 133-791, South Korea.

E-mail address: [ivana@hanyang.ac.kr](mailto:ivana@hanyang.ac.kr) (I. Karanovic).

In this regard, Yamaguchi and Endo (2003) used 18S rDNA to study the molecular phylogeny of Ostracoda, while Yu et al. (2005) used the same marker and a very limited number of taxa to study phylogenetic relationship within Cypridocopina. More recently Kong et al. (2014) aimed to resolve the position of the genus *Chrissia* Hartmann, 1957 within Cyprididae, also by studying 18S rDNA. Their analysis was so far the most extensive in terms of the number of taxa they used, and it strongly supported monophyly only of a few Cyprididae subfamilies, while it disputed Herpetocypridinae, Eucypridinae and Cyprinotinae.

Cyprinotinae comprises five genera and over 100 named Recent species (Martens and Savatnalinton, 2011) and as such it is one of the most specious Cyprididae subfamilies. Several species have a Holarctic distribution, and one, *Heterocypris incongruens* (Ramdohr, 1808), has a cosmopolitan distribution (Meisch, 2000), a variable carapace morphology and a high clonal diversity (Rossi and Menozzi, 1990). Although this species prefers shallow seasonal pools, it is very tolerant to various conditions, including high salinity (Meisch, 2000) and it displays a geographic parthenogenetic reproduction, sometimes with sexual and asexual populations inhabiting the same ecosystem (Rossi et al., 2007). As a result of its wide distribution, morphological variability, mixture of sexual and parthenogenetic reproduction, a number species may prove to be synonyms, but it is also possible that some of the records of *H. incongruens* actually apply to separate species. Studies to test a possible cryptic speciation in *Heterocypris incongruens* have never been carried out, but in another species, *Eucypris virens* (Jurine, 1820) with the same distribution, morphological variability and

peculiar reproduction modes, a numerous potentially cryptic species have been reported from around the globe with divergence rates between mtCOI sequences approaching 20% (see Bode et al., 2010). *Eucypris virens* belongs to Eucypridinae, which can be considered a sister group to Cyprinotinae. Representatives of the two subfamilies are indeed very similar and the two main differences are the presence of peculiar wart-shaped elevations on the frontal part of valves (“porenwarzen”) in at least part of the species and presence of a “c” seta on the fifth leg in Eucypridinae. In addition, there is a tendency of a slender uropodal ramus and short posterior seta on the same appendage in comparison to Cyprinotinae. However, at least one species, *Eucypris pigra* (Fischer, 1851), lacks porenwarzen, has a robust uropodal ramus and a longer posterior uropodal seta. This species is thus more similar to Cyprinotinae.

The systematics of Eucypridinae has gone through many changes in the recent years (see references above), thanks to the advances in the taxonomic research. This often led to the new systematic combinations for taxa described decades ago, especially when studying less explored regions of the world, such as Central and South America. For example, Díaz and Martens (2014) described a Eucypridinae genus, *Argentocypris* Díaz and Martens, 2014 to include one new South American species and two species previously assigned to *Eucypris* and *Cypris*. In this paper we describe a new genus to accommodate two new species collected from southern Mexico, plus *Eucypris cisternina* Furtos, 1936 described from the same region (Furtos, 1936), *Heterocypris punctata* Keyser, 1975 originally described from Florida (Keyser, 1975) and *H. antillensis* Broodbakker, 1982 from the Antilles (Broodbakker, 1982).

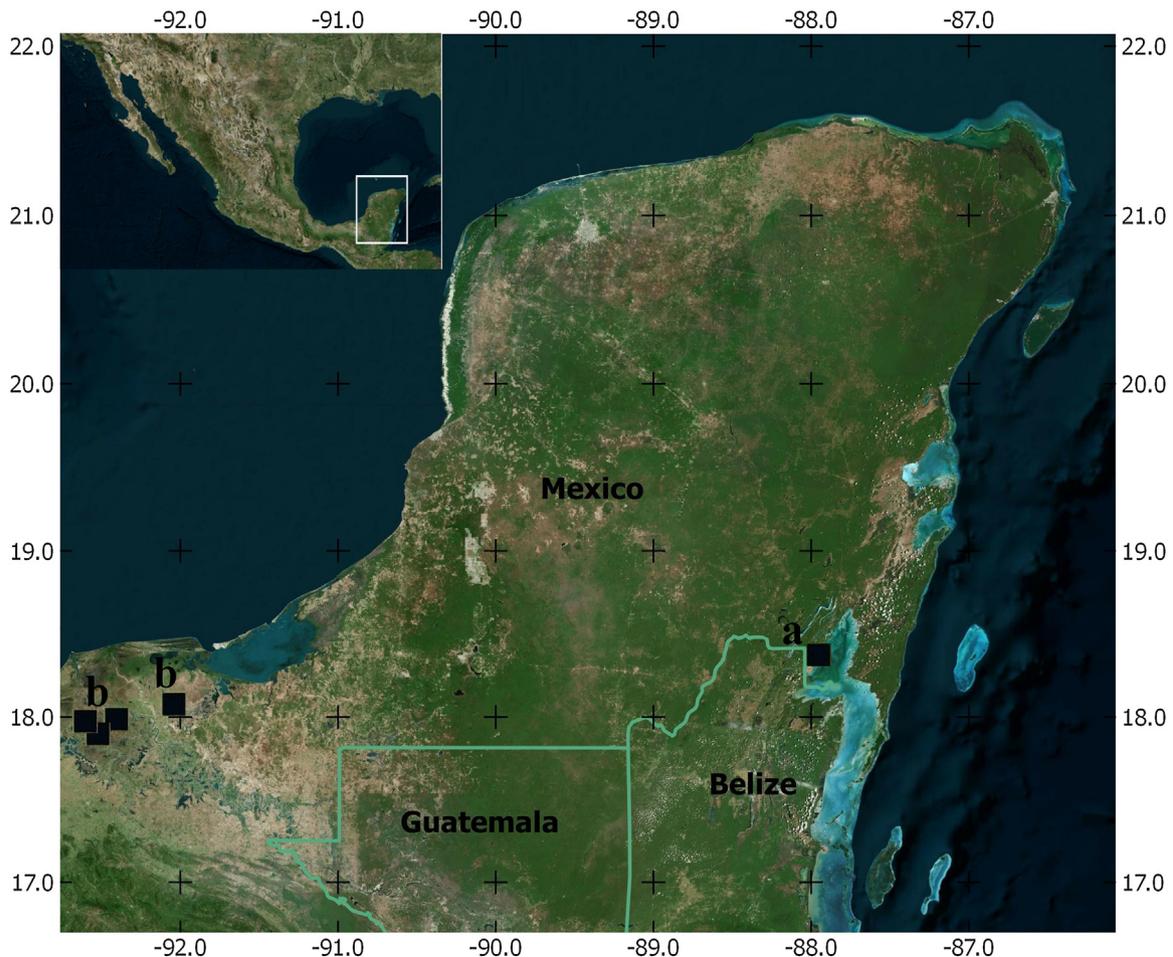


Fig. 1. Map of the study area. (a) Collection sites of *Manuelocypris chetumalensis* n. sp.; (b) Collection sites of *Manuelocypris tabascena* n. sp.

The characteristics of these five species place them into an intermediate position between Cyprinotinae and Eucypridinae and in order to test the position of the new genus we used two molecular markers, 18S rDNA and 28S rDNA. Besides the phylogenetic analysis this paper also provides a detail description of the two new species and redescription of *Eucypris cisternina* based on the type material deposited at the Invertebrate Collection of the Smithsonian Museum in Washington.

## 2. Material and methods

### 2.1. Sampling methods and taxonomy

Sampling campaigns were carried out in southern Mexico (Fig. 1) during 2011 and 2013. Lacustrine ecosystems were sampled with a hand net of 50  $\mu\text{m}$  mesh size. The net was thoroughly passed through the submerged vegetation on change with in the littoral zone. When a limnetic zone was developed, several vertical tows with a plankton net (50  $\mu\text{m}$  mesh size, 30 cm mouth) were taken. Samples from the Chetumal bay were collected using a net of 60  $\mu\text{m}$  mesh size at different points along the coast line by filtering at least 200 L of water. All samples were fixed with 96% ethanol and preserved in refrigeration until laboratory analysis. Ostracod specimens were extracted under a stereomicroscope Leica EZ4. Dissections of soft and hard part were carried out, in a mixture of distilled water and glycerin; dissected soft parts were mounted on microscope slides in Hidromatrix<sup>®</sup> mounting medium. Ostracod carapace photographs were taken with a Canon Powershot A640 digital camera attached to a Zeiss Axiostar-plus light microscope. Soft parts were examined and characterized with the aid of a camera lucida using the Leica DM 2500 compound microscope, equipped with NPlan objectives and a drawing tube attachment. Scanning Electron Micrographs (SEM) were taken with a Hitachi S-4700 scanning electron microscope at Eulji University (Seoul).

The terminology for the A1, Md, Mx1, L5 and L6 follows Broodbakker and Danielopol (1982), and for the L7 Meisch (1996). Here, the view of Meisch (2007) regarding the terminology and homology of the most posterior appendage on the ostracod body (“furca”) is accepted. Setal classification system follows Garm (2004). Kempf’s (1980a–d), Kempf’s 1991, Kempf’s (1997a–d) indexes and bibliographies of the freshwater ostracods have been extensively used in checking the availability of names and publications.

### 2.2. DNA amplification

In the first step of the DNA extraction specimens were kept for 2–3 h in distilled water. LaboPass Tissue Mini extraction kit (Cosmo Genetech Co., LTD, Korea) was used in all further steps of extraction, following the manufacturer’s protocol. Fragments of 28S rDNA were amplified using the primer pairs dd/ff, ee/mm, vv/xx from Hills and Dixon (1991), using a TaKaRa PCR Thermal Cycler Dice. PCR reactions were carried out in 25  $\mu\text{l}$  volumes, containing: 5  $\mu\text{l}$  of the DNA template, 2.5  $\mu\text{l}$ , 10x ExTaq Buffer, 0.25  $\mu\text{l}$  of TaKaRa Ex Taq (5 units/ $\mu\text{l}$ ), 2  $\mu\text{l}$  of dNDTP Mixture (2.5 mM each), 1  $\mu\text{l}$  each primer, and 13.25  $\mu\text{l}$  distilled H<sub>2</sub>O. The PCR protocol consisted of initial denaturation for 5 min at 94 °C, 40 cycles of denaturation for 35 s at 95 °C, annealing for 1 min at 50 °C, extension for 1 min at 72 °C. Final extension was at 72 °C for 5 min. Fragments of 18S rDNA were amplified using the primers from Yamaguchi (2003). For the initial amplification the F1/R9 primer set was used, which amplifies around 1800 base pair, if no band was apparent than internal primer pairs were used in the PCR reaction in which 1  $\mu\text{L}$  of the PCR product was used as the template. Only in the case of *Physocypria cf. biwaensis* the primer pare from Kato et al. (1997) was used. The PCR reaction was the same as for 28S rDNA. PCR settings for the amplification of 18S rDNA followed Yamaguchi (2003) and Kato et al. (1997) for each corresponding primer pair. The PCR products were

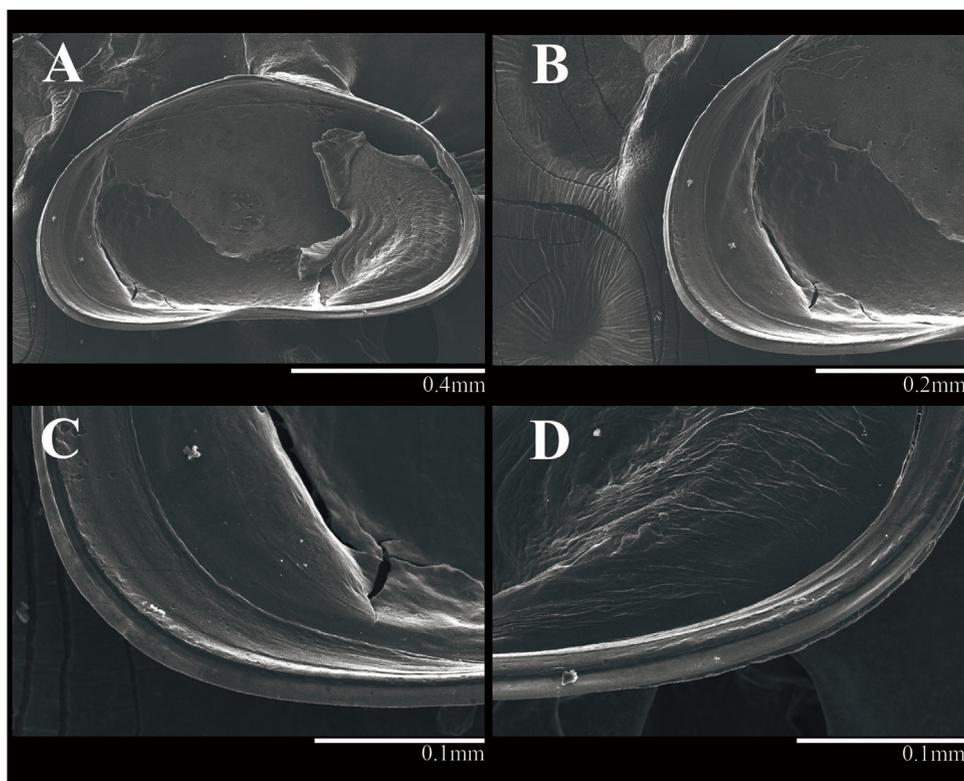


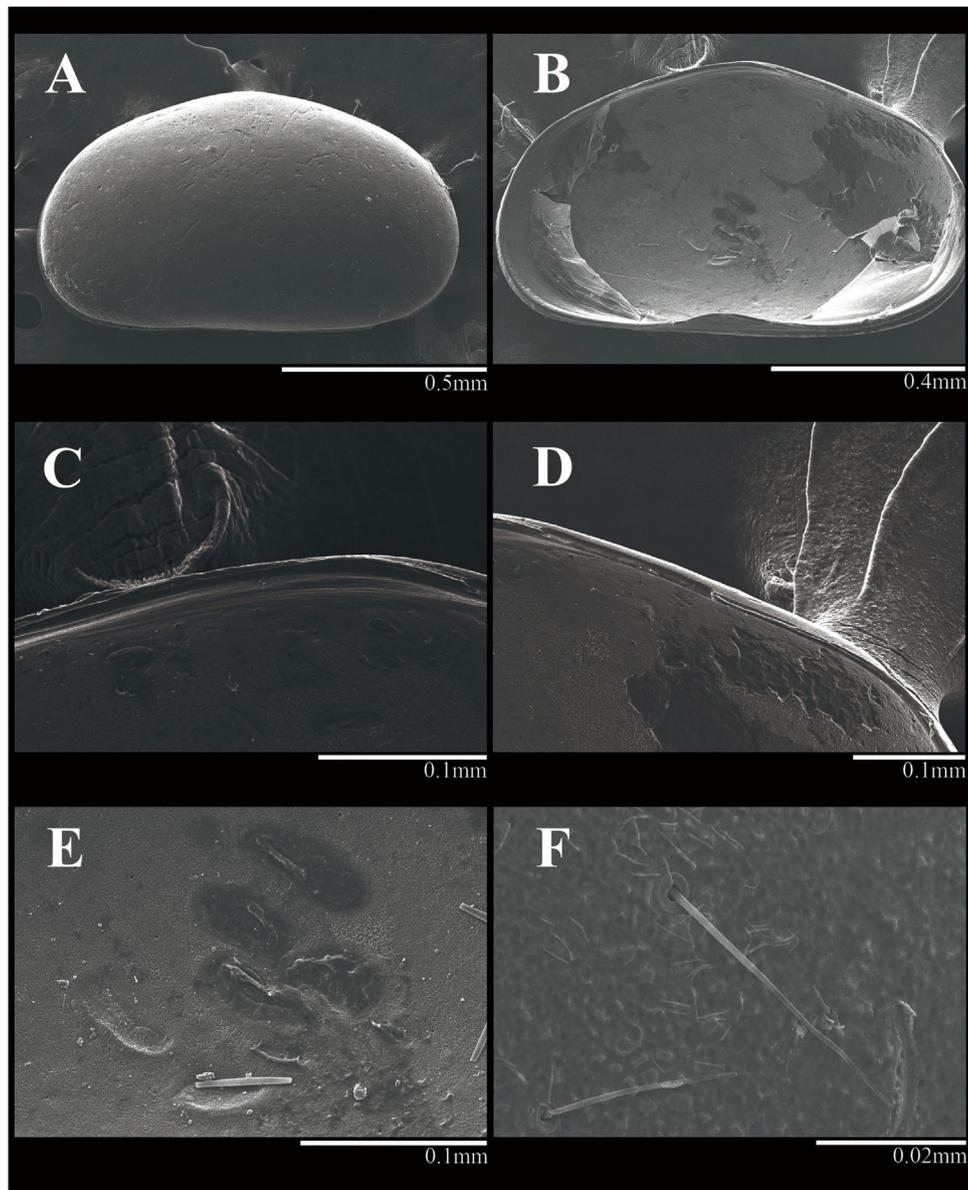
Fig. 2. *Manuelcypris chetumalensis* n. sp., SEM. Right valve adult male: (A) internal view, (B) Anterior margin internal view, (C) antero-ventral margin internal view, (D) Postero-ventral margin internal view.

electrophoresed on 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 (Macrogen, Seoul, South Korea) using the same set of primers.

### 2.3. Molecular data analysis

All obtained sequences were visualized using Finch TV version 1.4.0 (<http://www.geospiza.com/Products/finchtv.shtml>). Each sequence was checked for the quality of signal and sites with possible low resolution, and corrected by comparing forward and reverse strands. BLAST (Altschul et al., 1990) searches revealed that the obtained sequences were ostracod in origin and not contaminants. Each of the three 18S rDNA gene regions, as well as 28S rDNA sequences were aligned in MEGA 6 (Tamura et al., 2013) with ClustalW (Thompson et al., 1994) using default parameters and then concatenated. MEGA was also used for calculation of *p*-distances (Supplementary Tables S1 and S2 in the online version

at DOI: [10.1016/j.jcz.2016.09.003](https://doi.org/10.1016/j.jcz.2016.09.003)). The following phylogenetic analyses were performed: Maximum Likelihood (ML) and Maximum Parsimony (MP) using PAUP 4.0a136 (Swofford, 2002) and the best-fitting evolutionary model. For the best-fitting evolutionary model, the program jModelTest 2.1.6 (Darriba et al., 2012; Guindon and Gascuel, 2003) was used with Akaike information criterion (Hurvich and Tsai, 1989). In the ML and MP analyses the bootstrap values (Felsenstein, 1985) were calculated with 1000 pseudo-replicates. Although the three fragments of 28S rDNA were initially individually aligned, the analyses were performed on three fragments assembled in a single file. In both alignments the internal gaps were removed manually, and in 28S rDNA all sequences were trimmed to the same length, while in the 18S rDNA alignment sequences were only trimmed at the 3' end and not at 5' end (the 3' end was much more variable in terms of length). All sequences (in their original lengths) are deposited in the GenBank (Supplementary Table S3 in the online version at DOI: [10.1016/j.jcz.2016.09.003](https://doi.org/10.1016/j.jcz.2016.09.003)).



**Fig. 3.** *Manuelcypris chetumalensis* n. sp., SEM. Adult female: (A) Left valve outside view, (B) Right valve internal view, (C, D) Dorsal margin internal view, showing the hinge structure (E) Muscle scars imprints, (F) Surface sensilla exiting from rimmed pores.

### 3. Results

#### 3.1. Taxonomic description

Order Podocopida Sars, 1866  
 Suborder Cypridocopina Jones, 1901  
 Family Cyprididae Baird, 1845  
 Subfamily Cyprinotinae Bronstein, 1947

##### 3.1.1. Genus *Manuelcypris* gen. nov

###### 3.1.1.1. Type species. *Manuelcypris chetumalensis* sp. nov.

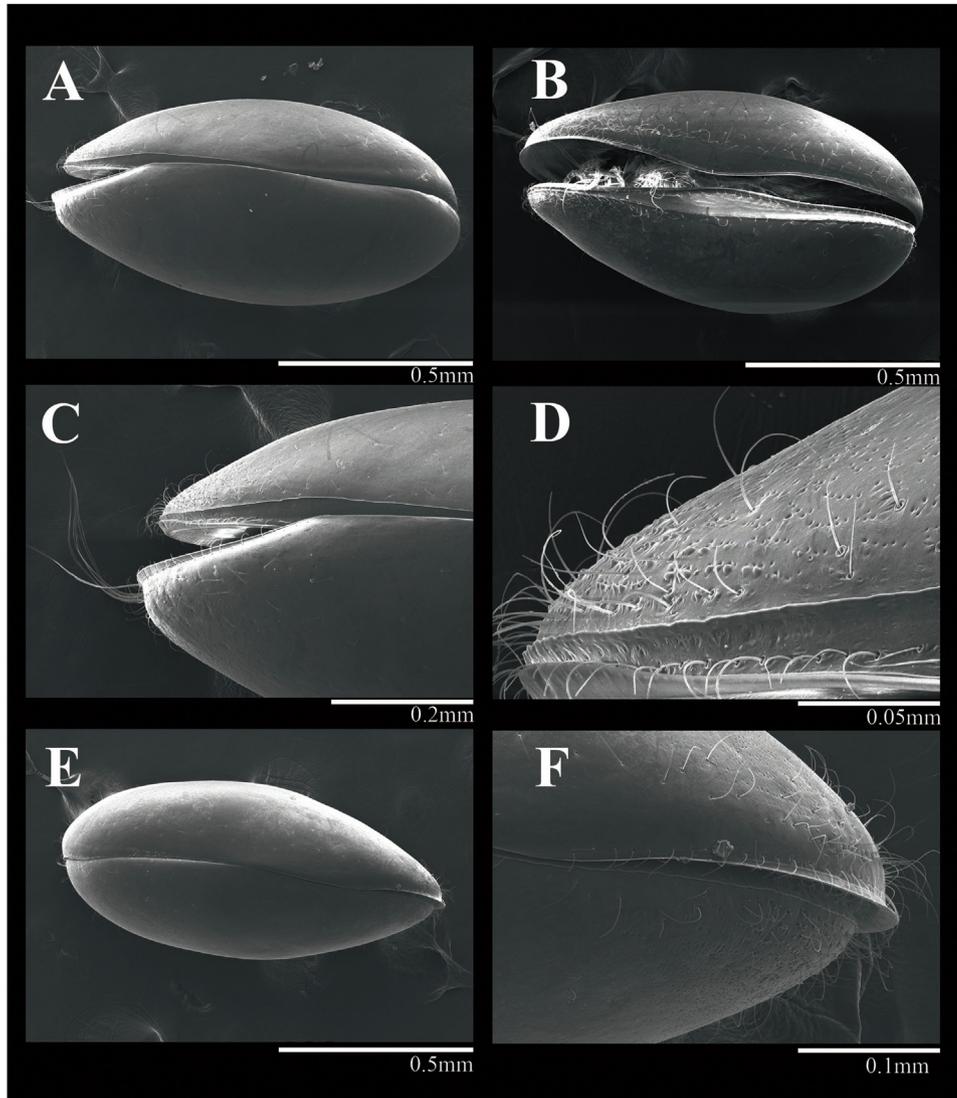
3.1.1.2. Other species. *M. antillensis* (Broodbakker, 1982) comb. nov., *M. cisternina* (Furtos, 1936) comb. nov., *M. punctata* (Keyser, 1975) comb. nov., and *Manuelcypris tabascena* sp. nov.

3.1.1.3. *Diagnosis.* Carapace stout between 0.5 and 1 mm long. In lateral view reniform with relatively low dorsal margin. Surface of the carapace smooth to poorly ornamented. RV sometimes with developed marginal tubercles. Selvage on both valves peripheral. Inner list only anteriorly present. Marginal pore canals relatively

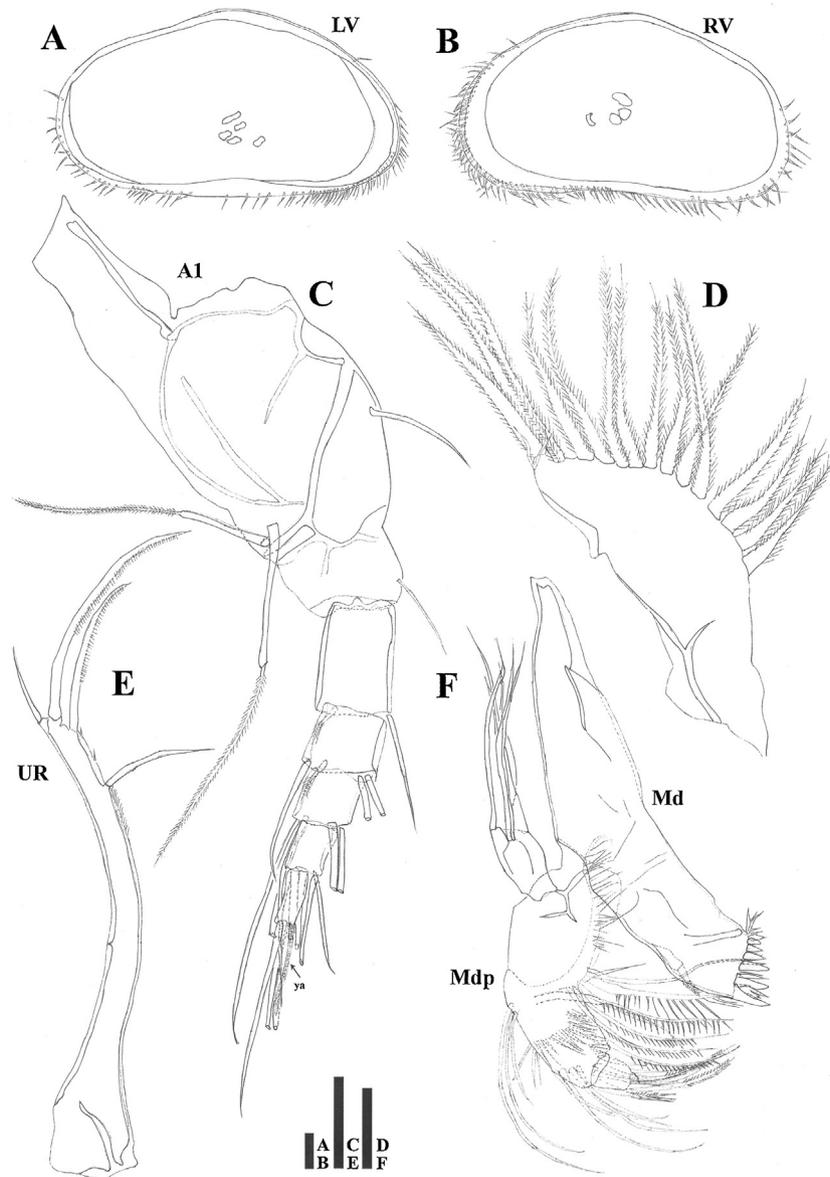
short and straight. A1–7 segmented, with small Rome's and Wouters organs; A2 sexually dimorphic with respect of terminal claws and setal elongation. A2 swimming setae at least reaching the tip of the claws. Maxillular palp with cylindrical terminal segment. L5 without "c" setae, but with "b" and "d" setae present. Setae "a" and "a'" sometimes missing as well. Prehensile palps asymmetrical with left palp being smaller than the right one. L6 5-segmented, without basal setae. L7 typical for the family. UR with both claws and both setae well-developed, the latter being long. Hemipenis with two shields: lateral and medial.

3.1.1.4. *Etymology.* The genus is named in honor of Dr. Manuel Elias-Gutierrez from "El Colegio de la Frontera Sur (ECOSUR)" (Chetumal, Mexico) for his continuous support and effort in the molecular studies of speciation in diverse crustacean taxa from the Neotropical region.

##### 3.1.2. *Manuelcypris chetumalensis* sp. nov (Figs. 2, 3, 4, 5, 6, 7, 8)



**Fig. 4.** *Manuelcypris chetumalensis* n. sp., SEM: (A) carapace dorsal view, (B) carapace ventral view, (C) carapace antero dorsally showing the differences in carapace size and overlapping, (D) anterior marginal pore canals ending with setae, (E) carapace ventral view, right valve overlapping left valve ventrally (F) Carapace anteriorly showing carapace overlapping.



**Fig. 5.** *Manuelcypris chetumalensis* n. sp. Adult male: (A) Left valve internal view, (B) Right internal view, (C) A1, (D) Respiratory plate, arrow showing distal short setae, (E) UR, (F) Md and Mdp. Scale bars = 0.05 mm.

**3.1.2.1. Type material.** Holotype: adult male dissected on one slide (ECO-CH-Z-09334), Allotype: adult female dissected on one slide (ECO-CH-Z-09335), Paratype: one adult male and one adult female dissected one slide each, approximately 15 specimens preserved in 99% ethanol, deposited at the Marine Biodiversity Institute of Korea (MABIK CR00235325).

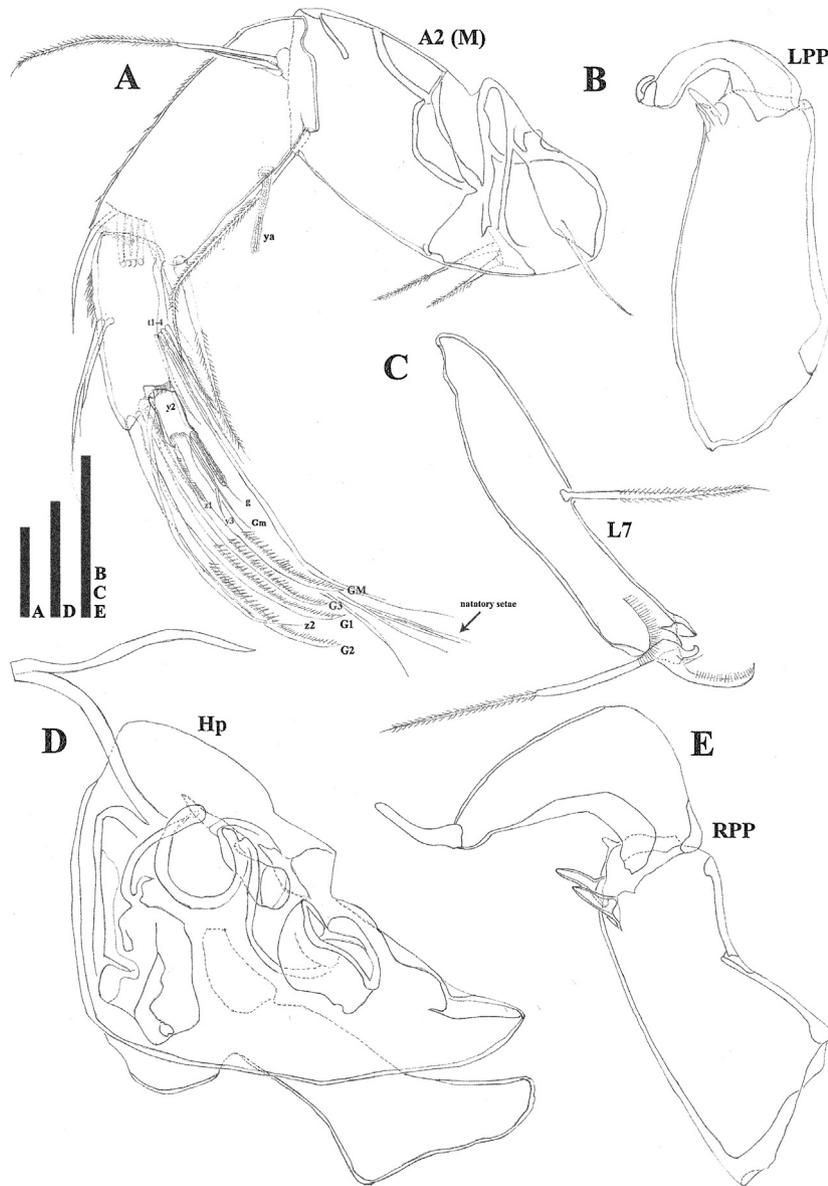
**3.1.2.2. Type locality.** Near mouth of rain water sewer, Chetumal bay, Chetumal, Quintana Roo, México, 18° 31' 40.65"N, 88° 15' 57.52"W (Fig. 1a).

**3.1.2.3. Etymology.** The species is named after Chetumal Bay (Mexico), from where it was collected.

**3.1.2.4. Diagnosis.** Carapace relatively small, not exceeding 1 mm in length. Dorsal margin of carapace slightly arched. Greatest height around middle but slightly toward anterior end. Anterior margins with abundant setaetae. Valves without marginal tubercles. A2 swimming setae by far exceeding tips of terminal claws. L5 with “b” and “d” setae present. L6 with basal segment bare and with e-seta

just reaching the distal end of the following segment. UR almost straight in female but strongly arched in male. Anterior claw reaching the middle of the ramus. Genital field round with one projection. Hemipenis with a-lobe slightly shorter than b-lobe.

**3.1.2.5. Description of male.** Carapace in lateral view elongated (Figs. 2A and 5A, B). Valves asymmetrical, LV overlapping RV anteriorly, posteriorly and ventrally (Fig. 4E, F). Carapace with dorsal margin slightly arched. Greatest height around middle, slightly toward anterior end. Anterior and posterior margins broadly rounded, anterior margin slightly narrower than posterior one. Ventral margin fairly covered by setaetae and slightly concave around middle. Calcified inner lamella shorter posteriorly than anteriorly. Anteriorly equaling 8.2% and posteriorly 6.1% of total length of the carapace. Marginal pore canals straight, setae more abundant anteriorly than posteriorly. Muscle scars imprints consisting of five grouped scars (Fig. 2A). Inner list well-developed anteriorly (Fig. 2B, C). Surface of carapace smooth, except anteriorly where small pits are present (Fig. 4F). Relatively prominent warts present on RV anteriorly (Fig. 4F), on the LV warts very



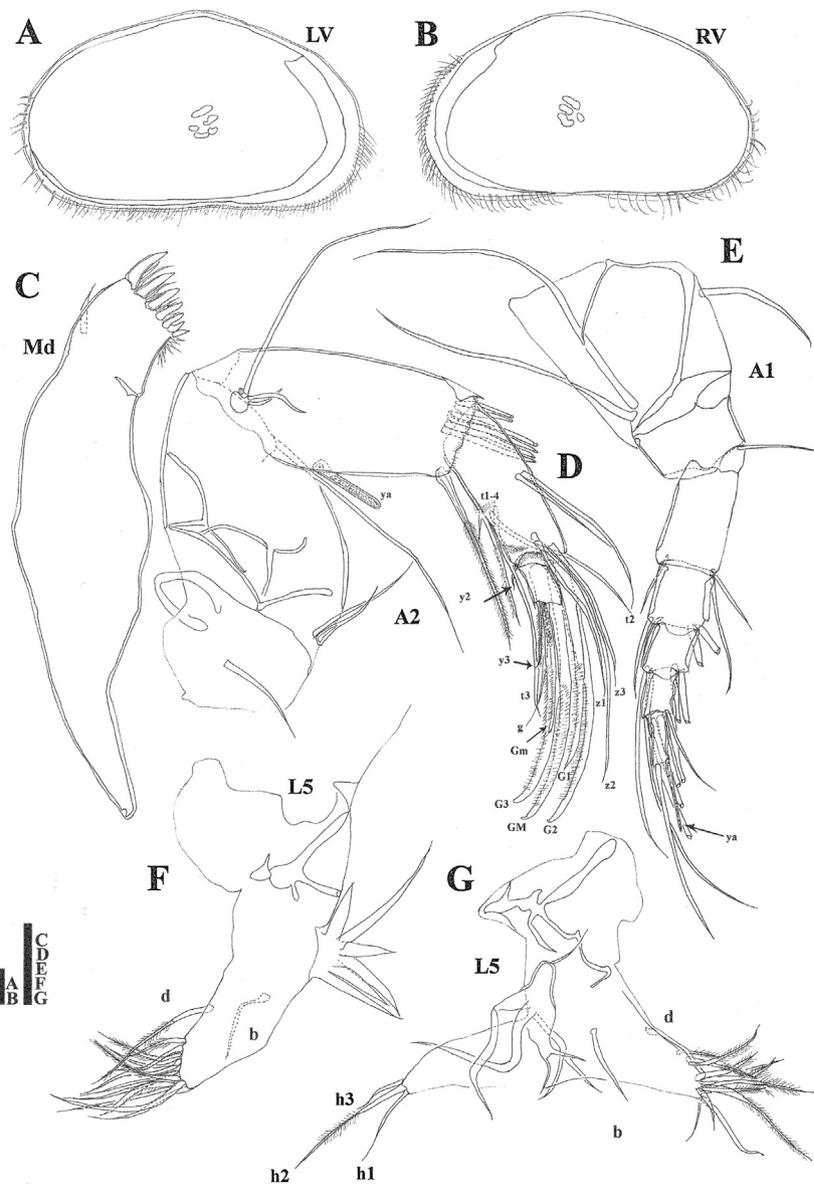
**Fig. 6.** *Manuelcypris chetumalensis* n. sp. Male: (A) A2; (B) left prehensile palp; (C) Distal section of L7; (D) Hemipenis; (E) Right prehensile palp. Scale bars = 0.05 mm.

small. Surface sensilla exiting from rimmed pores. No marginal tubercles present on either of valves. Length of valves (holotype): LV = 500  $\mu\text{m}$ , RV = 483  $\mu\text{m}$ .

A1 (Fig. 5C). 7-segmented. First segment anteriorly with one smooth seta that just reaches the distal end of the segment. Postero-distally, two unequally long serrulate setae. Longer one reaching the distal end of penultimate segment while shorter one just exceeding the distal end of fifth segment. Second segment with one antero-apical smooth seta that exceeds the middle of following segment. Rome's organ not observed. Third segment is carrying apically one anterior and one posterior seta. Anterior one just reaches the distal end of fifth segment. Posterior seta is serrulate and it exceeds the middle of following segment. Fourth segment with two long and bare setae antero-distally; postero-distally on this same segment, two unequally long setae, longer one bare and almost reaching the middle of terminal segment. Fifth segment antero-distally with two long and bare setae; postero-distally with two unequally long setae, both by far exceeding distal end of terminal segment. Sixth segment with five bare setae. The four most anteriorly located are long while the most posterior seta is short.

Terminal segment is carrying two long and one short setae in addition to the aesthetasc *ya*, which is 1.8 times longer than terminal segment. Length ratios of last five segment 1.8: 1.2: 1: 0.8: 1.

A2 (Fig. 6A) 5-segmented. Coxa is carrying medially one long and smooth seta that exceeds the distal end of the coxa. Postero-distally on the same segment, two unequally long serrulate setae not reaching the middle of following segment. Basis postero-apically with one long and serrulate seta that just exceeds the middle of penultimate segment. Exopod consists in a plate with three unequally long setae. Longer one is serrulate and just exceeds the distal end of following segment. The two most posterior setae are smooth and short not reaching the middle of following segment. First endopodal segment hirsute with aesthetasc Y relatively long, 1.5 times longer than terminal segment. Postero-distally on the same segment one serrulate seta is exceeding the distal end of terminal segment. Natatory setae are not equally long. The five most posteriorly located are smooth and long, exceeding the tips of the claws. Most anterior seta short, reaching the middle of following segment. Second endopodal segment with t-setae (t1–t4) unequally long. Antero-medially two smooth and unequally long setae. Seta z2 elongated but not



**Fig. 7.** *Manuelcypris chetumalensis* n. sp. Female: (A) Left valve internal view; (B) Right valve internal view; (C) Mandible; (D) Antenna: A2; (E) Antennula: A1; (F, G) First thoracopod: L5. Scale bars = 0.05 mm.

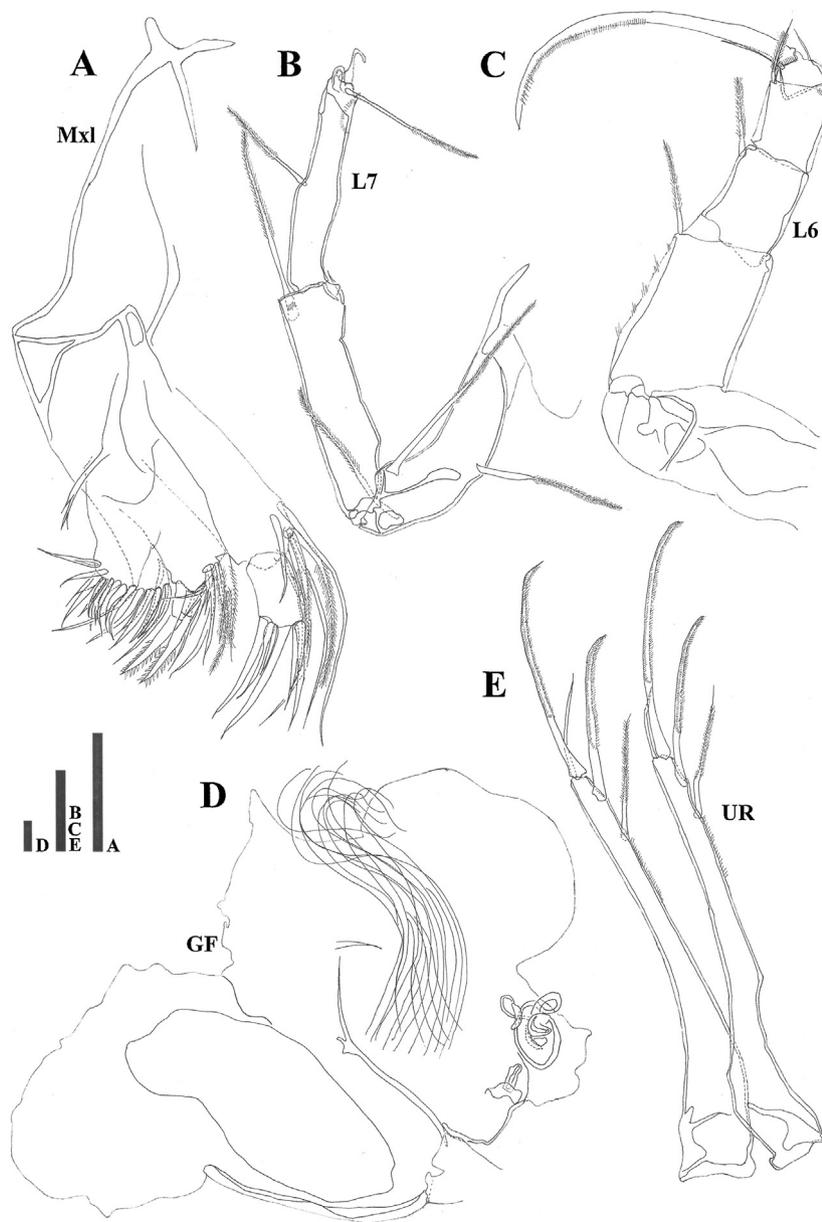
reaching the tips of terminal claws; seta z1 claw-like two times as long as terminal segment. Claws G1 and G2 subequally long, 5.2 times longer than terminal segment, both distally strongly serrate. Claw G3 slightly smaller than adjacent claws, five times longer than terminal segment, distally strongly serrated as well. Terminal segment carrying two smooth and equally long setae. Aesthetasc *ya* slender, 1.2 times longer than terminal segment. Gm claw strong and elongated with 5.1 times the length of terminal segment. GM claw seta-like.

Md. (Fig. 5F) Palp 4-segmented. First segment with one long and smooth seta that exceeds by far the distal end of terminal segment. Setae S1 and S2, thick and pappose,  $\alpha$ -seta slender, smooth and relatively long, reaching the middle of following segment. Respiratory plate with six smooth rays. Second segment dorso-distally with three unequally long setae, longer ones reaching the distal end of terminal segment while shorter one just exceeds the middle of following segment. Ventro-distally of this same segment, four approximately equally long setae are present, the most ventral ones pappose while the most dorsal one bare. Third segment hirsute

with a row of long setae medially. Dorso-distally with a bunch of four smooth and long setae, all of them overpassing by far the distal end of terminal segment. Apically on this segment four unequally long setae.  $\gamma$ -seta pappose and twice as long as terminal segment. Fourth segment (terminal segment) almost equally long as wide and carrying two bare and two pappose setae.

Mxl. Palp 2-segmented. First segment elongated and carrying six apical setae. Two of them serrulate while the other four smooth. Terminal segment 1.3 times longer than wide and carrying three long claw-like setae and three slender and smooth setae. Third endite with nine apical setae, three of them serrulate while the other six smooth. Teeth bristles strongly serrated distally. Second and first endite with eight and eleven smooth setae respectively. Branchial plate (Fig. 5D) with fifteen long and pappose rays followed by two small and smooth setae.

L5. Protopodite apically with twelve setae, three of them smooth and the others serrulate. Seta b- smooth and d-seta serrulate. Exopodite consisting in a plate with six rays. Palps asymmetrical and two segmented. First segment of right palp with three



**Fig. 8.** *Manuelcypris chetumalensis* n. sp. Female: (A) Mxl; (B) L7; (C) L6; (D) Genital field; (E) UR. Scale bars = 0.05 mm.

outgrowths forming small projections; terminal finger stout and strongly sclerified (Fig. 6E). Left palp carrying on first segment two small projections; terminal finger strongly curved (Fig. 6B). Fingers on both palps terminating in a nipple-like seta.

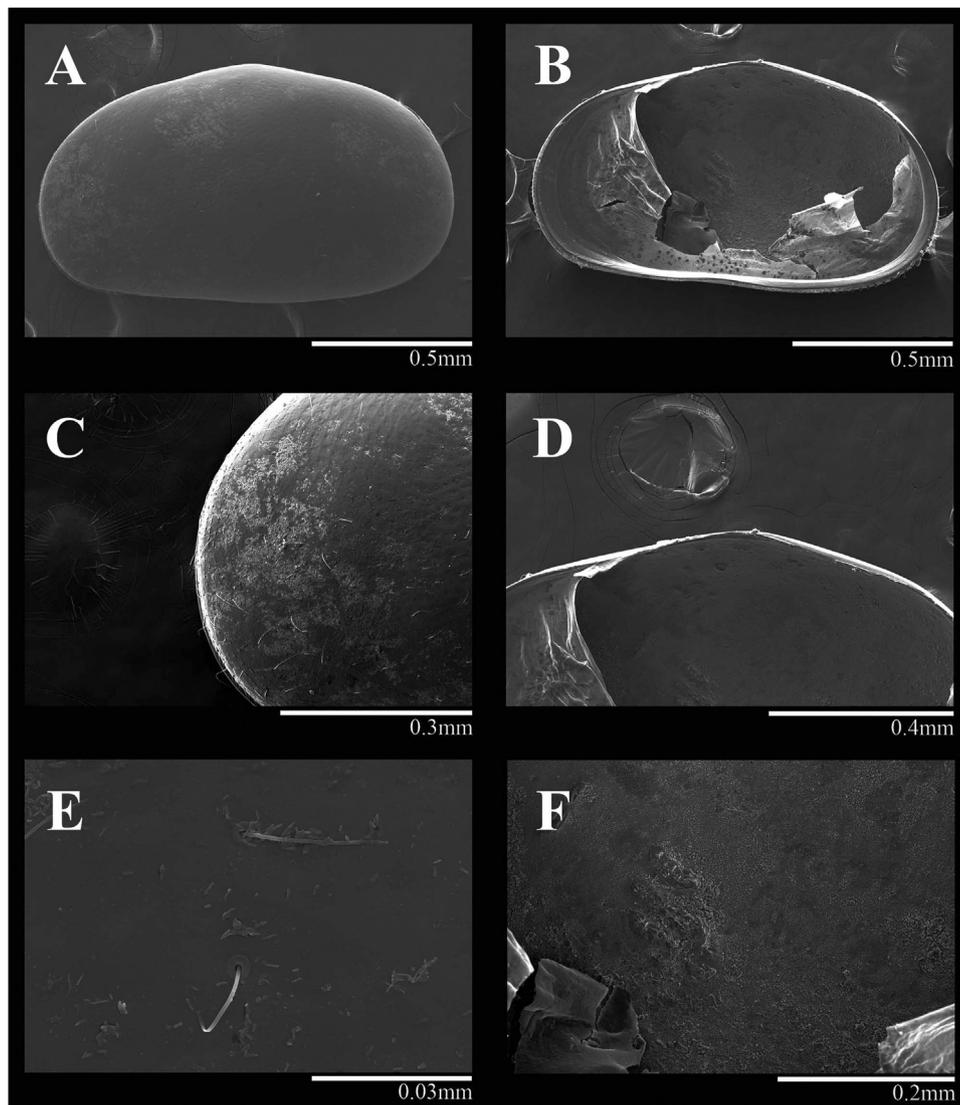
L6. 5-segmented. Basal segment bare. First endopodal segment slightly hirsute and with a relatively short and serrulate e-seta that not reaches the distal end of following segment. Second endopodal segment with f-seta serrulate and short, just reaching distal end of following segment. Third endopodal segment with g-seta short and serrulate. Terminal segment carrying h1 and h3 setae short and smooth. Terminal claw (h2-setae) equaling 1.3 times the length of last three segment combined.

L7 (Fig. 6C). 4-segmented. Basal segment with d1, d2 and d3 setae long and serrulate. Seta "e" exceeding the middle of following segment. Seta f- serrulate and just reaching distal end of the segment that carries it. Terminal segment with h1-seta missing, h2-seta claw-like and distally hairy and h3-seta serrulated and elongated.

UR (Fig. 5E). Ramus curved and postero-distally slightly hirsute. Anterior claw distally serrate and reaching the middle of the ramus. Anterior seta short and smooth, reaching one third of the anterior claw. Posterior claw curved and distally serrated, equaling one third of the length of the ramus. Posterior setae smooth, and slightly smaller than posterior claw. Length ratios between ramus, anterior and posterior claws equaling 2.8: 1.4: 1.

Hemipenis (Fig. 6D) with the a-lobe and b-lobe well developed. Lobe a, triangular shaped. Lobe b foot-like and slightly larger than a-lobe. Internal ducts only once coiled. Ejaculatory process simple.

**3.1.2.6. Description of female.** Carapace similar to male but slightly smaller. In lateral view elongated (Fig. 7A, B). Calcified inner lamella differing in length with male. Anteriorly equaling 11.2% and posteriorly 3.2% of total length of the carapace. Muscle scars imprints as in males consisting in five grouped scars (Fig. 7A, B), most posterior ones, elongated. Surface of carapace and valve overlap same as in male (Fig. 4A–D) smooth. Carapace size: LV = 487.5  $\mu$ m and RV = 475  $\mu$ m.



**Fig. 9.** *Manuelcypris tabascena* n. sp., SEM. Female: (A) Left valve outside view; (B) Right internal view; (C) Left valve anteriorly showing the small wart-like structures; (D) Dorsal margin internal view; (E) Carapace surface with setae exiting from a pore; (F) Muscle scars imprints.

A1 (Fig. 7E). Similar as in male with exception of some of the posterior setae (on the third and fourth segments) that are smooth instead of serrulate as in male. Third segment is carrying two antero-distally setae, at difference of male that is carrying one seta in the same segment. Rome's organ was not observed.

A2 (Fig. 7D). 5-segmented. Setae from coxa and basis are smooth at difference of males in which all setae are serrulate. t-seta (1–4) shorter than in male. All z-seta present, being slender and smooth. Seta z1, longer than the other z-setae. Postero-distally on penultimate segment there is one small short seta, that is absent in male. G1 and G3 claw subequally long, 5.6 times longer than terminal segment. G2 claw reduced, 4.8 times longer than terminal segment. Third endopodal segment (terminal segment) with GM seta rescues beyond the tips of G1 and G3. Gm claw, slender and equaling 2.8 times the length of terminal segment. Aesthetasc ya short and accompanied by two setae, longer one smooth, while shorter one serrulate.

Md (Fig. 7C), Mx1 (Fig. 8A) as in male.

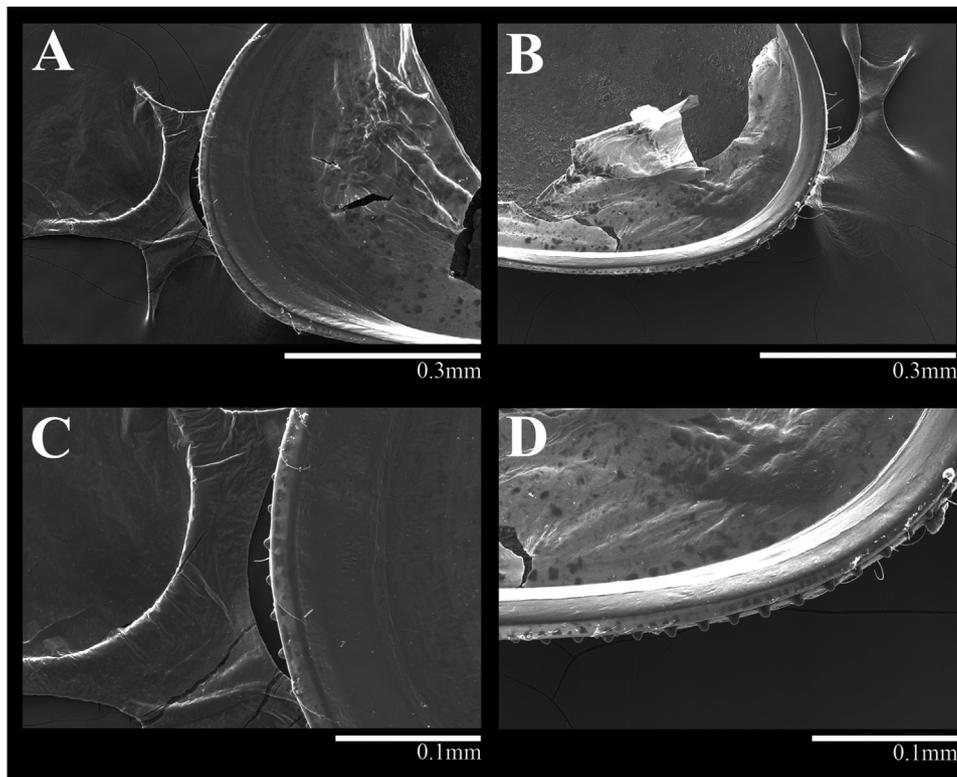
L5 (Fig. 7F, G). Endopodite with three apical setae, longer one serrulate and shorter ones smooth. Exopodite consisting in a plate with six rays. Protopodite with b-seta smooth and d-seta serrulate.

Apically with twelve setae serrulate, except three of them which are smooth.

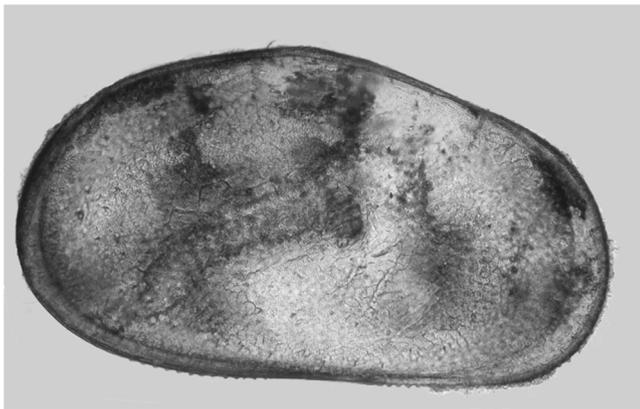
L6 (Fig. 8C) and L7 (Fig. 8B) as in male.

UR and genital process (Fig. 8D, E). Ramus (Fig. 8E) postero-distally slightly hirsute and straighter than in male. Anterior claw is slightly curved reaching one half of the length of the ramus. Posterior claw with tip curved and reaching slightly more than one third of the length of the ramus. Anterior seta smooth not reaching the middle of anterior claw. Posterior seta serrulate and slightly smaller than posterior claw. Length ratios between ramus, anterior and posterior claws are as follows: 2.5:1.4: 1. Genital field (Fig. 8D) mostly rounded but with one projection carrying sensorial organs.

**3.1.2.7. Remarks.** *Manuelcypris chetumalensis* and *M. cisternina* are the only two species in the genus without any marginal tubercles. *Manuelcypris cisternina*, unlike *M. chetumalensis* have neither of the two "a" setae on the L5, and has less elongated carapace in the lateral view. *Manuelcypris chetumalensis* differs from *M. tabascena* also by other carapace characteristics, such as more steeply inclined dorsal margin. Of the soft parts, the most prominent difference is



**Fig. 10.** *Manuelcypris tabascena* n. sp., SEM. Female Right valve: (A) antero-ventrally internal view showing the inner list; (B) postero-ventrally internal view; (C) detail of anterior marginal tubercles; (d) detail of posterior marginal tubercles.



**Fig. 11.** *Manuelcypris tabascena* n. sp., Female: Right valve internal view.

the number of swimming setae on A2 (six in *M. chetumalensis* and five in *M. tabascena*), in addition to the fine differences in the lengths of the L6 and L7 setae, and length ratio between anterior and posterior claw on the UR. *Manuelcypris chetumalensis* is also almost two times smaller than *M. tabascena*. *Manuelcypris chetumalensis* has similar hemipenis and prehensile pals like both *M. antillensis* and *M. punctata* with a little less pronounced “heel” part of the outer lobe on the hemipenis and less convex distal margin of the same part. In addition, the new species differs from *M. antillensis* in a considerably longer posterior seta on the UR, and longer setae on the L6, and from *M. punctata* in the absence of pits on the surface of the carapace.

### 3.1.3. *Manuelcypris tabascena* sp. nov (Figs. 9, 10, 11, 12, 13)

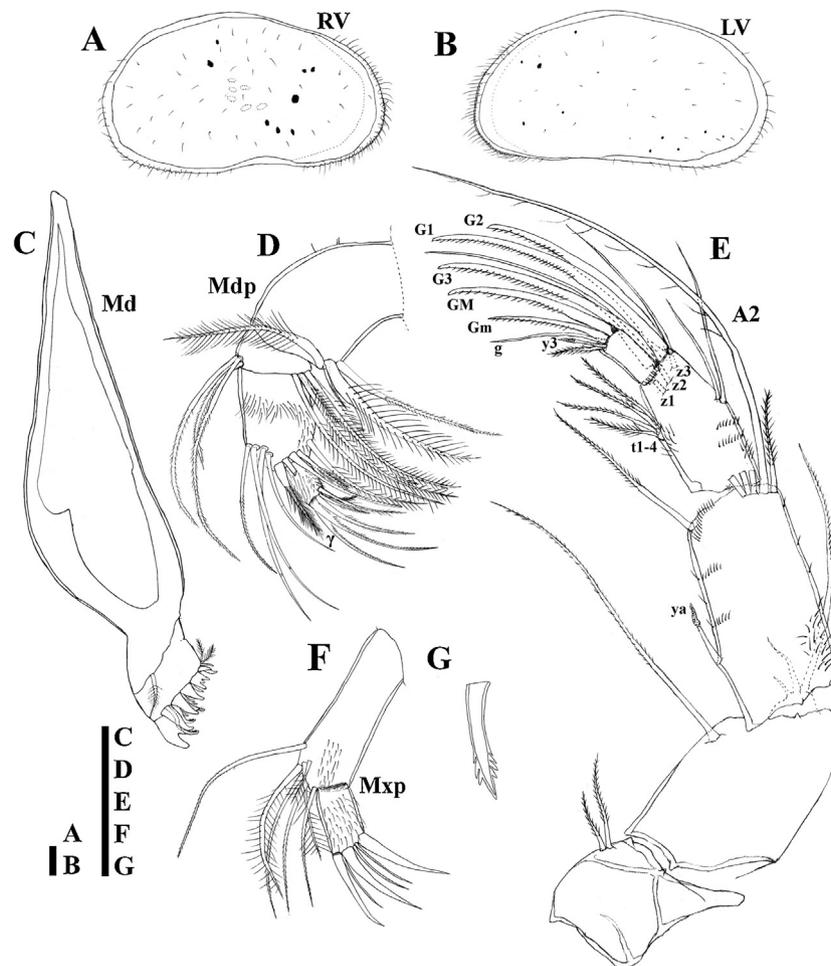
**3.1.3.1. Type material.** Holotype: adult female dissected on one slide (ECO-CH-Z-07708). Paratype: adult female dissected on one slide from Acayucan-Cosamaloapan wetlands (ECO-CH-Z-07709). Other material: 17 specimens from type locality, all females preserved in 70% ethanol (ECO-CH-Z-09333).

**3.1.3.2. Type locality.** Lake Vernet, Tabasco Mexico 17° 52' 22.8"N, 92° 32'45.1"W. Other localities Miguel Hidalgo wetlands (17°57'23.5"N, 92°25'48.7"W), Acayucan-Cosamaloapan wetlands (18°12'04.4"N, 95°36'23.3"W), Pond next Jonuta-Escárcega road (18°06'34.1"N, 92°04'41.3"W) (Fig. 1b).

**3.1.3.3. Etymology.** The name is after the Tabasco, Mexican state, where this species is mainly distributed.

**3.1.3.4. Diagnosis.** Medium size organisms, almost reaching 1 mm in length. Carapace with dorsal margin slightly arched. Greatest height located slightly around the middle but slightly toward posterior end. Right valve with marginal tubercles ventro-anteriorly and posteriorly. Five swimming setae on A2, longer ones exceeding the tips of distal claws. L5 with b- and d- setae. Basal segment on L6 bare and e-seta exceeding the distal end of following segment. UR straight with anterior claw reaching the half length of the ramus. Genital field round.

**3.1.3.5. Description of female.** Carapace in lateral view elongated (Figs. 9A, 11 and 12A, B). LV slightly larger than RV. Carapace in dorsal margin slightly arched, greatest height around middle but slightly toward posterior end (Fig. 9B, D). Greatest height equaling 57% of total length of carapace. Carapace with anterior and posterior margins broadly rounded, anterior margin narrower than posterior one. Ventral margin covered by sparsely setae and slightly concave around mid-length. Right valve in antero and postero-ventral margins with small tubercles (Figs. 10 and 11). Left valve



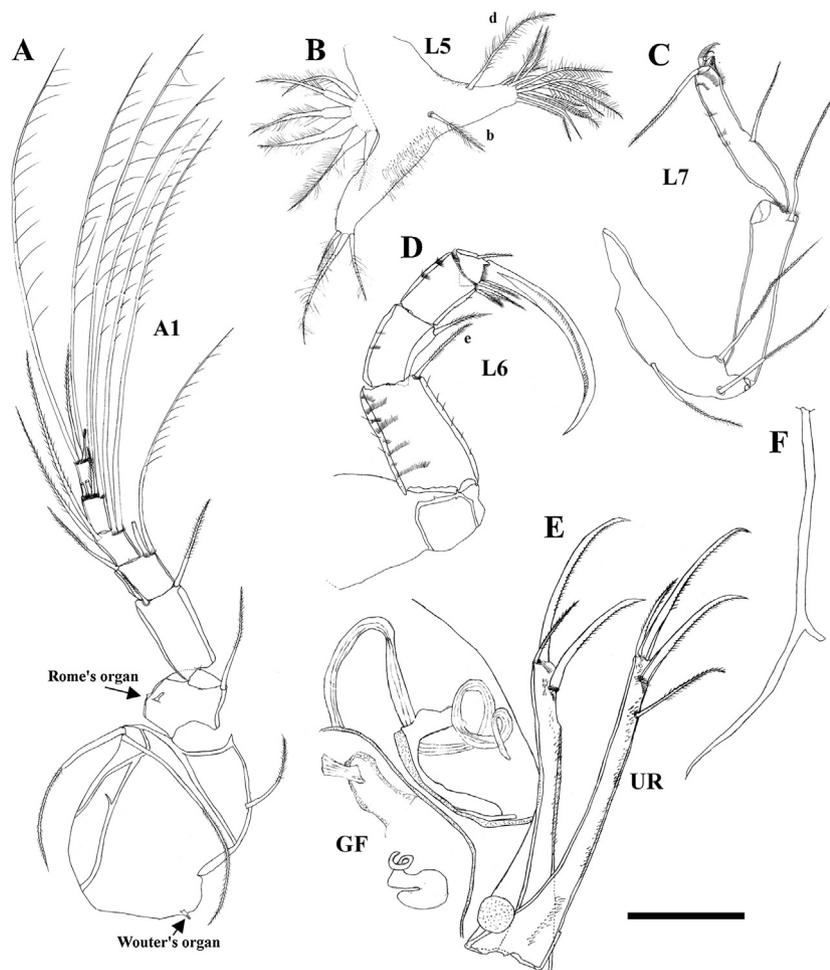
**Fig. 12.** *Manuelcypris tabascena* n. sp. Female: (A) Right valve external view; (B) Left valve external view; (C) mandible; (D) Mdp; (E) A2; (F) Mxp; (G) teeth bristle of the third endite of maxilla. Scale bars = 0.1 mm.

anteriorly with very small wart-like structures (Fig. 9C). Calcified inner lamella narrow, anteriorly equaling 9% and anteriorly 3.6% of total length of carapace. Marginal pore canals straight and denser anteriorly than posteriorly. Selvage peripheral. Muscle scars consisting on four compact scars and two elongated scars more antero-ventrally (Fig. 9F). Surface of carapace smooth, to finely pitted (Figs. 9E, 11 and 12A, B). Surface sensilla exiting from rimmed pores (Fig. 9E). Length of LV = 971  $\mu\text{m}$ , length of RV = 942  $\mu\text{m}$ .

A1 (Fig. 13A) 7-segmented. First segment is carrying postero-distally two unequal serrulate setae, longer one is just reaching the middle of the sixth segment while shorter one is just reaching the distal end of the fourth segment. Antero-medially on the same segment one serrulate and short seta is just reaching the distal end of the segment. Wouter's organ small, located proximally on the segment. Second segment, postero-medially with the Rome's organ short. Antero-distally a serrulate seta is just overpassing the distal end of following segment. Third segment is carrying two apical setae. The most anterior one is serrulate and reaching the distal end of fifth segment, while the most posterior seta is serrulate and short, not reaching the distal end of following segment. Fourth segment posteriorly with one serrulate seta that exceeds the distal end of penultimate segment; anteriorly two subequally long and hairy setae, 7.1 times longer than terminal segment. Fifth segment with two postero-distal serrulate setae, both by far exceeding the distal end of terminal segment. Antero-distally with two hairy and equally long setae, 11.2 times longer than terminal segment. Sixth segment hirsute distally and

carrying five apical setae. Four of them hairy and subequally long, 13.5 times longer than terminal segment. Shorter seta is serrulate and 1.2 times longer than terminal segment. Seventh segment (terminal segment) hirsute distally and bearing two long and hairy setae, 11.6 times longer than the segment; one short and serrulate seta, 3.8 times longer than terminal segment and the aesthetasc ya, which is about equally long than the terminal segment. Length ratios of last five segments are as follows 2.4:1.1:0.9:1.

A2 (Fig. 12E). 5-segmented. Coxa is carrying two postero-distally short and serrulate setae, longer one just reaching the middle of following segment while shorter one reaches one third of next segment. Basis with one long and serrulate seta reaching the middle of the penultimate segment. Exopod is reduced to a plate with three unequally long setae. Longer one serrulate and exceeding the distal end of the following segment. Medial seta is smooth and reaching one third of the following segment. Most posterior seta is very small. First endopodal segment hirsute with aesthetasc Y, 1.3 times longer than terminal segment. Postero-distally on this same segment a slender and serrulate seta is just exceeding the distal end of the following segment. Five natatory setae are located antero-distally on this segment, four of them are slightly setulae and equally long, overpassing the tips of the terminal claws. Shorter seta is serrulate and just exceeding the middle of the penultimate segment. Second endopodal segment hirsute with all t-setae present. Seta t1 just reaching the proximal end of the terminal segment, while all other t-setae exceeding the distal end of terminal segment. Antero-medially on this segment



**Fig. 13.** *Manuocypris tabascena* n. sp. Female: (A) A1; (B) L5; (C) L7; (D) L6; (E) UR and genital field; (F) UR attachment. Scale bar = 0.1 mm.

two smooth setae are overpassing the distal end of terminal segment. Setae z1 and z2 are slender and subequally long, exceeding the tips of terminal claws. Seta z3 short, 3.3 times the length of terminal segment. Claws G1 and G3 subequally long, five times the length of terminal segment. Claw G2, slightly smaller than adjacent claws, 4.2 times the length of terminal segment. Third endopodal segment (terminal segment) with GM claw long and distally serrulate, four times the length of terminal segment. Gm claw short, two times as long as terminal segment. Two accompanying setae are present as well on this segment, longer one smooth while shorter one serrulate. Aesthetasc ya, slender and slightly smaller than terminal segment.

Md (Fig. 12C). Palp 4-segmented (Fig. 12D). First segment ventrally with four setae. The most proximal one is long and serrulate. Setae S1 and S2 pappose and subequally long, overpassing the distal end of terminal segment.  $\alpha$ -seta slender and short, just exceeding the distal end of the following segment. Second segment dorso-distally with three unequal serrulate setae. Longer ones exceeding the distal end of terminal segment while shorter one not reaches the distal end of following segment. Vento-distally of this same segment with four pappose and equally long setae, five times the length of terminal segment.  $\beta$ -seta small and pappose, just exceeding the middle of following segment. Third segment hirsute with a row of setae medially. This segment is carrying dorso-distally four unequal setae, the most external one serrulate, while the other three smooth. Antero-apically on this segment with four dissimilarly long setae, all of them pappose and by far exceeding the distal end of terminal segment.  $\gamma$ -seta is strongly pappose

and elongated, by far overpassing the distal end of last segment. Fourth segment (terminal segment) hirsute, with 1.2 times longer than wide. This segment is carrying distally six setae, all of them serrulate but unequally long. Longer seta with 2.6 times the length of the segment.

Mxl. Palp 2-segmented (Fig. 12F). First segment hirsute and distally with four long serrulate setae and three pappose setae. Second segment hirsute with three claw-like setae subequally long, 1.2 times longer than terminal segment and other three smooth and slender setae. Third endite of maxilla with two large bristles apically strongly serrated (Fig. 12G).

L5 (Fig. 13B). Protopodite apically with twelve unequal long serrulate or plumose setae. Setae b- and d- hairy and long. Endopodite with three apical setae unequal long and pappose. Exopodite consisting of a plate and six pappose rays.

L6 (Fig. 13D). 5-segmented. Basal segment bare. First endopodal segments hirsute with e-seta serrulate and long, exceeding the distal end of the following segment. Second endopodal segment with f-seta serrulate and just reaching the distal end of following segment. Third endopodal segment apically with g-seta short and serrulate and one additional seta smaller and serrulate. Terminal segment carrying a strong claw (h2 seta), distally curved and serrated. Setae h1 and h2 slender and serrulate.

L7 (Fig. 13C). Basal segment with d1, d2 and dp setae long and serrulate. Second segment with e-seta serrulate and long, overpassing the half length of following segment. Third segment with f-seta serrulate and short, just reaching the distal end of the segment.

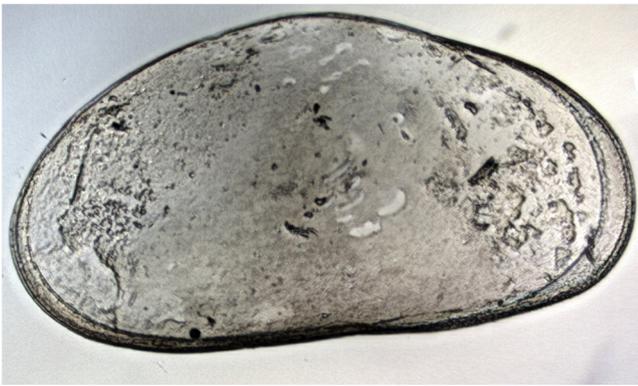


Fig. 14. *Eucypris cisternina* Furtos, 1936. Female: Right valve external view.

Terminal segment with h1-seta small and hook-like, h2-seta claw-like and distally serrulated, h3-seta long and serrulate.

UR and genital process (Fig. 13E). Ramus slender and hairy along posterior margin. Terminal claws slightly curved and distally serrulated. Anterior claw equaling one half of the length of ramus. Anterior seta serrulate and not reaching the half length of anterior claw. Posterior claw slightly serrulate and reaching two fifths of the length of the ramus. Posterior seta serrulate and exceeding the half length of posterior claw. Length ratios between ramus, anterior and posterior claws equaling 2.6: 1.2: 1. UR attachment distally bifurcated (Fig. 13F). Genital process rounded.

3.1.3.6. *Remarks.* *Manuelcypris tabascena*, possess marginal tubercles along the RV and because of this, it is more similar to *M. antillensis* and *M. punctata* than to the other two species. However, unlike, *M. antillensis*, this new species has neither of the “a” setae on the L5 what places it closer to *M. punctata* and *M. chetumalensis*. *Manuelcypris tabascena* also has only five swimming setae on the A2 and, unlike *M. punctata* does not have prominent pits on the surface. Additional difference from *M. antillensis* is a much longer posterior seta on the UR.

3.1.4. *Manuelcypris cisternina* (Furtos, 1936) *comb. nov*  
(Figs. 14, 15, 16, 17)

3.1.4.1. *Synonymy.* *Eucypris cisternina* n. sp. - Furtos (1936), p. 107, Figs. 124 -127.

3.1.4.2. *Material examined.* Holotype, adult female, SM - 67971. Champotón, Campeche, Yucatán península, México, 19°21'N, -90°43'W.

3.1.4.3. *Diagnosis.* Relatively small organisms, less than 1 mm in length. Carapace elongated with dorsal margin slightly arched. Greatest height located in the middle. Surface of carapace smooth. Ventral margin straight but little convex at mouth region. Swimming setae of A2 slightly overpassing the tip of claws. L5 with small and bare a-setae, serrulate b-seta and pappose d-seta. L6 with basal segment bare and e-seta relatively short, not reaching the distal end of following segment. UR straight with anterior claw not reaching the middle of ramus.

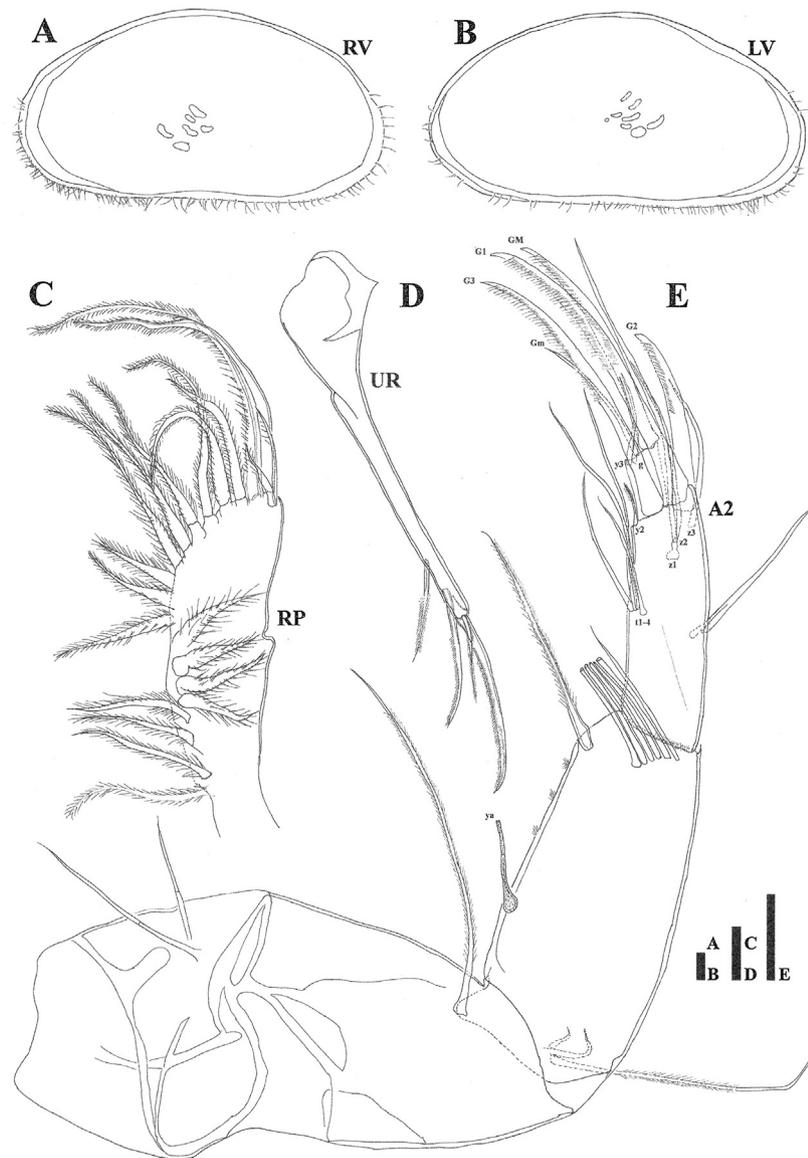
3.1.4.4. *Redescription of female.* Carapace in lateral view elongated (Figs. 14 and 15A, B). LV slightly larger than RV. Carapace in dorsal margin slightly arched, greatest high located around middle and equaling 50% of total length of the carapace. Anterior and posterior margins broadly rounded and covered by sparsely setae. Posterior margin slightly narrower than anterior one. Ventral margin almost straight, except in the mouth region where it is slightly

convex (Fig. 14). Calcified inner lamella is equaling 6.3% anteriorly and 3.2% posteriorly of the total length of carapace. Marginal pore canals straight and denser anteriorly than posteriorly. Muscle scars imprints consisting of four small and compact scars medially and two slightly elongated scars located more antero-ventrally. No marginal tubercles present. Surface of carapace smooth. Length of LV = 650  $\mu$ m. Length of RV = 620  $\mu$ m.

A1 (Fig. 16A). 7-segmented. First segment antero-medially with one bare seta just exceeding the distal end of the segment. Postero-distally with two setae that differ in length and appearance. Longer one is serrulate and almost reaching the end of penultimate segment; shorter seta is smooth and reaches the middle of fourth segment. Second segment with an antero-apical bare seta that not exceeds the middle of following segment. Rome's organ not observed. Third segment antero-distally with one seta that reaches the middle of sixth segment. Postero-apically on this same segment one slender seta not reaches the end of the following segment. Fourth segment with two long bear setae antero-distally. Postero-distally, two apical bare setae are unequally long. Longer one is almost reaching distal end of penultimate segment while shorter one is just exceeding the end of the following segment. Fifth segment carries anterior-distally two long bare setae and postero-distally two unequal setae, the last by far exceeding the distal end of last segment. Sixth segment is bearing two unequally long and bare setae postero-distally and two long setae medially. Terminal segment with two long and one relatively short setae; the last is at least three times longer than terminal segment. Aesthetascya missing. Length ratios of last five segments are as follows: 2.1:1.5:1.2:1:1.

A2 (Fig. 15E) 5-segmented. Coxa with two subequally long smooth setae; shorter one exceeding the distal end of the segment, while longer one is reaching the middle of following segment. Basis is carrying one long serrulate seta postero-distally, that exceeds the distal end of third segment. Exopod is consisting of a plate with three unequal setae. Longer seta is serrulate and reaching three fourths of following segment, middle seta is small, reaching one seventh of following segment and most anterior seta is very small. First endopodal segment with aesthetasc Y, elongated and equaling 1.4 times the length of terminal segment; Postero-distally on the same segment there is one long serrulate seta, which not reaches the distal end of the following segment. Swimming setae are unequally long, five of them are smooth and extended slightly beyond tip of terminal claws, while the most anterior setae is short and it just exceeds the middle of following segment. Second endopodal segment with t-setae (t1-t4 setae) smooth and unequally long, but all of them exceeding the middle of terminal segment. Antero-medially on the same segment two unequally long smooth setae. Postero-distally a small serrulate setae is present, not reaching the distal end of terminal segment. Setae z2 and z3 are slender and almost equally long, with 2.4 times longer than terminal segment. Seta z1 is slender with more than five times the length of terminal segment. Claws G1 and G3 subequally long, five times the length of terminal segment. G2 claw is reduced, being 2.6 times as long as the terminal segment. Third endopodal segment (terminal segment) short, with the GM claw slightly smaller than G1 and G3 claws. Gm claw is seta-like, with 2.1 times longer than terminal segment. All terminal claws strongly serrulated distally. Two additional small and smooth setae are present on the terminal segment, both are equally 1.2 times the length of terminal segment.

Md. (Fig. 16B). Palp 4-segmented (Fig. 16C). First segment ventrally with S1 and S2 setae pappose and extended to almost the end of terminal segment.  $\alpha$ -seta was not observed. Dorsally on this same segment the respiratory plate is carrying four plumose setae. Second segment dorsally with three unequally long setae; longer ones smooth and almost reaching the distal end of last segment. Ventrally on this same segment, four slender and pappose setae



**Fig. 15.** *Eucypris cisternina* Furtos, 1936. Female: (A) Right valve internal view; (B) Left valve internal view; (C) respiratory plate; (D) UR; (E) A2. Scale bars = 0.05 mm.

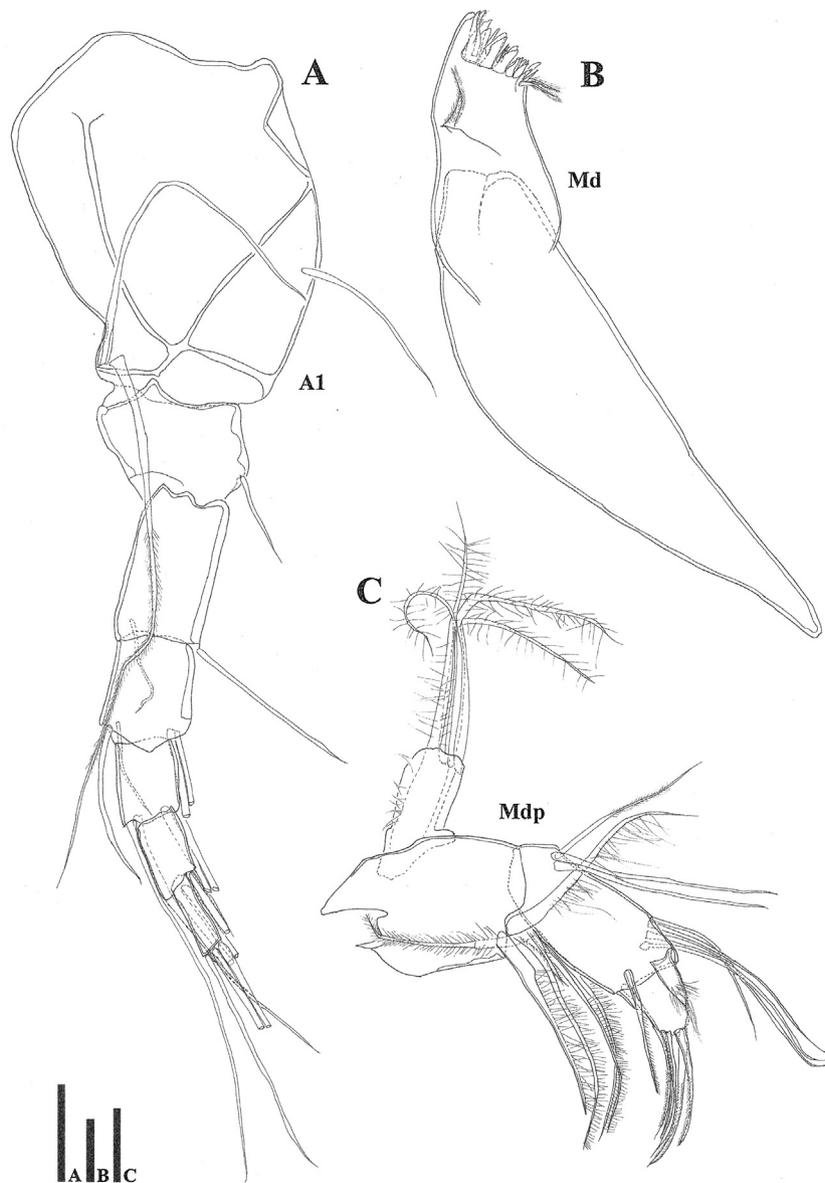
are equally long, exceeding the distal end of terminal segment.  $\beta$ -seta is thick at the base and pappose. Third segment is carrying dorso-distally four bare setae, all of them are exceeding the distal end of terminal segment. Ventro-distally two unequal setae are present; longer one is serrulate and it is by far exceeding the distal end of terminal segment; shorter seta is smooth and do not reach the middle of terminal segment.  $\gamma$ -seta, swollen and strongly pappose, it just exceed the distal end of terminal segment. Fourth segment (terminal segment) is carrying four elongated claw-like setae and two short and thin setae. Terminal segment is 1.3 times longer than wide.

Mxl. Respiratory plate (Fig. 15C) is carrying eleven short and pappose setae proximally, followed by eight long and pappose setae. Two additionally short and serrulate setae are present distally.

L5 (Fig. 17C) Palp with three unequally long setae, all of them distally slightly serrulate. Exopod consist in a plate with five rays, all of them smooth. Protopod with two short and smooth a-setae, a serrulate b-seta and a pappose d-seta. Protopod apically with fourteen almost equally long pappose or serrulate setae.

L6 (Fig. 17A) 5-segmented. Basal segment bare. First endopodal segment slightly hirsute, with e-seta serrulate and short, not reaching the distal end of following segment. Second endopodal segment with f-seta serrulate and just exceeding the distal end of penultimate segment. Third endopodal segment slightly hirsute and with g-seta serrulate and exceeding the distal end of terminal segment. A very small seta is located adjacent to g-seta. Terminal segment small and carrying a slender h1-seta, which is slightly serrulate and two times longer than terminal segment; h3-seta serrulate, 1.6 times as long as terminal segment and a strong claw (h2 seta) distally strongly serrulate, 5.7 times longer than terminal segment.

L7 (Fig. 17B) 4-segmented, Basal segment carrying the d1, d2 and dp setae. All of them serrulate. Second segment with e-seta serrulate and exceeding the middle of following segment. Seta f- is serrulate and just reaching the distal end of the segment that carries it. Seta g- missing. Penultimate and terminal segment not clearly divided. Terminal segment oblong with h1-seta short and hook-like; h2-seta claw-like and slightly curved distally; h3-seta long, more than 2.5 times longer than h2-seta.



**Fig. 16.** *Manuelcypris cisternina* (Furtos, 1936) comb. nov. Female: (A) A1; (B) Mandible; (C) Mdp. Scale bars = 0.05 mm.

UR (Fig. 15D) Ramus slender and carrying anteriorly a claw that almost reaches the middle of the ramus. Anterior seta slender and not reaching the middle of anterior claw. Posterior claw slightly exceeding one third of the length of the ramus. Posterior seta exceeding the middle of the length of posterior claw. Terminal claws slightly curved and fairly serrulate distally. Length ratios between ramus, anterior and posterior claws equaling 4.1:1.2:1.

3.1.4.5. *Remarks.* *Manuelcypris cisternina* seems to be most closely related to *M. antillensis*. They both have relatively elongated carapace in comparison to the other three species, both have “a” setae on the L5 and also carry a peculiar, additional seta on the L5. The postero-dorsal margin in *M. antillensis* is a bit more angular than in *M. cisternina*, and the RV carries marginal tubercles, which are absent in *M. cisternina*. Additionally, *M. antillensis* has a shorter posterior seta on the UR.

### 3.2. Key to species of the genus *Manuelcypris*

1. Setae “a” and “a” present on L5 ... 2
  - Setae “a” and “a” absent on L5 ... 3

2. Posterior seta on UR not reaching 1/3 of the L of the posterior claw, marginal tubercles present on the RV ... *M. antillensis* (Broodbakker, 1982) comb. nov.
  - Posterior seta on the UR reaching 1/2 of the L of the posterior claw, marginal tubercles absent on the RV ... *M. cisternina* (Furtos, 1936) comb. nov.
3. Marginal tubercles present along the RV ... 4
  - Marginal tubercles absent along the RV ... *M. chetumalensis* n. sp.
4. Surface covered with prominent pits ... *M. punctata* (Keyser, 1975)
  - Surface almost smooth ... *M. tabascena* n. sp.

### 3.3. Molecular analysis

The total length of the 18S rDNA alignment was 1660, while that of 28S rDNA was 1561 base pairs long. Table 1 summarizes the results of the analysis on both alignments. Based on Akaike information criterion (Hurvich and Tsai, 1989) evolutionary best fit model for the 18S rDNA alignment was TIM2+I+G (transition

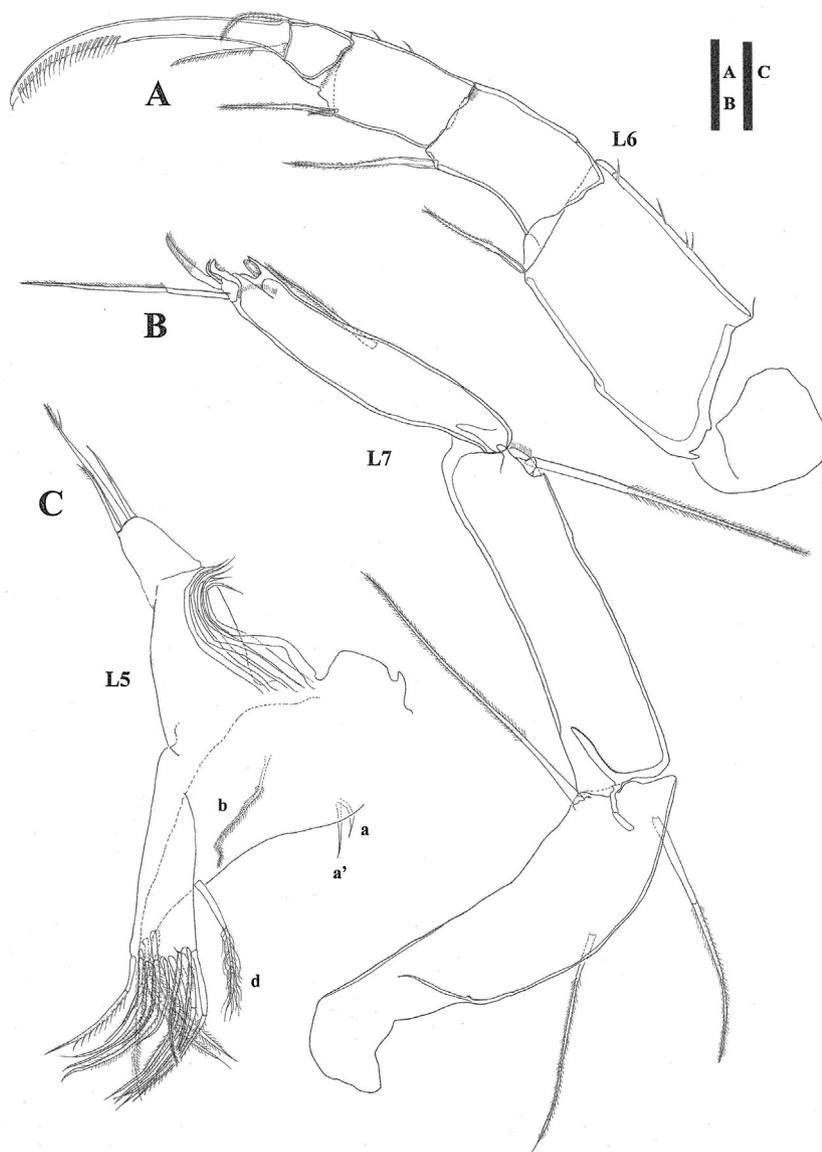


Fig. 17. *Manuelcypris cisternina* (Furtos, 1936) comb. nov. Female: (A) L6; (B) L7; (C) L5, arrow is showing additional seta on endopodite. Scale bars = 0.05 mm.

**Table 1**  
Summary of the molecular analyses results.

marker	L of alignment	constant characters	parsimony informative	parsimony uninformative	no of most parsimonious trees	L of tree	consistency index	retention index
18S rDNA	1660	1418	140	102	1	392	0.769	0.769
28S rDNA	1561	1220	240	101	1	722	0.632	0.682

model) and for 28S rDNA it was GTR+I+G (Rodríguez et al., 1990). The trees obtained from both ML and MP methods (Figs. 18 and 19) on both alignments were rooted with three sequences belonging to Cyclocypridinae: *Cypria exsculpta*, *Physocypris nipponica*, and *P. cf. biwaensis*. The first two were downloaded from the GenBank while the last was obtained in this study (Supplementary Table S4 in the online version at DOI: [10.1016/j.jcz.2016.09.003](https://doi.org/10.1016/j.jcz.2016.09.003)). Only the ML analysis of both genes has a high bootstrap support (100) for the ingroup taxa. On all obtained trees the support for the new genus, *Manuelcypris*, is very high (99 or 100), while the support for it and its closely related genera (*Heterocypris*, *Cyprinotus*, and *Eucypris*) ranged from 96/97 (of the 18S rDNA) and 72 and only 29 for the 28S rDNA. The lowest support for this group was obtained by the

ML analysis (Fig. 19B). In order to check if the GenBank sequence of *Dolerocypris ikeyai* caused such a low support for the group, this sequence was removed and the new alignment was analyzed again, but the obtained trees did not change the topology or the support significantly. We have also tried to see if the removal of the GenBank sequences belonging to *Heterocypris vandouwei* would change the topology of the genus *Heterocypris* on the 18S rDNA tree (Fig. 18A, B) or the support for it, but this also did alter neither topology nor support. *Heterocypris*, in fact appears polyphyletic on all obtained trees. Other groups that received a high bootstrap support were *Bradleycypris/Tanicypis* and *Chrissia/Stenocypris*. The group containing *Eucypris/Cyprinotus* was strongly (100/99) supported only on the 28S rDNA trees.

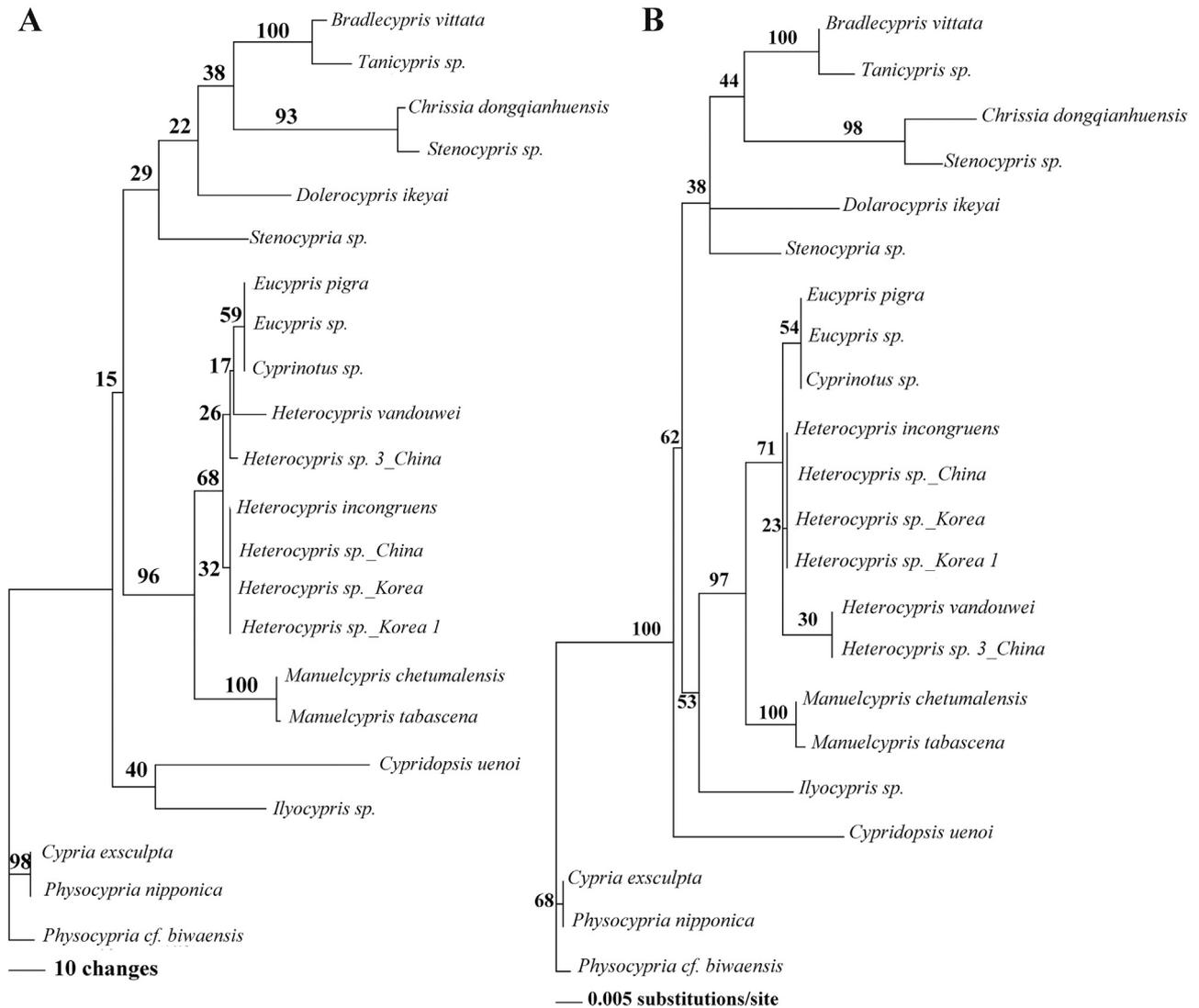


Fig. 18. Molecular analysis of taxonomic position of *Manuelcypris* n. gen. using the 18S rDNA gen (A) Maximum Parsimony tree topology; (B) Maximum Likelihood tree topology. Numbers above branches represent bootstrap values.

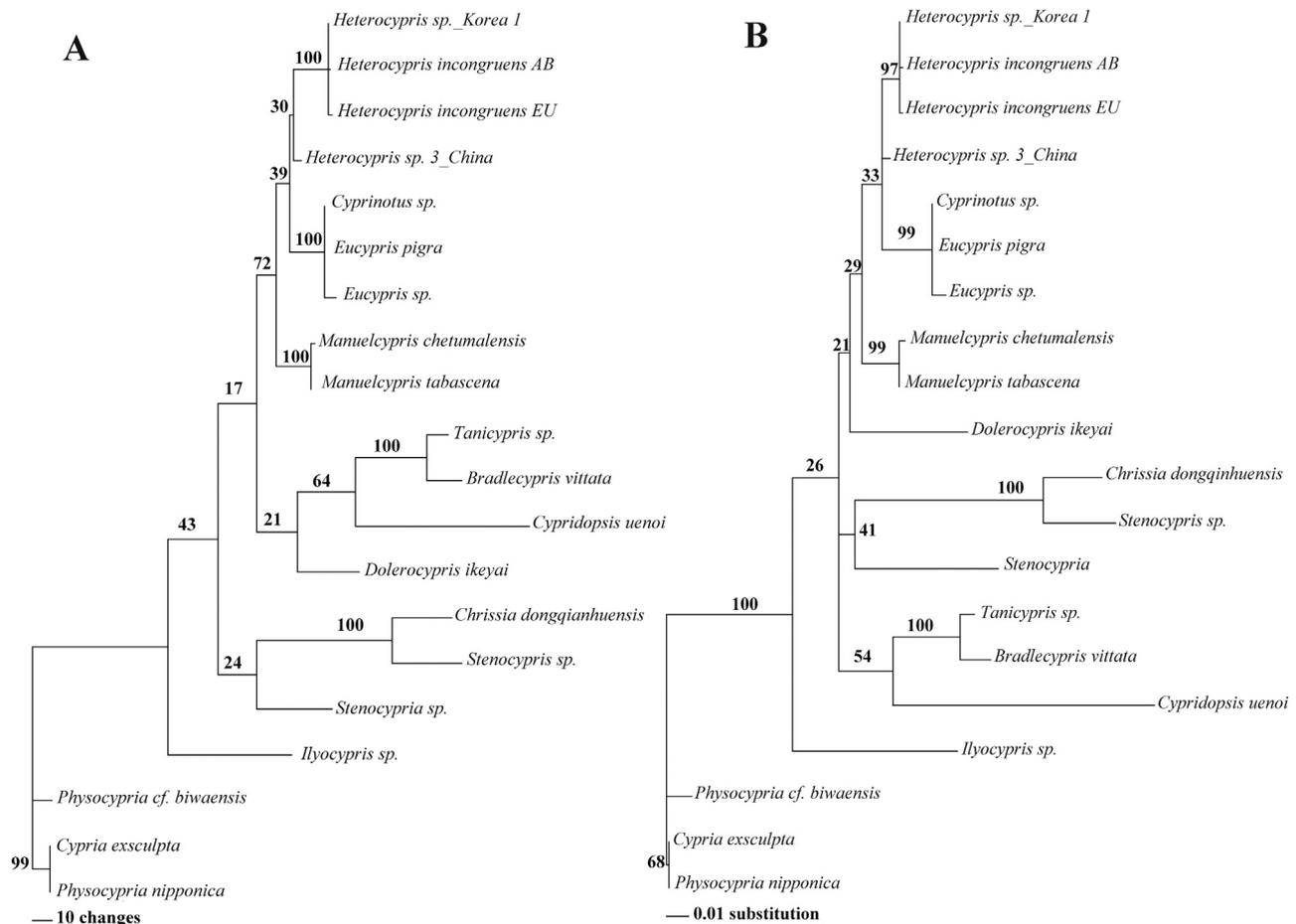
#### 4. Discussion

*Manuelcypris* has an isolated position within Cyprinotinae, and it shares several characters with Eucypridinae. For example, the carapace shape is more Eucypridinae than Cyprinotinae like, what probably prompted Furtos (1936) to place *M. cisternina* into the genus *Eucypris*. However, even though the frontal parts of the valves, at least in the type species (*M. chetumalensis*), carries prominent pores, those are not the same as the pores usually found in Eucypridinae (“porenwarzen”). Also, the other two *Manuelcypris* species do not have any peculiar frontal pores. It has to be pointed out that even within Eucypridinae the “porenwarzen” are not always present (such as, for example, in *Eucypris pigra*). Another reason why *Manuelcypris* was not included in Eucypridinae is the absence of “c” seta on the L5 in all three species and a longer posterior seta on the UR in comparison to most of the Eucypridinae species described so far.

Cyprinotinae at the moment, beside the new genus, includes five additional Recent genera: *Cyprinotus*, *Hemicypris* Sars, 1903, *Heterocypris*, *Homocypris* Sars, 1924; and *Riocypris* Klie, 1935. *Manuelcypris* differs from all of them by the absence of any seta on the walking leg, and three out of five species also do not possess “a” setae on the

L5. While representatives of *Homocypris* and *Riocypris* do not have any tubercles along the valve margins, tubercles are present along the LV margin in *Hemicypris* and right valve margin in *Cyprinotus* and *Heterocypris*. Taxonomic importance of the marginal tubercles on the valves has to be taken with caution when Cyprinotinae is in question because in *Manuelcypris* three species have them, while in the other two are absent. In addition, variability in the marginal tubercles has also been reported for *Heterocypris incongruens* (see Meisch, 2000). On the other hand, the chaetotaxy of the walking leg seems to be taxonomically very important in various Cypridoidea ostracod groups (see genera diagnoses in Karanovic, 2012). In Eucypridinae even the length ratio between two setae (d1 and d2) is important on the genus level (see Martens, 1989; Martens et al., 1992), while, only *Candelocypris Baltanás, 2001* from Eucypridinae lacks any seta on the basal segments of L6 (see Baltanás, 2001).

All our phylogenetic analysis and both genetic markers strongly support an isolated position of *Manuelcypris* within Cyprinotinae (see Figs. 18 and 19). This is in congruence with the results of Kong et al. (2014) who only had the 18S rDNA sequences of *M. chetumalensis* (“New Genus Mexico” on their phylogenetic tree). It has to be pointed out that this latter analysis, same as the present



**Fig. 19.** Molecular analysis of taxonomic position of *Manuelcypris* n. gen. using the 28S rDNA, (A) Maximum Parsimony tree topology; (B) Maximum Likelihood tree topology. Numbers above branches represent bootstrap values.

ones, includes only two representatives of Eucypridinae: *Eucypris pigra* and *Eucypris sp.* which always cluster with *Cyprinotus sp.* sequence. Such topology may question the validity of Eucypridinae, but since *E. pigra* is indeed a morphologically unusual representative of Eucypridinae such results only point out the importance of further studies. Another important result of our phylogenetic analysis is also the fact that *Heterocypris* does not appear monophyletic. With the exception of *H. vandouwei*, the sequence of which was downloaded from the GenBank, other *Heterocypris* sequences have been obtained for the purpose of this study and the study of Kong et al. (2014) and the animals were morphologically identified by one of us (I.K). Our initial identification was *H. incongruens* so the results of our analysis comes as a surprise, since we use very conservative, slowly evolving genes and still *Heterocypris* appears as a polyphyletic taxon (i.e. *H. sp. 3* 18S sequence clusters with *H. vandouwei*, see Fig. 18). Although the bootstrap support for this group is low, it clearly indicates that *Heterocypris incongruens* may represent a species complex which needs further study.

While *Homocypris* comprises only one species, restricted to South Africa (Sars, 1924), and the new genus is at the moment restricted to the northern Neotropics, other genera have a wider geographic distribution. *Cyprinotus* has been recorded from North and Central America, Middle East, East Asia and Indo Pacific Region, but most of the species reported from the Americas have a doubtful position in the genus (see Karanovic, 2012). *Hemicypris* has been recorded mostly from the Southern Hemisphere, but, like in *Cyprinotus*, many species will undergo systematic changes in the future. *Heterocypris* has a global distribution and *Riocypris* has

been recorded so far from Australia and South America (Díaz and Martens, 2014; Karanovic, 2008).

The results of our study clearly indicate that the use of slowly evolving genetic markers can be very helpful in establishing new genera, even with limited number of taxa. It also shows that the Cyprididae is in need of a revision which should to include extensive use of various genetic markers, because the level of morphological taxonomy, although much higher than a couple of decades ago, still leaves the position of different taxa unresolved. Finally, our study also points out that *Heterocypris incongruens* may represent a complex of species, which has already been postulated by Rossi and Menozzi (1990); Rossi et al. (2007).

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