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A new species of *Cavernocypris* (Ostracoda) from Texas (U.S.A.) with a taxonomic key

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Cavernocypris reddelli n. sp. is a new species of the genus *Cavernocypris* collected from spring waters of Texas, U.S.A.. This is the sixth species of the genus described so far. It can be distinguished from the other species of the genus by the shape and length of carapace, presence of robust marginal pore canals on right valve, number and length of setae on second antenna, shape of hemipenis, numbers of whorls on the Zenker organ, and differences in other parts of chaetotaxy. The new species was compared with other species and a new taxonomic key for the genus is presented for future studies.

Keywords: bisexual population, distribution, Podocopida, spring waters, Texas

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Introduction

The subfamily Cypridopsinae includes about 19 genera (Savatenalinton, 2018; Meisch *et al*., 2019), with *Cavernocypris* being one of them. Some of the diagnostic characteristics (but also see taxonomic details and discussion below) of the genus (after Smith *et al*., 2017) include a small carapace (ca. 0.8 mm), elongate to triangular shape in external view, left valve larger than right valve, dorsal margin of left valve distinctly arched, valve margins with peripheral selvage and well-developed fused zones, inner lamella broad to very broad anteriorly and posteriorly, lists on posterior end of inner calcified lamella of one or both valves, short swimming setae on second antennae barely extending about proximal margin of penultimate segment, distal segment of maxillular palp elongated, and uropod flagellum-like in female but absent in males. Hartmann (1964) was the first to revise the genus *Cavernocypris*, but it was Marmonier *et al*. (1989) who made the first detailed review on the genus along with describing *C. wardi* Marmonier *et al*., 1989 from Colorado, U.S.A. At that time, there were only three species (*C. coreana* (McKenzie, 1972) with two subspecies (*C. c. coreana* and *C. c. elongata*), *C. subterranea* (Wolf, 1920), *C. wardi*). Of the species, only the males of *C. coreana* were known. Approximately 10 years later, Külköylüoğlu and Vinyard (1998; 2000) reported the first males of *C. subterranea* from spring waters of Idaho and Nevada (U.S.A.), while parthenogenetic populations of *C. wardi* were also reported from March to November of 1999 from a cold rheocrene spring in Nevada (Külköylüoğlu, 1999). Up to now, *C. subterranea* and *C. wardi* are both known from U.S.A., where of *C. wardi* tends to be more common than *C. subterranea*, although *C. subterranea* has broader global distribution (Smith *et al*., 2017). Meisch (2000) included *C. subterranea* in his classical work from parts of Europe while Smith and Horne (2016) reported *C. wardi* from relatively cold waters. Smith (2011) described a new species, *C. cavernosa*, from South Korea. Karanovic (2012) did not mention about the males of the species but provided a key for the three species described until that time. Recently, Smith *et al*. (2017) described another new species (*C. danielopoli* Smith and Kamiya, 2017) from Gosu Cave (Yongsugol), South Korea. The authors discussed the biogeographical distribution of the members of the genus along with comparing their new species with others. They also provided one unnamed species as *Cavernocypris* sp. that is similar to *C. danielopoli*. Readers should consult their work for details about species distribution, habitat preferences, and carapace-environment relationship. Although previous studies (e.g., see Schornikov and Trebukhova, 2001; Schornikov, 2004; Smith *et al*., 2017) noted that *Cavernocypris* has a wide global distribution with rare abundance, this genus does not have a cosmopolitan distribution due to its specific habitat preferences (see discussion below). The aim of the present study is to describe *Cavernocypris reddelli* n. sp. as a new species from Texas, U.S.A. and present a new taxonomic key for *Cavernocypris*.

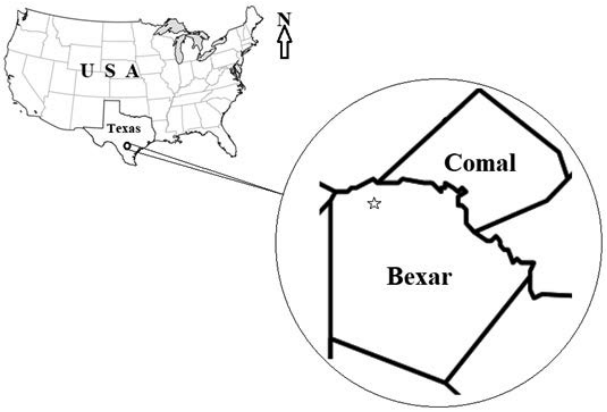

Fig. 1. Sampling site of *Cavernocypris reddelli* n. sp.

Materials and Methods

Samples were collected with a hand net from the type locality (Jelly Bean Spring) and kept in 70% ethanol in situ (36°59′N, 128°22′E) (Fig. 1). This spring seeps from the base of a 1-m-high bluff at the head of an unnamed valley on Briese Hill, Bexar County, Texas (U.S.A.). At the time of the sampling, water with the temperature of 19.9°C was clear and flowing at a visually estimated rate of 5 cm^3 per second. The spring discharges from the contact or just below the contact of intervals A and B of the Upper Member of the Glen Rose Formation (Dr. James Reddell, pers. comm.). Individual specimens were sorted with a pipet in the laboratory and kept in 70% ethanol in glass vials. Each sample was coded and numbered with the catalog number of the Texas Memorial Museum, Invertebrate Zoology Collection. Species identification was done after individuals were measured and dissected in lactophenol solution. All individual slides were numbered. Soft body parts and carapace structures were used during identification. Drawings of the soft body parts were done with the aid of a camera lucida (Olympus U-DA) attached to an Olympus BX-51 microscope before they were digitized using Illustrator CS5 software (Adobe). Taxonomic keys of Meisch (2000) and Karanovic (2012), and related literature (Marmonier *et al*., 1989; Smith, 2011; Smith and Kamiya, 2017; Smith *et al*., 2017) were used for species description.

Systematic Accounts

Order Podocopida Sars, 1866
Suborder Cypridocopina Jones, 1901
Superfamily Cypridoidea Baird, 1845
Family Cyprididae Baird, 1845
Subfamily Cypridopsinae Kaufmann, 1900

Genus *Cavernocypris* Hartmann, 1964
Type species. *Cavernocypris subterranea* (Wolf, 1920)

***Cavernocypris reddelli* n. sp. (Figs. 2–4)**

Type specimen. Holotype: Male (OK-TX-BeCo-01), dissected in lactaphenol solution with soft body parts and sealed in a glass-cover. Valves stored dry in a micropalaeontological cavity slide (OK-TX-BeCo-03). Collected from the type locality on 17 April 2001.

Diagnosis. During the present work, the amended diagnosis of Smith *et al*. (2017) was followed. Carapace elongate, similar to the type species of the genus but larger. Height more than half of the length. Posterior margin more rounded than anterior margin. Left valve overlaps right valve. Dorsal view anterior margin more pointed than posterior margin. Valve surface with shallow and small pits.

Type locality. Jelly Bean Spring (8A-1), Camp Bullis. Bexar County. Texas. U.S.A. 17 April 2001. Collected by James Reddell and M. Reyes from Texas Memorial Museum Invertebrate Zoology Collection (catalog number: 36,863).

Allotype. Female (OK-TX-BeCo-02) dissected in lactaphenol solution with soft body parts. Valves of the allotype preserved in micropalaeontological cavity slide (OK-TX-BeCo-04).

Paratypes. Two females (OK-TX-BeCo-05; OK-TX-BeCo-06) and two males (OK-TX-BeCo-07; OK-TX-BeCo-08), mounted and sealed in glass slides, collected from the type locality on 17 April 2001; twelve females and four males and nine juveniles collected from type locality on 31 May 2007.

Additional material examined. 11 females and five males collected on 25 July 2007 and 12 females and four males and three juveniles on 02 October 2007 collected from Copperhead Spring Cave. Many adults collected from Cute Chick Spring on 31 March 2010.

All materials are kept in 70% ethanol and deposited at the Limnology Laboratory, Bolu Abant İzzet Baysal University, Department of Biology, Hydrobiology Subdivision, Bolu, Turkey.

Etymology. The species is named after James R. Reddell, who kindly provided the samples, and for his continuous contribution to the knowledge of invertebrates and insects in Texas.

Description of male. Measurements (based on mid-length): L = 0.75–0.77 mm, H = 0.37–0.40 mm, W = 0.37–0.4 mm (Average: L = 0.767 mm, H = 0.383 mm, W = 0.396 mm [n = 3]). H/L = 0.487–0.501 (Average: 0.499 mm); W/L = 0.487–0.545 (Average: 0.519 mm) (Fig. 2A–C). LV overlaps RV (Fig. 2C). Carapace elongate in shape. Marginal pore canals more prominent ventrally in RV than LV. Fused zones present. Selvage

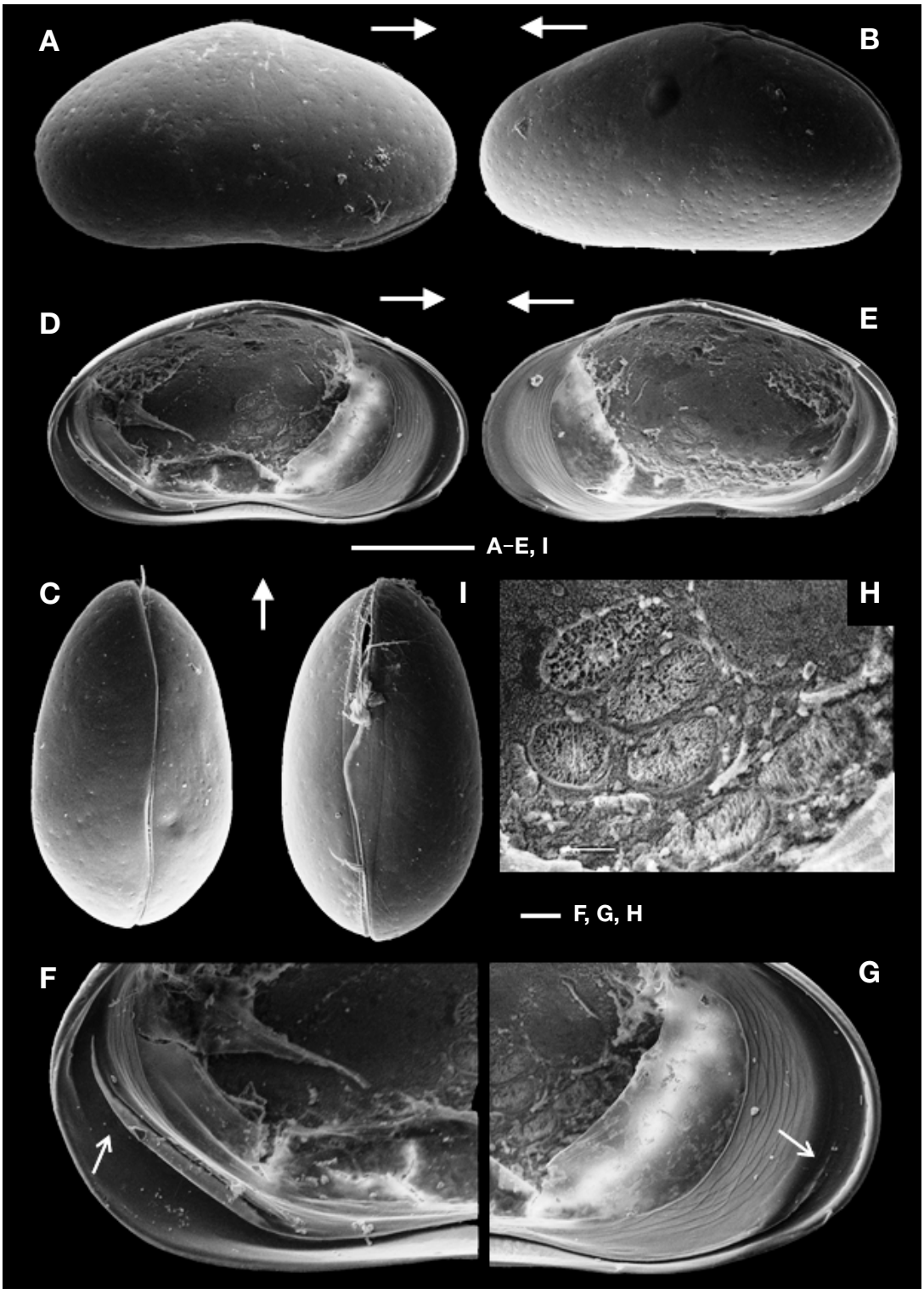

Fig. 2. *Cavernocypris reddelli* n. sp. Male: A, RV external view; B, LV external view; C, dorsal view; D, LV internal view; E, RV internal view; F, posterior detail of LV; G, anterior detail of LV; H, muscle scars; I, ventral view of female. Arrows point the list. Scale: A–E, I = 100 μm; F, G = 25 μm; H = 10 μm.

well-developed on both valves. Dorsal margin slightly arched. Greatest height approximately at center. Valve surface with small shallow pits and thin setae. In dorsal view (Fig. 2C), posterior margin more rounded than an-

terior margin. Lateral lines slightly concave posteriorly. Calcified inner lamella wider in anterior margin than posterior in both valves (Fig. 2D, E). Posterior list present in LV (Fig. 2F, G), absent in RV. Six big muscle scars located closer to center (Fig. 2H). Eye visible with small black pigment. Color greenish to blueish.

Antenulle (A1): 7 segmented (Fig. 3A): First segment with an x-shaped articulation, and a finely plumosed seta medium in length on dorsal margin, and two unequally long finely plumosed setae on ventral margin (anterior one slightly shorted than posterior). Wouters organ (WO) short with slightly plumosed terminal end. Second segment with one small dorsal-apical seta in about 1/4th of the same segment, Rome organ short about the same size with WO on ventral margin. Third segment with a thin and long dorsal-apical seta reaching almost to the end of terminal segment. Fourth segment with two long dorsal-apical setae, and one short and thin ventral-apical seta. Fifth segment with two long dorsal-apical setae, and one short ventral-apical setae. Sixth segment with three long setae. Terminal segment with two long setae and one medium-length aesthetasc ya.

Antenna (A2): 4 segmented (Fig. 3B). First segment with a strong base, and a long thin dorsal-apical seta slightly exceeding the next segment. Second segment with one long and two small setae on exopodial plate, and with six short natatory setae on inner edge of the segment, 6th seta shorter, longest one barely exceeding half way of the segment, and one well developed plumosed dorso-apical seta slightly exceeding the terminal segment. Aesthetasc Y short in about 1/4 of the segment. Third segment with two medium sized setae in dorsal edge, t1 and t2 setae plumosed in about similar size, t3−4 setae not seen (compare with female). G1 and G2 claws well developed in about the same size, G3 very short seta-like about 1/3 of G1. Seta z1 small, z2 seta-like plumosed slightly shorter than G1 claw, z3 thin seta-like slightly longer than G1. Terminal segment with a well-developed GM claw, Gm claw very short about 1/4 of GM, and a very short y3. Setae y1−2 not seen. All claws serrated and slightly curved at the end.

Mandible (Md) (Fig. 3C): Coxa with 5 well developed teeth and a medium-sized plumosed seta internally. Palp 4 segmented. First segment with four setae (S1, S2, alpha and a smooth setae). S1 and S2 plumosed and about equal in length. S2 seta slightly s-shaped. Alpha seta plumosed about 1/3 of S1 seta. Vibratory plate with 6−7 well developed setae, first one smaller. Second segment with a group of 3 smooth setae internally and slightly smaller than S1 seta. Beta seta tapering to hirsute distal end, about 1/2 of S2 seta. Two long smooth reaching end of terminal segment and one short hirsute seta about size of this segment located distally. Third (penultimate) segment with four slightly plumosed externodistal setae exceeding terminal segment. Gamma seta slightly plumosed with setules thin similar in size with other 3 setae located anterodistally. Length size gamma > beta > alpha. Terminal segment with unequally long 3 claws, longer one slightly hirsute. L ratios of 4 segments measured in middle 2.5 : 1.25 : 3.25 : 1.

Maxillula (Mxl) (Fig. 3D): Three endites and a two-segmented palp with a well-developed vibratory plate with 12 plumosed setae. First, second, and third endites with 6 (2 long 4 short), 4 similar in size and 6 (4 smooth 2 bristle-like [Zahnborsten] setae), respectively. Base of first endite with two medium-sized slightly plumosed setae. First segment of Mx-palp with 5 setae (2 long smooth, 2 medium-sized plumosed and 1 short seta). Terminal (second) segment rectangular (length 2 × width) with 4 claw-like and a smooth setae in about equal in size.

Rake-like with 7 teeth (Fig. 3E).

Hypostome wide with hirsute opening (Fig. 3F).

First thoracic leg (T1): Prehensile palps, almost similar in size, ending with hooked-like fingers modified into clasping organs. Right finger (Fig. 3G) stronger and more robust than left one (Fig. 3H). Endite (masticatory process) with 8 plumosed apical setae in about equal size. Two unequally long "a" setae (short seta 1/2 of long seta) and a medium-sized "d" setae present, setae "b" and "c" absent. Vibratory plate with four hirsute medium-sized setae.

Second thoracic leg (T2) (Fig. 4A): Five segmented with a medium-sized d2 seta on first segment (d1 and dp setae missing). Second, third and fourth (penultimate) segments with e, f, g setae plumosed, respectively. Length ratio: e > f > g. Terminal segment subrectangular, with a short and slightly plumosed h1 seta and very long claw (h2) reaching about half of second segment.

Third thoracic leg (T3) (Fig. 4B): Three segmented with almost equally long and slightly hirsute d1, d2 and dp setae on first segment. Second and third segments with slightly plumosed "e" and "f" setae, respectively. Seta "f" located from middle of third segment and about 3x shorter than "f" seta. Same segment ending with pincer organ, h1 very short, h2 hooked-like and h3 seta-like extending about halfway of third segment.

Hemipenis (Fig. 4C): Medium in size, lobe "a" (outer lobe) with pointing protrusion (diagnostic character), lobe "b" not clearly seen, lobe "h" small semirectangular.

Zenker organ (Fig. 4D): with 16 (14 + 2) whorls ending with sperm canal.

Description of female. Carapace similar in shape and length of male (Fig. 2I). Female L = 0.75−0.77 mm, H = 0.374−0.375 mm, W = 0.375−0.420 mm. Average: L = 0.76 mm, H = 0.375, W = 0.398 mm (n = 3). H/L = 0.480−0.500 mm (Average: 0.495 mm); W/L = 0.500−0.545 mm (Average: 0.525 mm). T1 (Fig. 4F) normally developed in about same size with male clasping organs, endopod with 2 long and one short subequal h1−3 setae

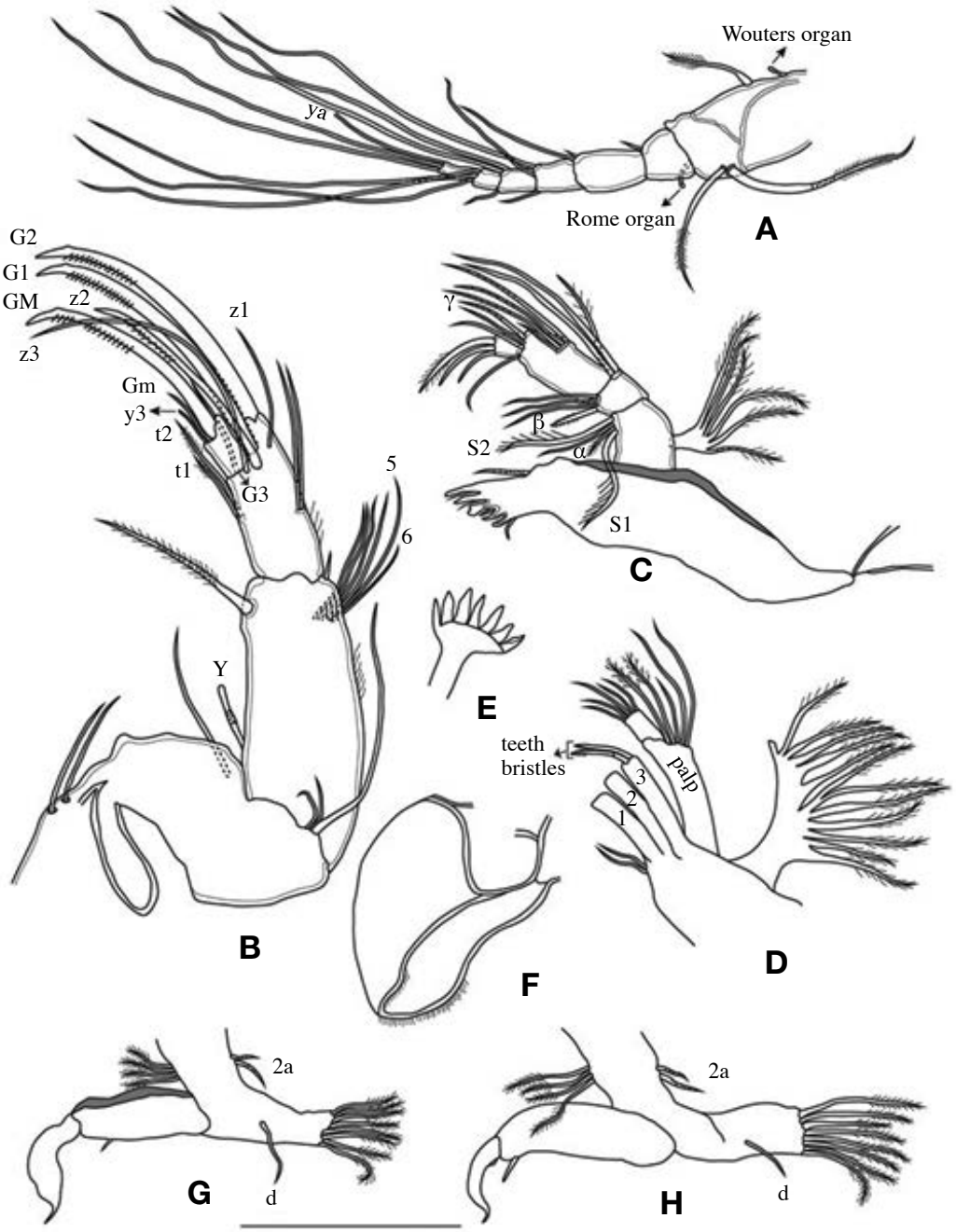

Fig. 3. Male: A, Antennule (A1); B, Antenna (A2); C, Mandible (Md); D, Maxillule (Mx1); E, Rake-like organ; F, Hypostome; G, Right clasping organ; H, Left clasping organ. Scale: 100 μm.

(h1 > h2 > h3). Seta h3 1/3 of h2. All plumosed. Endite with 10 apical setae. G-claws (length ratio G1 ≈ G3 ≈ GM > G2 > Gm) present (Fig. 4E). Setae z1−3 thin slightly exceeding halfway of terminal claws. Uropod (Fig. 4G): not seen in males, flagellum type (cypridopsine type) with an elongated base and one posterior seta. Genital part rounded and without a copulatory appendages. All other soft parts similar to that of male.

Key to the species of *Cavernocypris*

1. A1, 6 segmented ····· 2
– A1, 7 segmented ····· 3
2. *C. c. coreana* (McKenzie, 1972), *C. c. elongata* (McKenzie, 1972)
3. 6th swimming seta of A2 longer, Md-palp with 4 setal group ····· 4

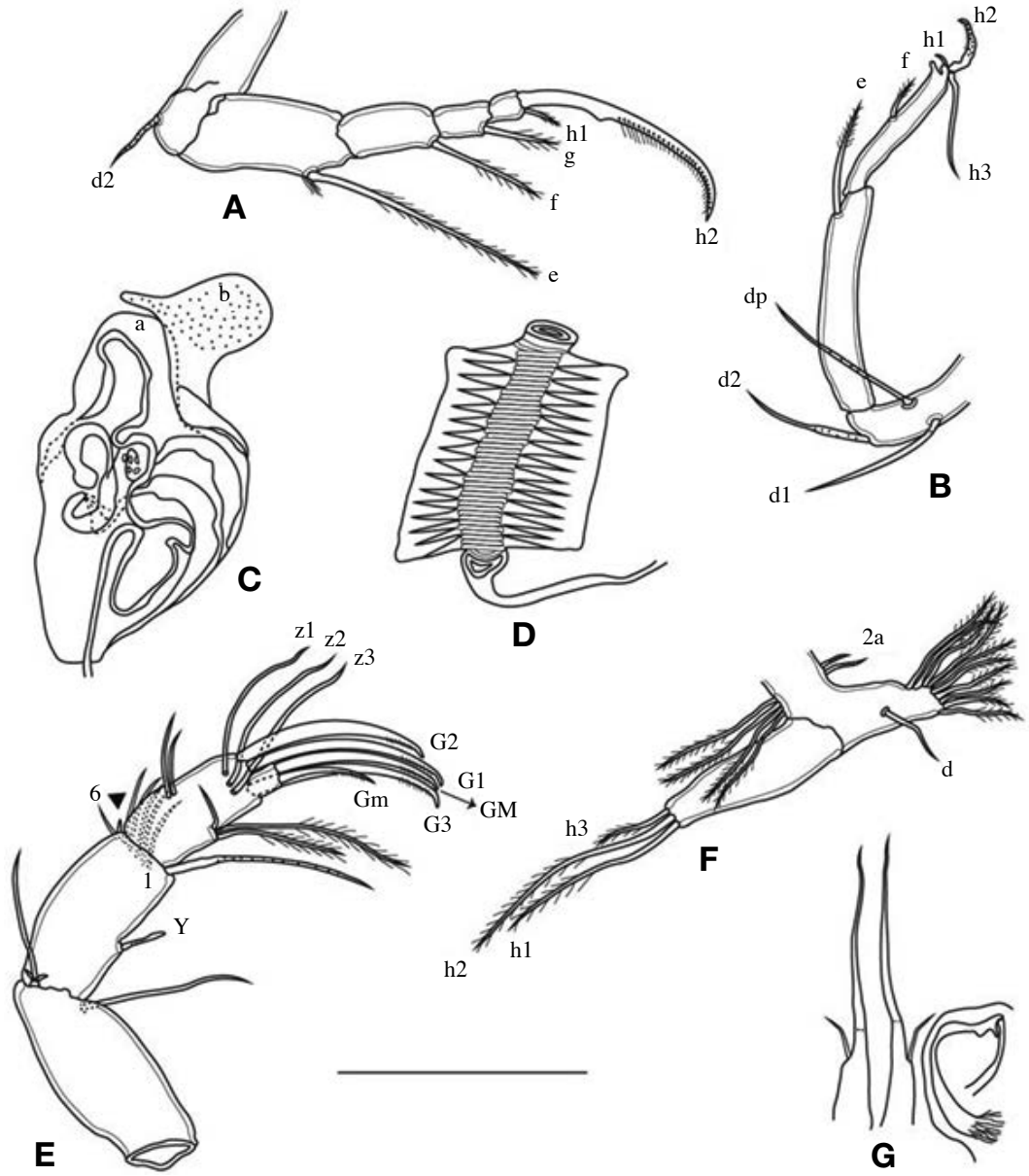

Fig. 4. Male: A, T2; B, T3; C; Hemipenis; D, Zenker Organ; E, Female: Antenna (A2); F, T1; G, Uropod of female with genital organ. Scale: 100 µm.

– 6th swimming seta of A2 shorter, Md-palp with 3 setal group····· *C. reddelli* n. sp.
4. Seta b absent on T1 ····· *C. cavernosa* Smith 2011
– Seta b present ····· 5
5. T1 vibratory plate with 1 seta ····· ····· *C. danielopoli* Smith and Kamiya 2017
– T1 vibratory plate with 2 or more setae ····· 6
6. T2 setae (e, f, g) and T3 basal setae (d1, d2, dp) smooth ····· *C. subterranea* (Wolf, 1920)
– These setae plumosed····· ····· *C. wardi* Marmonier et al. 1989

Discussion and Conclusion

As indicated above, *Cavernocypris reddelli* n. sp. has several different characteristics compared other species of the genus. However, three of these characteristics need to be discussed at species and genus levels such as (i) length ratios (H : L : W) of the carapace, (ii) occurrence and/or shape of inner list in LV, and (iii) length of swimming setae on A2. This is because these characters are mostly used in taxonomic keys and species identification in the literature (e.g., see Marmonier *et al*., 1989; Meisch, 2000;

Karanovic, 2012; Smith *et al.,* 2017).

(i) Length ratios: H:L ratio of C. *reddelli* n. sp. is about 0.50. In general view, carapace length is more tumid (globular) in *Cypridopsis* than *Cavernocypris* which is elongate. As indicated by Smith *et al.* (2017) intraspecific variation in carapace length was possible between the species of the genus (e.g., cf. *Cavernocypris* sp. and *C. danielopoli*). This is also true at generic level. For example, *Cypridopsis* and *Cavernocypris* are the two close genera but are thought to be different in length ratios. According to Marmonier *et al.* (1989), carapace ratio of *Cavernocypris* (H : L ≈ 0.50) and *Cypridopsis* s. str (H : L > 0.50) suggests that the former is elongate and the latter is subovate in shape. However, Karanovic (2012) using H : W (not H : L) ratio stated that the ratio was about 1/2 of L in *Cypridopsis*. Similarly, length ratio is also used to separate *Cavernocypris* from another genus *Kapcypridopsis* McKenzie, 1977. The ratio (H : L = 0.60 and ca. 0.50) suggests a subovate and more elongate carapace shapes in *Kapcypridopsis* and *Cavernocypris*, respectively (Marmonier *et al.,* 1989). If so, length ratios of *Cypridopsis* and *Kapcypridopsis* overlap, but this makes the usage of the ratio difficult and confusing between these genera. Although carapace length ratio is commonly used in taxonomic keys, it is also problematic when we compare the species within or between different genera. For example, Cole (1965) stated that the ratio of both H and W were about one half of the L in *Cypridopsis arhiga*, suggesting that the species is elongate in shape. In another example, Smith *et al.* (2017) provided detailed length ratios (H : L : W) of the species of *Cavernocypris*, in which the H : L ratio in *C. cavernosa*, *C. danielopoli* and *Cavernocypris* sp. are 0.52, 0.66, and 0.60, respectively. Considering the ratios stated above, if we accept the length ratio as a useful character, these taxa can be classified in other genera such as the genus *Cavernocypris* or *Cypridopsis*, respectively. One can be aware of that a single character should not be used as good enough for taxonomic identification, although there is no rule for that. Therefore, such a character should be carefully selected when used in future studies (also see below).

(ii) Occurrence and shape of inner list: According to Marmonier *et al.* (1989), the list in *Cypridopsis* is well developed as a double folding shape within the narrow inner lamella of the posterior margin. In contrast, the list is absent or weakly developed (not a double fold) on the posterior and posteroventral margin in *Cavernocypris*, in which, inner lamella is broad to very broad posteriorly. Smith *et al.* (2017) suggested that presence or absence of inner list on LV in *C. danielopoli* and in other species of the genus may indicate different species lineages. This is actually similar scenario in double fold issue between these two genera and species of them. However, some species of *Cavernocypris* with or without a double fold are still considered under this genus. In the new species, *Cavernocypris reddelli* n. sp., the situation resembles to this one discussed above that the double list is present in both sexes. Although the character has been used due to its considerable importance, there seems to be no common consensus about the levels of double fold or its shape. If such a character is really important and differs among the groups, several other species in both genera (*Cavernocypris* and *Cypridopsis*) can be separated into different (and possibly new) genera. However, the character may be used in different ways for the separation between the genera. For example, when the fold or a single line is present, it is somewhat oval or rounded in shape from ventral to posteroventral margin almost parallel to posterior margin of the LV in *Cavernocypris*. In contrast, double fold is not oval or rounded in *Cypridopsis* and it makes a somewhat straight crossing from ventral to almost about the mid-posterior part of the LV. This can also be useful for the species of other genera of the subfamily. In my opinion, this issue is still controversial and needs to be further studied.

(iii) Length of swimming (natatory) setae on A2: The length of the swimming setae on A2 is one of the other characters commonly used during taxonomic identification. Marmonier *et al.* (1989) clearly indicated that these setae are very short and barely reach beyond the proximal end of the penultimate segment of A2. Of the six setae, only the sixth one extends to the middle of the next joint. Meisch (2000), in his taxonomic key for the genera of Cypridopsinae, stated that these setae are well developed and reach about the middle of the terminal claws in *Cypridopsis*, but they are very short or strongly reduced and not reaching the base of the terminal A2 claws. Although there is a slightly different interpretation between these two definitions, the idea is that *Cavernocypris* spp. have short swimming setae on A2 while *Cypridopsis* has longer. The problem herein comes with the fact that there is no value of the length of these setae. Therefore, how long or short these setae is not clear and still open for discussion. Indeed, there are some species formerly belonging to the genus *Cypridopsis* or other genera known with short (e.g., Klie, 1943) or setae absent. For example, swimming setae are absent in *Plesiocypridopsis newtoni* (Brady and Robertson, 1870) (formerly known as *Cypridopsis albida* (Vávra, 1897)) and in *Cy. minima* Klie 1935. Similarly, *Cy. horai* Klie 1927 (now *Klieopsis horai*), *Cy. clathrata* Klie 1936 (now *Pseudocypridopsis clathrata*), and *Cy. alluaudi* (now *Plesiocypridopsis alluaudi*) have short swimming setae but now are placed into different genera. Indeed, there are at least six more species with short setae (*Cy. acanthodes* Rome 1962, *Cy. herpestica* Cole 1965, *Cy. arhiga* Cole 1965, *Cy. malcolmi* Löffler 1968, *Cy. mawenzii* Löffler 1968, *Cy. brevisetosa* Klie 1943) included in the genus *Cypridopsis*.

Three parthenogenetic species (*Cy. reptans*, *Cy. herpestica* and *Cy. arhiga*) of the genus *Cypridopsis* were earlier described from USA by Cole in 1965. She clearly highlighted (most probably, she is the only one doing this) the approximate length measurement of setae on A2 as (pages 138, 140) "*…not extending beyond the proximal one-third of the penultimate segment*". This ratio is actually similar to the setae in the species of *Cavernocypris* (see, Marmonier *et al*. 1989, p. 238). However, Marmonier *et al*. (1989) also indicated that the genus *Kapcypridopsis* has very short setae. The point here is that such a ratio can differ within (and between) the species of the same genera; therefore, great caution and attention should be paid when the character is used during identification. In our case, *Cavernocypris reddelli* n. sp. has males different than any other species of the genus *Cavernocypris* (and *Cypridopsis* as well). Its setae extend about 1/2 to 2/3 of the penultimate segment but never reach the base of terminal claws. This suggests that the new species belongs to the genus *Cavernocypris*. Besides, another important point should also be highlighted about these setae. While the sixth seta is shorter in the new species, this seta is the longest in all other species of *Cavernocypris*. This difference may also be another example of intraspecific variation among the species as suggested by Smith *et al*. (2017).

Finally, as noted above that there are some problems with the taxonomic usage of the characters, review of the genera (if not the subfamily) is necessary. Nevertheless, *Cavernocypris reddelli* n. sp. is described as a new species of the genus *Cavernocypris* based on the differences described above.

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