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Cypria lacrima sp. nov. A New Ostracoda (Candonidae, Crustacea) Species from Texas, U.S.A.

Okan Külköylüoğlu[1,*], Derya Akdemir[2], Mehmet Yavuzatmaca[1], Benjamin F. Schwartz[3, 4], and Benjamin T. Hutchins[5]

[1]*Department of Biology, Faculty of Arts and Science, Abant İzzet Baysal University, Bolu, Turkey. E-mail: yavuzatmaca_m@ibu.edu.tr (Yavuzatmaca)*
[2]*Merdivenköy Mah. Şair Arşi Cad. No:16/14, 34732, Kadıköy, İstanbul, Turkey. E-mail: deryadiyar@gmail.com*
[3]*Edwards Aquifer Research and Data Center, Texas State University, San Marcos, TX 78666, USA*
[4]*Department of Biology, Texas State University, San Marcos, TX 78666, USA. E-mail: bs37@txstate.edu*
[5]*Texas Parks and Wildlife, 4200 Smith School Road, Austin TX 78744, U.S.A. E-mail: ben.hutchins@tpwd.texas.gov*

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Okan Külköylüoğlu, Derya Akdemir, Mehmet Yavuzatmaca, Benjamin F. Schwartz, and Benjamin T. Hutchins (2017) A new ostracod species, *Cypria lacrima* sp. nov. (Cyclocypridinae) is proposed from an artesian well in Hays County, Texas, U.S.A. The species differs from its conspecies with the following characteristics: unique shape and pit ornamentation on carapace, absence of “e” and “g” setae on both walking and cleaning legs, long gamma seta on Mandibular palp, asymmetric clasping organs and differences in the shape and size of other cheatotaxial parts. A detailed comparison among the species of the genus is provided along with ecological implications. This report increases the geographic distribution of the genus in the southern USA.

Key words: Cyclocypridinae, New species, Taxonomy, Artesian well, Hays County.

BACKGROUND

There are three subfamilies of the family Candonidae Kaufmann, 1900. Two of them (Candoninae Kaufmann, 1900, Cyclocypridinae Kaufmann 1900) cover non-marine ostracods while the third one (Paracypridinae, Sars 1923) comprises mostly marine forms (Meisch 2000; Karanovic 2011; Martens et al. 2013). The main difference between these two is the presence of swimming setae on second antenna (A2) in Cyclocypridinae and absence of these setae in Candoninae (Bronshtein 1947; Hartmann and Puri 1974; Meisch 2000; Karanovic 2012). Also, the members of the former subfamily have a two segmented clasping organ on T1 while the latter has an unsegmented clasping organ. Cyclocypridinae includes about 6 recent genera (*Cypria* Zenker, *Cyclocypris* Brady and Norman, *Physocypria* Vávra, *Allocypria* Rome, *Keysercypria* Karanovic, *Kempfcyclocypris* Karanovic), in which there are about 110 species known worldwide (Karanovic 2011). Two of the genera, *Allocypria* (endemic to Lake Tanganyika) (Rome 1962) and *Kempfcyclocypris* (endemic to Australia with one species *K. australis*) (Karanovic 2011) are so far known with limited geographical distribution. In contrast, *Keysercypria* erected from some South and Central American species of the genera *Physocypria* or *Cypria* covers wider geographical range (Karanovic 2011). Rest of the four genera (*Physocypria*, *Cypria*, *Cyclocypris*, *Keysercypria*) are known from North America. The aim of the present study is to describe a new species *Cypria lacrima* sp. nov. from Texas.

*Correspondence: E-mail: kulkoyluoglu_o@ibu.edu.tr

MATERIALS AND METHODS

Ostracods were collected with a drift-net (100 μm mesh size) placed over the well outflow pipe and preserved in 95% EtOH at the type locality, San Marcos artesian well (SMAW) in the Edwards Aquifer, which is known for its high stygobiontic species diversity (Holsinger and Longley 1980; Hutchins et al. 2014; Külköylüoğlu pers. obs.).

Ostracod specimens were sorted from detritus under a stereomicroscope (Olympus SZ X7) and kept in ethanol for taxonomic description. Each individual specimen was first measured before dissection in lactophenol solution. Empty valves were deposited in micropalaeontological slides for Scanning Electron Microscope (JEOL 6335 F SEM) photography at TÜBİTAK-MAM Institute (Gebze, Turkey). Additional SEMs were also provided at Abant İzzet Baysal University, Faculty of Arts and Science, Physics Laboratory.

During the soft body parts drawings and digitizing, a camera lucida (Olympus U-DA model) attached to an Olympus BX-51 microscope and Adobe Illustrator CS5 program were used, respectively. The following references (Hartmann and Puri 1974; Broodbakker and Danielopol 1982; Martens 1987; Meisch 2000; Karanovic 2011) were mainly used for species identification and description of chaetotaxy. Abbreviations used in the text and figures are as follows: A1, first antenna (antennula); A2, second antenna (antenna); d1-3 and dp, setae on the basal segment (protopodite) of the second (T2, walking leg) and third (T3, cleaning leg) thoracopods when they are present; Exp, exopodite; G1-G3, Gm, GM, terminal claws of A2; H, height; L, length; LV, left valve; Md, mandibula; Mdp, mandibular palp; Mxl, maxillula; Pr, protopodite; RV, right valve; T1 (first), T2 (second), T3 (third) thoracopods; t1-4, setae on the penultimate segment of A2; UR, uropod; W, width; Y, ya, y1-y3, aesthetascs; z1-3, setae on the second segment of A2. All materials (OK-TX-AW053:01-07) can be found at the Limnology Laboratory of the Biology Department, Abant İzzet Baysal University (Bolu, Turkey) and can be available upon request.

RESULTS

***Cypria lacrima* sp. nov.**

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(Figs. 1-4)

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Material Examined: Holotype: Adult male with soft body parts dissected in lactophenol and sealed with translucent nail polish; valves kept in a micropalaeontological slides for SEM photography (OK-TX-AW053:01-02). Collected from type locality by Benjamin F. Schwartz and Benjamin T. Hutchins on February 12, 2013.

Allotype: One complete ♀ with soft body parts dissected and sealed in a slide (OK-TX-AW053:03-04); valves kept in a micropalaeontological slides for SEM photography.

Paratypes: One ♀ (OK-TX-AW053:05) and one ♂ (OK-TX-AW053:06) dissected and sealed in slides and their valves kept in micropalaenotological slides. Nine other specimens (2 males, 6 females, 1 broken LV) collected from the type locality were kept in 70% ethanol. Additionally, 67 individuals were collected from the type locality at different times as;
19 Nov. 2013. 2 individuals.
25 Nov. 2013. 1 individual.
02 Dec. 2013. 10 individuals, (1F and 1M mounted).
20 May 2013. 54 individuals.

Deposition of types: All specimens collected from the type locality deposited separately in the vials at the Abant İzzet Baysal University, Department of Biology, Subdivision of Hydrobiology (Bolu, Turkey).

Type locality: Artesian Wells, Edwards Aquifer, San Marcos, Hays County, Texas State University, Texas (U.S.A). 29°53'22"N, 97°56'11"W, ~177 m ASL.

Etymology: The species is named after a latin noun “lacrima” due to its “teardrop” shape. Gender feminine.

Diagnosis: This new ostracod species can be distinguised from other members of the genus by the following features: 1) shape and unique ornamentation on carapace; 2) presence of tiny tubercles on posterior margin of RV seen internaly; 3) absence of “e” and “g” setae on both T2 and T3; 4) presence of a long gamma seta on Md-palp; 5) asymmetric clasping organs; and 6) differences in the shape and size of other cheatotaxial parts. A detailed comparison among the species of

the genus is provided along with ecological implications. This new report increases geographic distribution of the genus in the southern USA.

Description: Male: A small (L = 0.54 mm, H = 0.31 mm, W = 0.18 mm (*n* = 2)), subovoid to trapezoidal species in lateral view (Figs. 1A-C, E). In external view, carapace surface with pentagonal and squarish ridges and pits with normal pore openings. LV overlaps RV on all sides. Hinge adont (Fig. 1A). Greatest H anterior to the mid-length. Anterior and posterior margins of the valves without tubercles. Anterior margin more rounded than posterior (Figs. 1B-C, E). In internal view, LV with tiny tubercles on the inner lamella extending from postero-ventral to postero-dorsal part (Figs. 1C, G). RV with a short salvage postero-ventrally (Figs. 1E, D). Inner lamella narrow in both margins. LV and RV with a node and a short node-like fringe antero-ventrally, respectively (Figs. 1C, E, F). Marginal zones of hinge area strongly curved inward in RV than LV. In dorsal view (Fig. 1A), carapace lateral line slightly convex, posterior end rounded, anterior end beak-shaped. Central muscle scars typical of the family (Fig. 1B). Color opaque white.

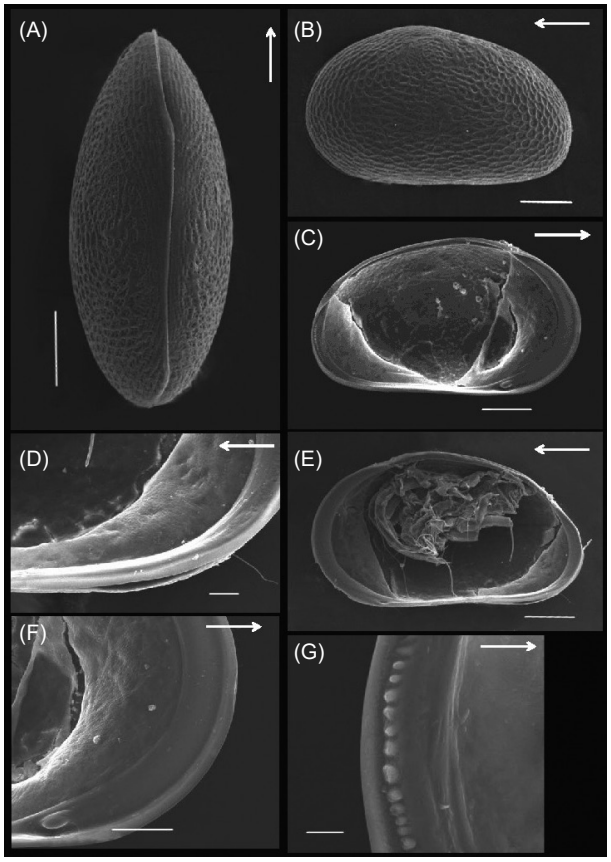

Fig. 1. *Cypria lacrima* sp. nov. A: Dorsal view, B: External and C: internal view of LV, D: Posterior end of RV, E: RV internal view, F: Internal views of anterior, and G: posterior ends of LV. Male (A-C, F, G); Female (E, D). Scale bars: 100 μm for A-C, E; 50 μm for F; 20 μm for D, 10 μm for G. Arrows show anterior end.

A1 (Fig. 2A): Seven articulated segments present. Two long slightly plumose apical setae on first segment well developed ventrally, longer one almost reaching to the end of terminal segment and shorter seta extending to the base of terminal segment. Two dorsal setae of the first segment with different size, the proximal one long reaching to the end of third segment and the distal short barely reaching to the next segment. One medium sized slightly plumose seta present medio-dorsally on the second segment. Rome-organ short tubular. Wouters organ absent. Third segment slightly longer than its width and with a short dorso-apical seta. Fourth segment with two unequally long smooth setae, dorso-apical seta long extending beyond the next segment and ventro-apical seta short in about 1/2 of the next segment. Fifth segment with two smooth sub-equally long dorso-apical setae, longer than the size of all segments of A1. One medium size seta on ventro-apical side, about the size of penultimate (6th) segment. Penultimate segment with 5 unequally long setae. One of the two smooth ventro-apical setae short in about 1/2 of the terminal segment, another long extending beyond the end of the terminal segment. Two thin antero-distal setae very long. One well-developed plumose dorso-apical seta in about 2 times the length of the terminal segment. Terminal segment with one ya aesthetasc 2x longer than the terminal segment and three long setae.

A2 (Fig. 2B): Five-segmented. Exopodial plate with one long and two very short setae. Second segment with a two segmented short aesthetasc Y placed medially on ventral margin. Hyaline portion about 50% of total length. The same segment with two long postero-proximal and postero-distal setae, former reaching to the end of third segment and the later plumose seta extending about half way of GM claw. Six natatory setae present; first five equally long exceeding the tips of terminal claws by about twice the length of the claws, sixth seta very short about one fifth of the third segment. Third segment with one well-developed claw-like seta dorso-anteriorly (t4?), one smooth seta and two t2 and t3 bristles. t2 straight and t3 slightly curved, both extending beyond terminal segment. Penultimate segment with all G claws present (length: G2 > G3 > G1); G1 claw short about 1/3 of G2. G3 claw slightly shorter than G2. Three z-setae (z1-z3) present, z1 seta claw-like and about the

length 1/2 of G2, z2 and z3 setae short and about the length of terminal segment. GM and Gm claws on terminal segment different in size; GM slightly shorter than G3 claw, Gm very short about the size of the terminal segment. Aesthetasc y1 and y2 not observed, aesthetasc y3 plumose slightly extending tip of terminal segment. All claws slightly serrated terminally.

Md (Figs. 2C, D): Md-coxa well developed ending with 8-10 coxal teeth and one slightly plumose, medium sized dorsal seta (Fig. 2D). Md palp 4-segmented (Fig. 2C): vibratory plate on the first segment with 6 almost subequally long pappose setae dorso-medially. S1 seta thin and

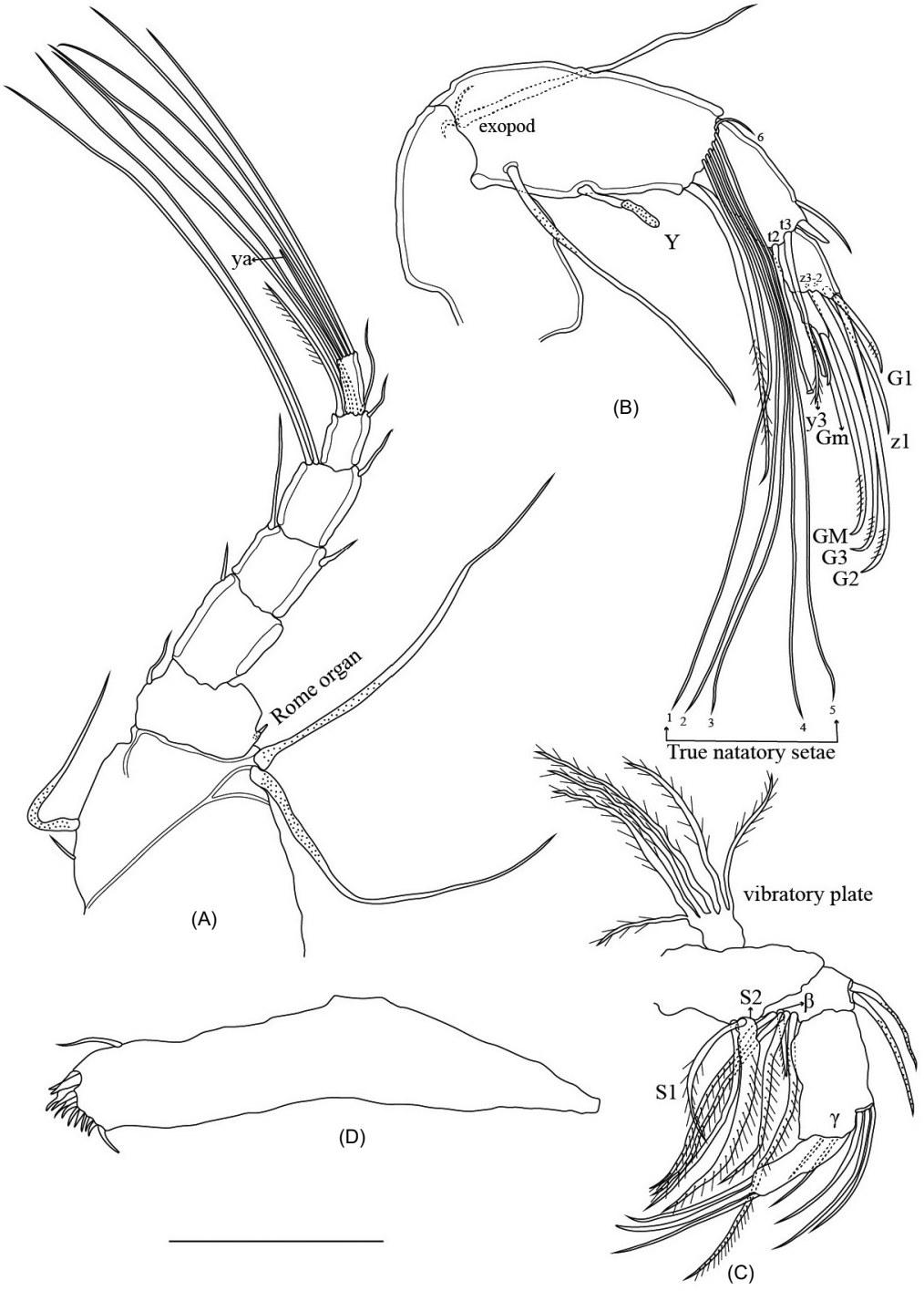

Fig. 2. *Cypria lacrima* sp. nov. Male: A: Antennule (A1), B: Antenna (A2), C: Mandibular palp, D: Mandibular coxa. Scale bar = 10 μm.

shorter than S2 seta in about 2/3 of its length. S2 seta well-developed reaching to the tips of terminal claws. Both setae plumose. Another seta on the same segment smooth equally long with S2 seta. Alpha seta not seen. Second segment dorsally with two subequally long setae, longer one about the size of penultimate segment, shorter barely extending halfway of this segment. Four plumose setae present internally (ventrally) almost reaching tips of terminal claws. Beta seta short and plumose. Penultimate segment with three smooth dorso-lateral setae almost reaching to the end of terminal segment. Two smooth setae (Gamma and a short seta) present antero-distally, Gamma seta longer extending to the tip of terminal segment. Terminal segment with three strong claws, two not fused with the segment, the middle one fused and slightly plumose. Terminal segment at least 4 times longer than wide.

Mxl (Fig. 3A): Three endites normally developed with 5, 4 and 4 setae, respectively. Third endite with two bristles and two short setae. One of the bristles slightly plumose, the other thin and smooth. No seta observed at the base of the first endite. First segment of the Mxl-palp with two smooth setae (apical seta slightly claw-like about the size of the terminal segment, shorter seta half of the segment) antero-distally and one smooth seta about the size of the terminal segment postero-distally. Terminal segment squarish with three slightly plumose claws. Vibratory plate well developed with 14 plumose long setae.

Rake-like organ (Fig. 4B): with a weakly serrated hammer shape.

T1 (Figs. 3B, C): asymmetric clasping organs with robust hook-like pointing finger. Right prehensile palp wider than the left palp, finger strongly curved downward, ending with a short claw-like process, lower part of trunk with a strong and sharp pointing process. Left palp with a short hook-shaped, curved finger. Vibratory plate with 5 well developed unequally long plumose setae. Two short, unequally long a setae and one d seta present, c and b setae not seen (but see discussion). Masticatory process with about 9-12 plumosed setae.

T2 (Fig. 3D): Five-segmented without basal (d1) seta. Seta e absent on the second segment. Seta f slightly plumose and short about 1/4 of the third segment. Penultimate segment without g seta (but see discussion below). Terminal segment with one long slightly serrated h2 claw about the length of the last three segments, and with h1 and h3 very short setae.

T3 (Fig. 3E): Four-segmented. First segment with slightly plumose d1, d2 and dp setae. dp seta long extending to the half way of penultimate segment. d1 seta about 1/2 of the d2. Second segment without setae. Penultimate segment with a medium sized f seta. e and g setae absent. Terminal segment subrectangular, about two times longer than wide with three setae (length: h3 > h1 ≈ h2). h3 seta smooth reaching to the halfway of the second segment.

UR as in female (Fig. 3F): Normally developed with two slightly serrated claws, equally long anterior and posterior setae short about 1/6 of the anterior claw. Ramus robust 2x longer than anterior claw. UR attachment simple with one ventral branch.

Zenker organ (Fig. 3G): with 7 (5 + 2) whorls of spines, ending with 11-corrugated opening.

Hemipenis (Fig. 4A): Lobe a longer than lobe b. Lobe a subtriangular and distally rounded. Lobe b short, distally slightly noded and rounded.

Female (Figs. 3F, 4C-E): Female similar in shape but slightly longer (L = 0.55 mm, H = 0.32 mm, W = 0.16 mm (*n* = 4)) than male. A2 (Fig. 4C) 4-segmented. t1-3 setae thin, barely reaching to half way of terminal segment. z1-3 setae short and smooth slightly exceeding end of terminal segment. G claws present (length ratio: G3 ≈ G1 ≈ GM > G2 > Gm), G1-G3 slightly serrated. T1 normally developed with five well developed plumose setae on vibratory plate and with two short unequally long slightly plumose a setae. Setae b and d short (but see discussion). Palp of T1 ending with 3 very short h-setae (h2 > h1 ≈ h3) (Fig. 4D). Masticatory process of T1 ending with about 12 setae. Genital organ simple without appendages (Fig. 3F). All other parts are similar to that of holotype male. Hypostome (Fig. 4E) similar to male with rounded base with weak attachment.

Distribution: Known only from the type locality.

DISCUSSION

The genus *Cypria* consists of about 35 species (Meisch 2000), 38 species (Martens et al. 2013), 37 species (Karanovic 2011) or 50 living species (Karanovic 2012) worldwide but there are several species whose taxonomic status are not clear (Karanovic 2012). In North America, there are about 20 species of the genus (Külköylüoğlu pers. obs.). It is however known that almost all the species are distributed in both lotic and lentic freshwater habitats including cave waters, littoral

lake zones, ditches, ponds, spring, and creeks. The genus *Cypria* is basically diagnosed with a short laterally compressed carapace. Both valves with smooth margins (Meisch 2000) or sometimes with tubercles (Karanovic 2012). A1 with seven segments. A2 of males with bristles. Terminal segment of Md-palp several times longer than broad. Mxl palp with square terminal segment. T1 palps asymmetrical. Seta d1 of T2 absent. All three basal setae present in T3. Setae h1 and h2 short and h3 long in T3. UR well-developed. Hemipenis with two processes. Zenker Organ with

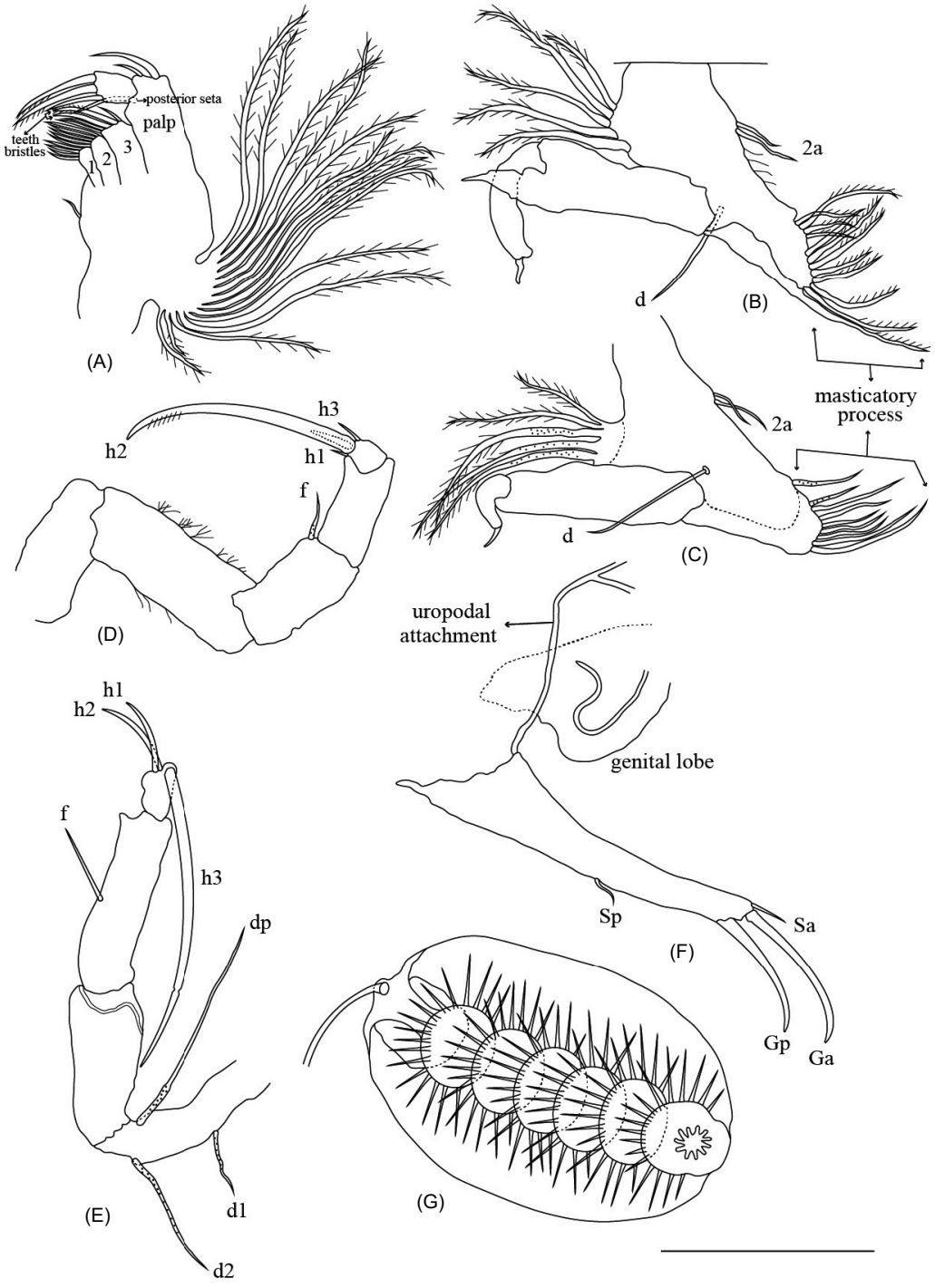

Fig. 3. *Cypria lacrima* sp. nov. A: Maxillule, B: right clasping organ, C: left clasping organ, D: walking leg (T2), E: cleaning leg (T3), F: female UR with genital organ, G: Zenker organ. Male (A-E, G). Scale bar = 10 μm.

5 + 2 whorls of spines. These main characters of the genus are similarly found in the new species. However, *Cypria lacrima* sp. nov. has some distinguishing characters from its conspecies as described above. One of the most important of these in the strongly ornamented carapace surface with squarish and pentagonal structures. Additionally, the presence of tiny tubercles on the internal posterior margin of RV and a node located on antero-ventral side of both valves are also

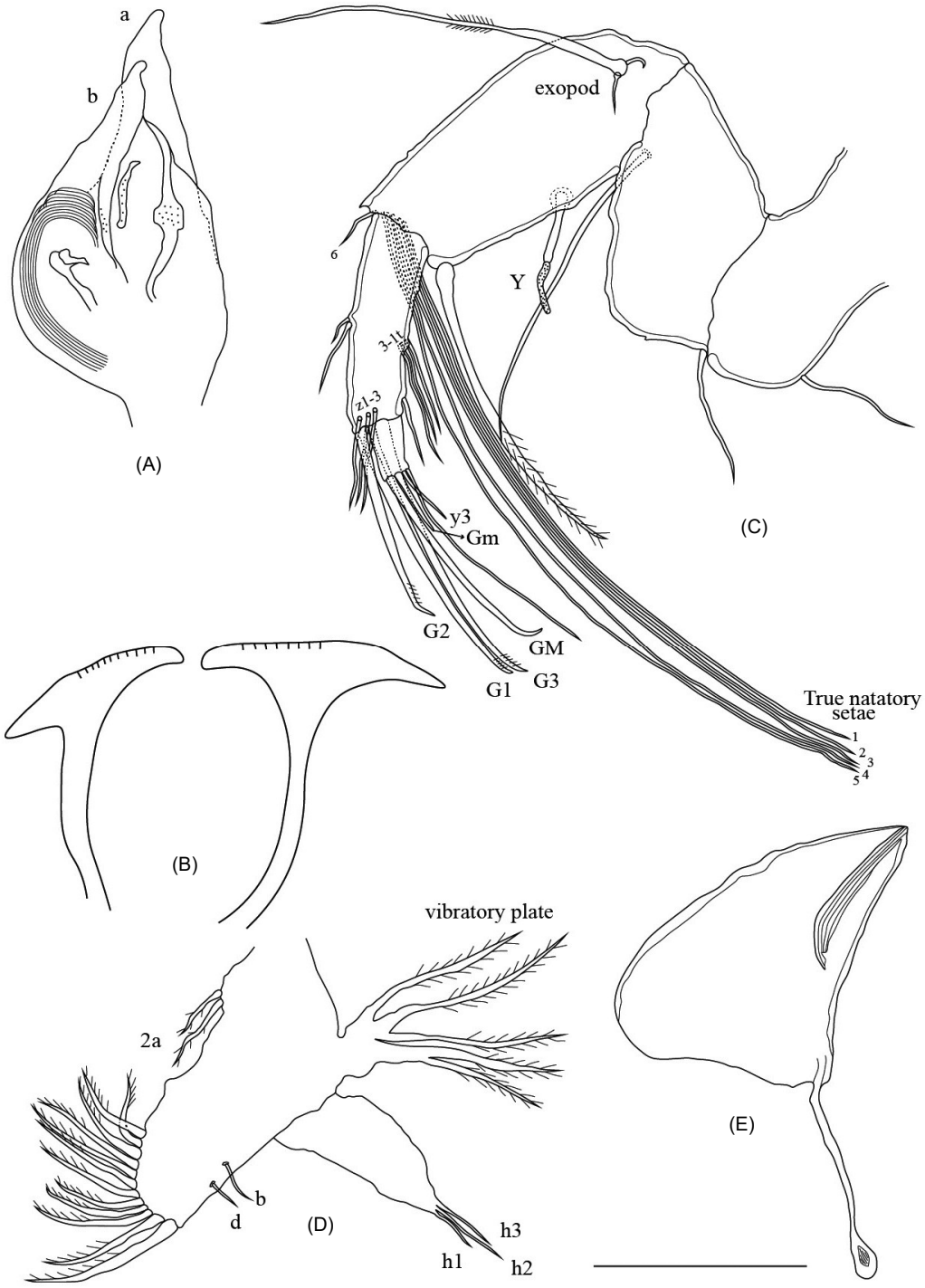

Fig. 4. *Cypria lacrima* sp. nov. A: Hemipenis of male, B: rake-like organ of male, C: A2, D: T1, E: hypostome. Female (C-E). Scale bar = 10 μm.

unique to the species. Compared to other species of the genus, the carapace structure is indeed different in this species. However, this is not surprising since variability in carapace morphology can be induced by environmental factors (Sywula 1974, Petkovski 1976, Pipík and Bodergat 2003). Hence, as listed above, all the characters not known in other species of the genus can be considered as diagnostic characters of the new species.

Cypria lacrima sp. nov. carries long swimming setae on A2 extending about twice the length of terminal claws. Based on the length of swimming setae on A2, Wagenleitner (1990) suggested three species groups in *Cypria*: 1) The *C. ophtalmica*-group, with long natatory setae, exceeding the extremities of the terminal claws (*e.g.*, *C. ophtalmica* (Jurine, 1820), *C. sketi* Petkovski, 1976, *C. subsalsa* Redeke, 1936, *C. cavernae* Wagenleitner, 1990, *C. karamani* Petkovski, 1976); 2). The *C. reptans*-group, with short natatory setae, not exceeding the extremities of the terminal claws (*e.g.*, *C. reptans reptans* Bronstein, 1928, *C. obliqua* Klie, 1939); 3) The *C. exsculpta*-group, including species with large size (longer than 0.75 mm) (Petkovski, 1976), consisting of *C. exsculpta* (Fischer, 1855), *C. curvifurcata* Klie, 1923. About four years later, Petkovski and Meisch (1994) reported a new species *C. bicolor* from Izice-spring in Ljubljana (Slovenia). These authors considered two groups of the genus *Cypria* and their new species was included in the second group with short swimming setae on A2. According to Karanovic (2012), these setae are short and not reaching (or barely reaching but not exceeding) the end of the terminal claws in several species (*C. reptans*, *C. curvifurcata*, *C. konishii* Smith and Kamiya, 2006, *C. polessica* Kovalenko, 1982, *C. brevisetigera* Cole, 1965, *C. bicolor* Petkovski and Meisch, 1994, *C. osburni* (Furtos, 1933)). Unlike these seven species, other members of the genus *Cypria* usually have long swimming setae exceeding the length of terminal claws one to three times.

Cypria lacrima sp. nov. has two small "a" setae and only one "d" seta on T1 with asymmetric prehensile palps. Other setae (b, c) are absent in males. However, in addition to "d" seta, another short seta of about equal size was observed in one female. After a detailed search on T1 of many ostracods, one of us (OK) considers that the location of seta (a-d) can differ between male and females of the same species. Smith and Kamiya (2006) providing nice drawings of T1 in the female *C. konishii* showed two small "a" setae and one long setulous setae on inner edge, and one longer setulous subapical seta on endite. The authors did not name these setae but if we consider them as "c" and "d" setae, one may consider the absence of "b" seta because of its location as is the case in our one female. However, this view can only be applied for this character and not to others because *C. konishii* has a shorter swimming setae on A2. Comparing to the type species of the genus (*C. ophtalmica*), absence of these ("a", "b", "c" and/or "d") setae herein can be considered as diagnostic difference. Unfortunately, chaetotaxy of T1 has not been focused on as well as clasping organs in most (if not all) of those earlier taxonomic keys and descriptions. For example, setae "a-d" of T1 of the genus *Cypria* were not even drawn or not mentioned those previous publications (*e.g.*, see Furtos 1933; Tressler 1937; Hoff 1942; Okubo 1990; Wagenleitner 1990; Petkovski and Meisch 1994; Meisch 2000; Karanovic 2011). This eventually creates difficulties for comparison of all chaetotaxy of T1 among the species. We therefore suggest future studies with better taxonomic keys including these setae.

Cypria lacrima sp. nov. displays a medium size "f" seta on T2 and T3 but both "e" and "g" setae are absent from both of these legs of the males (diagnostic characteristics). However, in one female, a very short "g" seta was observed as well. In contrast, the genus *Cypria* exhibits a short (*e.g.*, *C. ophtalmica*) or long (*e.g.*, *C. javana*) (Victor and Fernando 1981) "e" seta on T2 while T3 with a long "e" and "f" setae, and a very short or absent "g" seta. During the first description of *C. spinifera*, Tressler (1937) underlined the presence of a long heavy bristle reaching slightly beyond the base of the terminal claw of T3. He actually mentioned that this structure separates the species from *C. javana*. As far as we know, such a character is not known in any members of the genus and unique to *C. spinifera*.

Cypria lacrima sp. nov. has two almost equally long setae (h1 = h2) on terminal segment of T3. Seta h3 is long and slightly lobed in about one-third of its length. These setae differ in size among the species. They are unequal (*e.g.*, see *C. brevisetigera*, *C. mediana*, *C. obesa*, *C. matzkeae*) or almost equal (*e.g.*, compare *C. palustera*, *C. pellucida*, *C. spinifera*, *C. maculata*, *C. javana*, *C. conchensis*) in some species (Furtos 1935, Tressler 1937, Hoff 1942, Cole 1965, Smith and Janz 2008). Length of the setae can be tentatively classified into three groups: 1) h1 seta is shorter

than h2 (*e.g.*, *C. mediana*-group), 2) h1 can also be longer (*e.g.*, *C. matzkeae*-group) (see details in Smith and Janz 2008) and 3) h1 is almost equal to h2 (*e.g.*, *C. maculata*-group). Having almost equal h1 and h2 setae, our new species can be in the last group. Such grouping of these setae are actually used to define one of the main characters of the genera of the subfamily Cyclocypridinae. For example, Karanovic (2011) pinpointed that one of the major differences of her new genus *Keysercypria* was the size differences in h1 and h2 setae where h2 seta is at least two times longer than seta h1. This application suggests the importance of these setae and can also be applied for use at species level as is the case in our new species.

CONCLUSIONS

Karanovic (2011) published her revised work on the subfamily Cyclocpridinae introducing two new genera (*Kempfcyclocypris* and *Keysercypria*). In which, she also underlined the difficulties of using a few morphological characters (*e.g.*, presence/absence of tubercles on RV or LV in *Cypria* and *Physocypria*) during taxonomic identification. Similarly, as suggested, addition of some other characters - especially the soft body parts- can improve the accuracy of ostracod species' taxonomic level (Meisch 2000). There are several examples where ostracod species have been placed into wrong taxonomic levels or named erroneously (*e.g.*, see *C. petenensis* - *P. globula*, *C. osburni* - *C. fontana*, etc.) (Ferguson et al. 1964; Pipík and Bodergat 2003; Okubo 2004; Smith and Janz 2008; Karanovic 2011; Cohuo et al. 2017; Külköylüoğlu pers. obs.). In one of the most current examples, Cohuo et al. (2016) were able to revise several topotypes of *C. petenensis* from Lake Petén Itzá and specimens from surrounding areas (northern Guatemala). They showed that *P. globula* and *C. petenensis* were actually two different species although once these two were considered as synonymous due to presence of pustules in valves and similarities in the male copulatory organ (Pérez et al. 2010). We believe that such confusion comes from the variability of the valve structures in the genus. Indeed, Pipík and Bodergat (2003) assumed that the environment could be an influential factor on the external outline of the *Cypria* valves. Accordingly, triangular and subtriangular shapes could be related to stable environmental conditions. This corresponds to the idea of Danielopol (1978) in Candoninae species in Europe and of Meisch (2000) who suggested that species (*e.g.*, *C. ophtalmica*) with several synonyms may be based on a broad morphological variability of the species. Therefore, following these views, we consider that the subovoid to trapezoidal shape of *C. lacrima* sp. nov. may increase its adaptation to groundwater conditions, from which the species was found.

Except for the characters discussed above, *C. lacrima* sp. nov. has similar characteristics as other species in the genus. Finally, we propose *Cypria lacrima* sp. nov. as a new species of the genus *Cypria*.

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