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Front Cover: Iron oxide crusts. See Lazaridis et al in this issue.

CONTRIBUTION TO THE OSTRACODA (*CRUSTACEA*) FAUNA OF THE KARSTIC REGION OF TEXAS WITH THE DESCRIPTION OF A NEW SPECIES

Okan Külköylüoğlu^{1,C} and Alaettin Tuncer²

Abstract

A total of 27 ostracod taxa were collected from 1995 to 2017 from 61 aquatic bodies in the karstic region of Texas (USA) and 1 in Mexico, including from caves, springs, spring-fed pools, and springs/drips in karstic rock-shelters. Among these taxa, *Dolerocypris reyesi* sp. nov. is a new species collected from a shallow pool connected to the springs above a rock-shelter. Three species (*Penthesilenula aotearoa*, *P. incae*, and *Vestalenula marmonieri*) are reported for the first time from North America. In addition, *Typhlocypris* cf. *prespica* and 4 taxa (*Microdarwinula zimмери*, *Cypridopsis* cf. *herpestica*, *Pseudocandona* cf. *parallela*, *P.* cf. *pratensis*) are new records of ostracod fauna of the US and Texas, respectively. The phylogenetic results based on 31 taxa and 30 morphological characters revealed 4 subgroups belonging to 6 main clustering groups within the family Darwinulidae. Including the new species reported here, the total number of non-marine ostracods in Texas is increased to 115 species. The results indicate that the total number of species is far below the true diversity and richness of the state.

INTRODUCTION

The members of the class Ostracoda are found in different kinds of aquatic habitats varying from freshwater to marine habitats. Furthermore, they are widely distributed in almost all natural and artificial aquatic systems of the world due to their broad range of tolerance to different environmental conditions, where each species may prefer species-specific conditions (Delorme and Zoltai, 1984; Külköylüoğlu, 2004; Akdemir et al., 2016). In addition to their extensive geographical distribution, ostracods are also one of the most diverse taxonomic groups (Bronshstein, 1947). Along with these characteristics, they are frequently obtained from geological records as fossils since the Ordovician Period (ca. 480 Ma) due to their calcified carapace structure (Siveter, 2008; Williams et al., 2008). Ostracods are good indicator species for determining and understanding palaeoecological conditions and for illustrating possible historical relationships between fossil and recent species (Hoff, 1942; Wise, 1961; Delorme, 1982; Yavuzatmaca et al., 2015). According to Meisch et al. (2019), 2330 subjective non-marine ostracod species in 270 genera are known worldwide. Out of these, more than 442 ostracod species are known from North America (NA) (Meisch et al., 2019; Külköylüoğlu et al., 2021). More than 100 species have already been reported from Texas where different previous works (Külcöylüoğlu et al., 2011; Külcöylüoğlu et al., 2017a, Külcöylüoğlu et al., 2017b, Külcöylüoğlu et al., 2017c, Külcöylüoğlu et al., 2017d, Külcöylüoğlu et al., 2017e; Külcöylüoğlu, 2018, Külcöylüoğlu, 2019), and ongoing studies have contributed a considerable amount of knowledge on ostracod species diversity and distribution for the last 10 years (Külcöylüoğlu et al., 2021). Along with these studies on ostracods, other studies showed that species diversity and richness are much higher than the current knowledge (Holsinger and Longley, 1980; Ponder, 2004; Reddell and Cokendolpher, 2004; Hutchins et al., 2014; Hutchins, 2018; Nissen et al., 2018). Therefore, it is suggested that unique species diversity may be related to the variety of habitats in Texas that require extensive study. The aim of the present study is to propose a new non-marine ostracod species *Dolerocypris reyesi* sp. nov. and to discuss the occurrences of the first records of other species in Texas, the US, and NA.

MATERIALS AND METHODS

The specimens examined during the present study (Appendix 1) were collected from karst systems in central Texas (USA) (Fig. 1), with 1 exception from Mexico noted in Appendix 2. Collections were made from April 8, 1995, to February 27, 2017, by James Reddell (University of Texas Insect Collection) and Marcelino Reyes (formerly University of Texas, Texas Memorial Museum), unless otherwise indicated. Samples were collected with a 100% cotton mophead placed in springs, cave streams, and spring-fed pools in rock-shelters, and kept in 70% ethanol in glass vials. Ostracod specimens were separated in the laboratory and stored in 70% ethanol. Samples were coded and numbered with the catalog number of the Texas Memorial Museum, Invertebrate Zoology Collection (now the University of Texas Insect Collection).

A standard procedure was followed during species identification (Külcöylüoğlu, 2020). Accordingly, after measurements of the individual species were made, specimens were dissected in lactophenol solution on slides and sealed with nail polish. We used both carapace and soft body parts during identification. Soft body parts were drawn with a

¹ Department of Biology, Faculty of Arts and Science, Bolu Abant İzzet Baysal University, Bolu, 14280, Turkey

² Department of Geological Engineering, Hacettepe University, Ankara, Turkey

^C Corresponding author: kulkoyluoglu_o@ibu.edu.tr

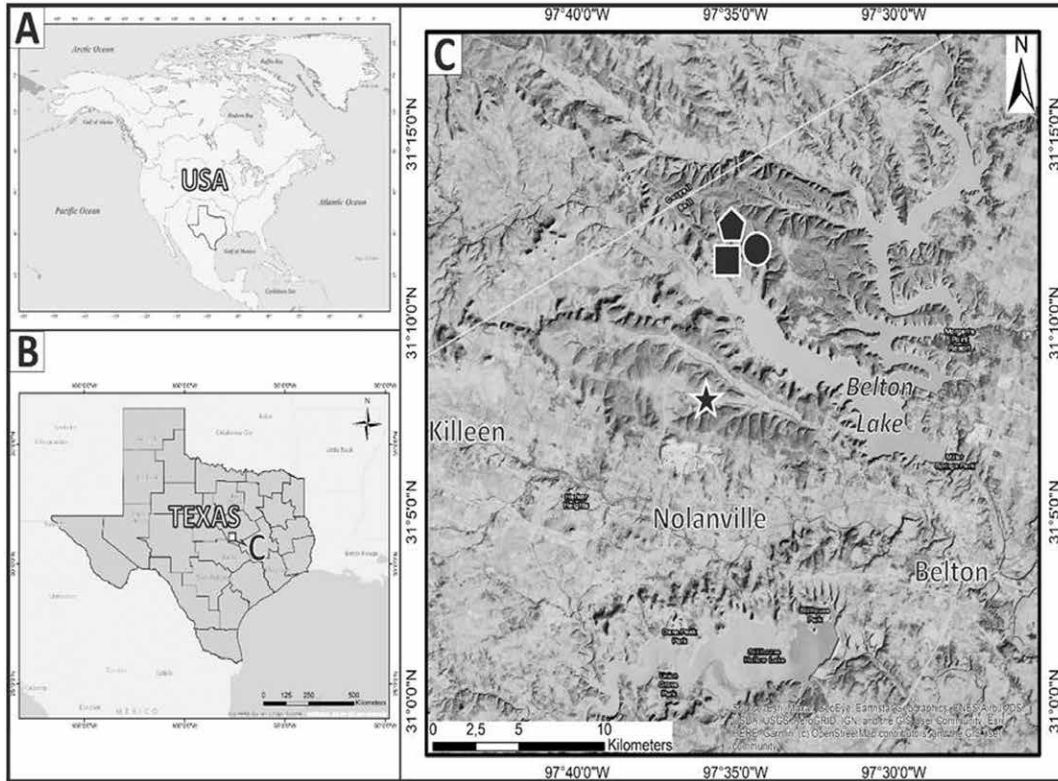


Figure 1. Location maps. (A) Map of the United States of America and North America showing the State of Texas. (B) Map of the State of Texas showing the study area (C and arrow). (C) Map of the study area showing the sampling localities of *Dolerocypris reyesi* sp. nov. (star), *Penthesilenula incae* and *Microdarwinula zimmeri* (pentagon), *Penthesilenula aotearoa*, *Microdarwinula zimmeri* (circle), and *Vestalenula marmonieri* (square).

deposited at the Texas Memorial Museum as described below, are stored in glass jars of 70 % ethanol at the Limnology Laboratory of Department of Biology, Bolu Abant İzzet Baysal University, Bolu, Turkey. They are available upon request from corresponding author.

Cluster Analyses

To illustrate clustering relationships among 36 known taxa of the family Darwinulidae, we used WinClada software version 1.00.08 (<https://www.winsite.com/Development/Components-Libraries/WinClada/>) and NONA software together. During the analyses, the data matrix in Appendix 5 was prepared for each taxa/species as presence or absence of the characters along with the character states shown in Appendix 4, which were equally weighted by 1. We used a heuristic approach in unconstrained searching with the following conditions: 200 replications, 1 tree to hold per iteration, 3 characters for sampling, 10 random constraint levels, with the “amb-poly” option. This was applied when collapsing a branch if the ancestor and descendant characters had different states under the same resolutions of multistate characters or if character states are unknown (amb), and treating trees are assumed as collapsed (poly). Along with the data used, tree length, computation of consistency, and retention value were also recorded. In addition, as indicated in the WinClada software, the method of branch-swapping (tree bisection and reconnection, TBR + TBR) was applied to search the trees. In addition to the reasons listed in previous studies (e.g., Martens et al., 2005; Rossetti et al., 2011), the genus *Darwinula* was chosen as an outgroup because (1) it is the type genus, and (2) it is the most ancient form within the family (Martens et al., 1997; Yavuzatmaca and Külköylüoğlu, 2019) with unique diagnostic characters. We used 30 morphological characters based on those previously proposed by Martens et al. (2005) and Rossetti et al. (2011), and other related references were also used for some species (e.g., Smith and Kamiya, 2006; Artheau, 2007; Smith and Janz, 2009). We ran the analyses with 31 taxa after excluding some taxa/species due to lack of morphological information about them. In the meantime, species described recently and/or not used in those previous studies were added into the analyses, as detailed in the discussion section.

Abbreviations

- A1, first antenna;
- A2, second antenna;

camera lucida (Olympus U-DA) attached to a microscope (Olympus BX-51) and were digitized with CoreIDRAW software. SEM photographs of carapaces and valves were acquired by a Zeiss EVO50 scanning electron microscope at the Department of Geological Engineering, Hacettepe University, Turkey. Terminology from Broodbakker and Danielopol (1982) and Martens (1987), the main taxonomic keys of Rossetti and Martens (1998), Meisch (2000), and Karanovic (2012), and related literature (e.g., Danielopol, 1968; Smith and Kamiya, 2006; Savatentalinton and Suttajit, 2016; and others as noted) were used during species description. The materials listed in the present study, but not

db, dorsal branch of uropodal attachment;
 H, height of valves;
 L, length of valves;
 ls, lateral shield;
 LV, left valve;
 Md, mandibula;
 ms, medial shield;
 Mxl, maxillula;
 Ro, Rome organ;
 RV, right valve;
 T1, first thoracopod;
 T2, second thoracopod;
 T3, third thoracopod;
 Ur, uropod;
 vb, ventral branch; and
 W, width of carapace.

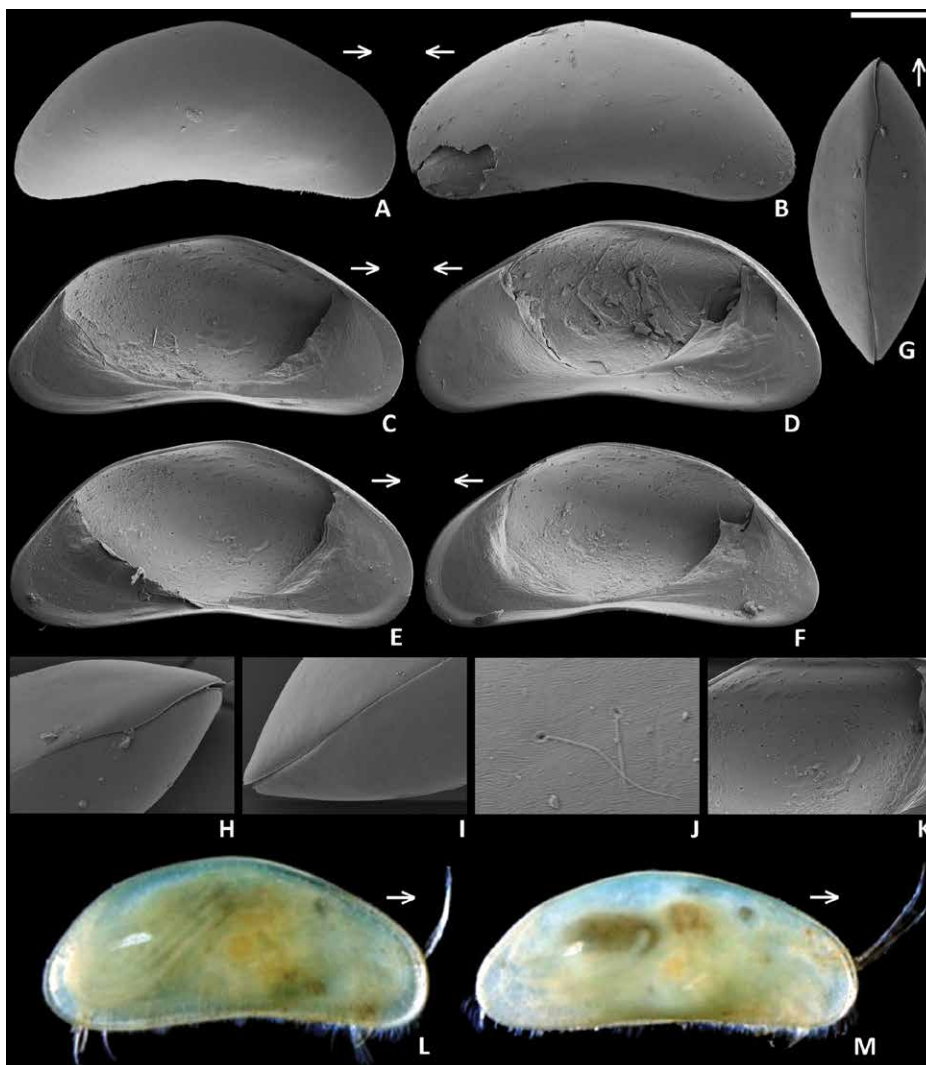


Figure 2. *Dolerocypris reyesi* sp. nov. Arrows point to anterior. (A) RV external view of female. (B) LV external view of male (note the damaged antero-ventral margin). (C) LV internal view of male. (D) RV internal view of male. (E) LV internal view of female. (F) RV internal view of female. (G) Dorsal view of male. (H) Anterior margin, dorsal view of male. (I) Posterior margin, dorsal view of male. (J) Pore openings with setae of male. (K) Muscle scars of male. (L) RV external view of male. (M) RV external view of female. Note 2 "striations" on the valve surface in Fig. 2J. The scale bar represents 225µm for Fig. 2A–F and L–M and 60µm for Fig. 2H–K.

RESULTS

A total of 27 non-marine ostracod taxa were identified from a diversity of karstic aquatic habitats at 61 sites in central Texas and 1 in Mexico (Appendices 1 and 2). *Dolerocypris reyesi* sp. nov. is proposed as a new species (Figs. 2–5, Table 1). Three species (*Penthesilenula aotearoa*, *P. incae*, *Vestalenula marmonieri*) are newly reported for the ostracod fauna of NA (Appendix 3), 1 species (*Typhlocypris* cf. *prespica*) is newly reported for the ostracod fauna of the US, and 4 other taxa (*Microdarwinula zimmeri*, *Cypridopsis* cf. *herpestica*, *Pseudocandona* cf. *parallela*, *P.* cf. *pratensis*) are newly reported for the ostracod fauna of Texas. Moreover, *Physocypria exquisite* was reported from an additional sample collected from Mexico. Reporting these species from Texas expands their geographical distributions to NA. With these new reports, the number of non-marine ostracods in Texas has now reached 115 species. Overall, our most recent findings continue to suggest that the true species diversity and richness of Texas are underestimated and deserve future studies.

Systematics

Class Ostracoda Latreille, 1802
 Subclass Podocopa Sars, 1866
 Order Podocopida Sars, 1866
 Suborder Cypridocopina Baird, 1845

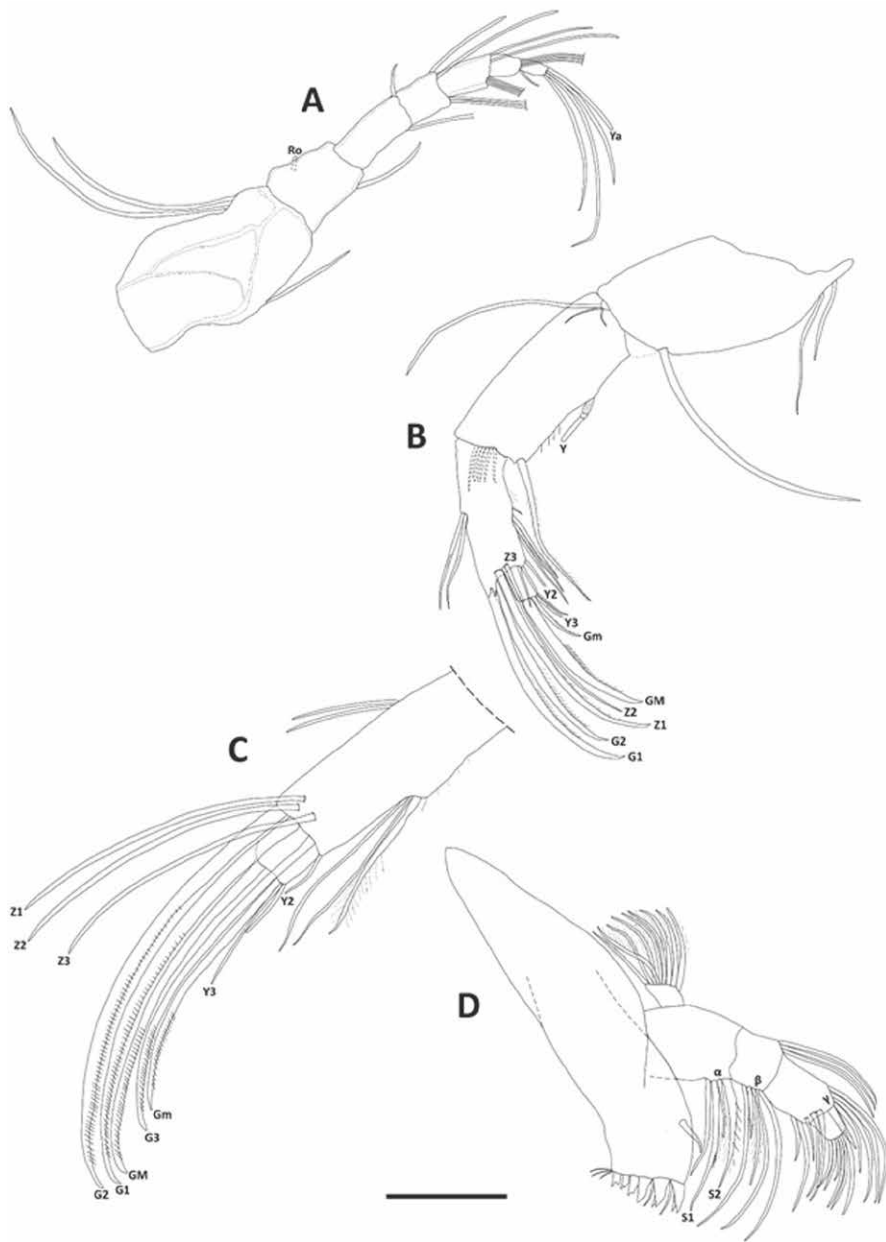


Figure 3. *Dolerocypris reyesi* sp. nov. (A) A1 of male. (B) A2 of male. (C) Detail of female A2. (D) Md of male. The scale bar represents 100 μ m for Fig. 3A, B, and D and 50 μ m for Fig. 3C.

March 16, 2010, by James Reddell and Marcelino Reyes; 3 females, 1 male, and 4 juveniles also collected from the type locality and kept in 70% ethanol.

Type Locality. Hidden Rock-Shelter (F 35-11) (Appendix 2, site 23), Fort Cavazos (formerly Fort Hood), Bell County, Texas, USA, lat 31.1330307 N, long 97.5987080 W.

Description (Male). Measurements based on mid-length. L 1.33–1.64 mm; H 0.55–0.68 mm; W 0.44 mm ($n=4$). H/L 0.42; W/L 0.30. LV overlapping RV anteriorly and posteriorly (Fig. 2B). Carapace elongate and crescent, anterior margin narrowly curved and apex lower down (Fig. 2C–D, L–M). Carapace surface smooth (striations seen in higher magnification) with normal pore openings and thin setae (Fig. 2J). Dorsal margin slightly arched anteriorly. Greatest height approximately in front of center. In dorsal view (Fig. 2G–I), anterior margin more rounded than posterior margin. Calcified inner lamella wide anteriorly and posteriorly (Fig. 2C–D), anterior margin with diamond-shaped ornamentations internally. Six muscle scars located at center and 5 to 6 small scars seen dorsally (Fig. 2K). LV with 2 keels ventrally. Eyes visible with black pigment (Fig. 2L–M). Color translucent to greenish.

Antennule (A1). Seven segmented (Fig. 3A). First segment (base) with V-shaped articulation, a medium-sized,

Superfamily Cypridoidea Baird, 1845
 Family Cyprididae Baird, 1845
 Subfamily Dolerocypridinae Triebel, 1961

Genus *Dolerocypris* Kaufmann, 1900

Diagnosis (Genus). Carapace elongated ($L > 2H$), compressed in dorsal view. $RV > LV$ in lateral view. Calcified inner lamella wide in both margins. Selvage on RV peripheral, inwardly placed in LV, or rarely the opposite. Terminal palp on Mx1 short, cylindrical. Basal segment on T2 with d1 and d2 setae. Ur well developed; posterior seta displaced close to posterior terminal claw. Ur attachment simple (Meisch, 2000).

Dolerocypris reyesi sp. nov.

Figs. 2–5

Etymology. The species is named after Marcelino Reyes for his kind help with sampling and his contribution to the knowledge of the cave and spring invertebrates of Texas.

Holotype. One male dissected in lactophenol solution with soft body parts (slide no. OK-TX-JR-1) sealed with translucent nail polish and valve (slide no. OK-TX-JR-2). Collected from the type locality on March 16, 2010, by James Reddell and Marcelino Reyes. Deposited at Texas Memorial Museum, Invertebrate Zoology Collection, Catalog no. 72.550.

Allotype. One female dissected in lactophenol solution with soft body parts from the type locality (slide no. OK-TX-JR-3). Collected from the type locality on March 16, 2010, by James Reddell and Marcelino Reyes.

Paratypes. Two males (slide no. OK-TX-JR-4) and 3 females (slide no. OK-TX-JR-5) mounted and sealed in glass slides, collected from the type locality on

slightly plumose seta on dorsal margin, and 2 unequally-long, smooth setae on ventral margin. Wouters organ absent. Second segment with Ro short on ventral margin, dorsal-apical seta medium in size and smooth. Third segment with a long, dorsal-apical seta extending to the end of the aesthetasc ya, and 1 short and smooth ventral seta. Fourth segment with 2 long dorsal-apical setae, and 2 unequally-long, ventral-apical setae. Fifth segment with 3 long dorsal setae and 2 medium-sized, ventral-apical setae. Sixth segment with 3 long and smooth setae and 1 very-short, dorsal seta. Terminal segment with 3 long and smooth setae and 1 medium-sized aesthetasc ya.

Antenna (A2). Four segmented (Fig. 3B). First segment with 2 medium-sized, smooth dorsal-apical setae reaching half of base and 1 long seta extending to the 2nd segment. Exopodial plate on 2nd segment with 1 long and 2 small setae and with 6 short, natatory setae on inner edge of the segment; 6th seta longer, barely reaching a third of the segment, and 1 well-developed, plumosed, ventral-apical seta exceeding the terminal segment. Aesthetasc Y short, 2 segmented. Third (penultimate) segment with 2 medium-sized setae in dorsal edge, t1–t3 setae unequally long and slightly plumosed; t1 seta thicker than t2–3 setae; t4 seta reduced or very thin. Y2 seta very short 1/3 of terminal segment. G1 and G2 claws well developed and unequally long; G3 very short, seta-like, and slightly exceeding terminal segment. Z1 seta well developed, longer than the claws; z2 seta-like, smooth, similar in size to the G2 claw; z3 seta thick, stout, and slightly longer than the terminal segment. GM claw well-developed on terminal segment; Gm claw short about 1/3 of GM; short y3 and short seta. Claws (G1, G2, GM) and z1 seta serrated and slightly curved at the end.

Mandible (Md). Coxa with 6 well-developed, robust teeth and medium-sized setae internally, and 1 short, stout dorsal seta (Fig. 3D). Palp 4 segmented. First segment with vibratory plate with 8–9 well-plumosed setae; S1 and S2 plumosed and unequally long. Alpha seta slightly plumosed about 1/3 of S1 seta. Second segment with a group of 3 smooth and 1 plumosed setae internally, beta seta slightly hirsute tapering to distal end, about size of alpha seta. Three equally-long and smooth, external setae reaching end of terminal segment. Third (penultimate) segment with 5 unequally-long and smooth setae, 2 antero-distal setae exceeding twice the terminal segment, 1 short seta slightly longer than terminal segment, 2 internal setae unequally long and smooth, short one about 1/3 of long (claw-like) seta. Gamma seta stout and slightly hirsute. Four antero-distal setae smooth, reaching about tips of terminal setae. Terminal segment with unequally-long, 3-claw-like setae, and 3 short setae.

Maxillula (Mxl). Two-segmented palp and 3 endites well developed (Fig. 4A), vibratory plate with 20–21 plumosed setae. First, 2nd and 3rd endites with 7 setae (2 long, 5 short), 6 setae similar in size, and 6 setae (4 smooth and 2 smooth bristle-like (Zahnborsten)), respectively. Base of 1st endite with 2 medium-sized slender setae. First segment

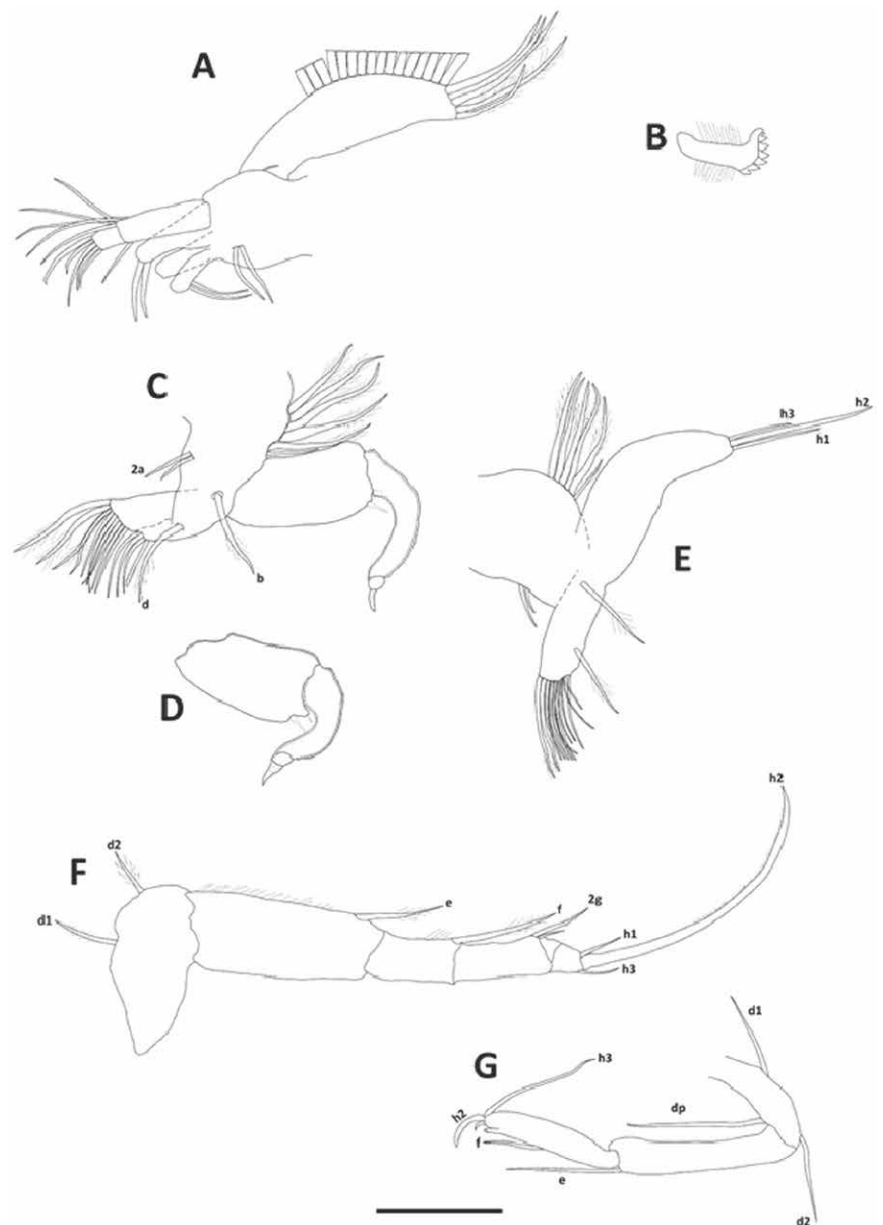


Figure 4. *Dolerocypris reyesi* sp. nov. (A) Mxl of male. (B) Rake-like organ of male. (C) Right T1 of male. (D) Left palp of male. (E) T1 of female. (F) T2 of male. (G) T3 of male. The scale bar represents 100 μ m for Fig. 4A and C–G and 25 μ m for Fig. 4B.

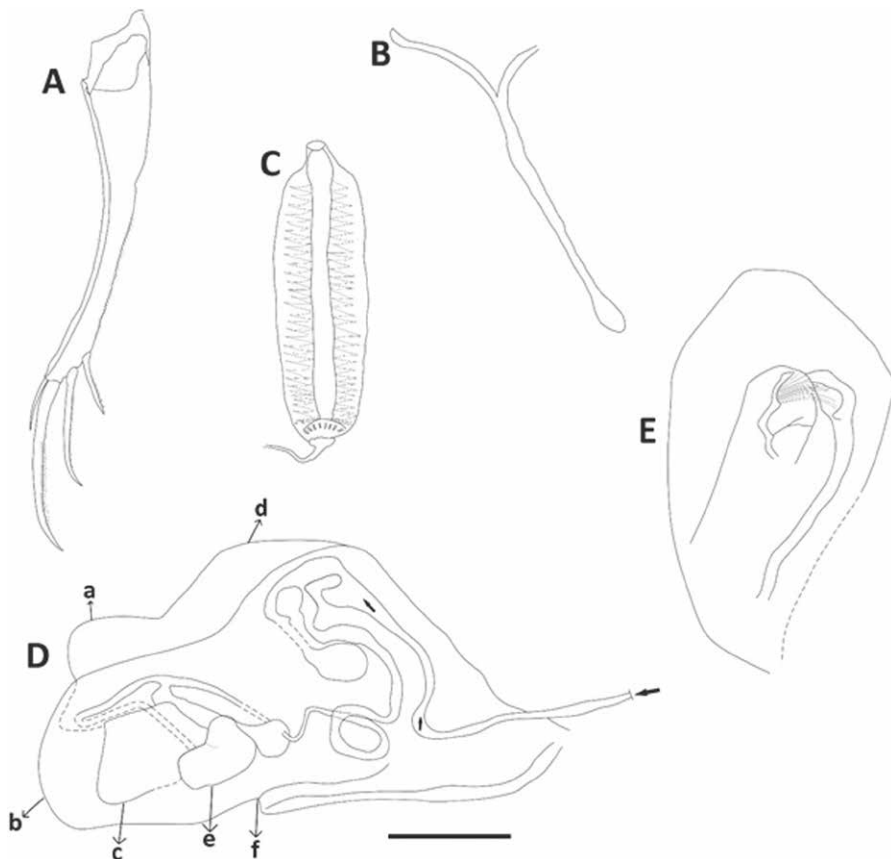


Figure 5. *Dolerocypris reyesi* sp. nov. (A) Ur of male. (B) Ur attachment of male. (C) Zenker's organ. (D) Hemipenis with lobes a, b, c, d, e, and f. (E) Genital organ of female. The scale bar represents 100 μ m for Fig. 5A and D–E and 50 μ m for Fig. 5B–C.

half of long seta. Length ratio $f > e > g$. Terminal segment subrectangular, seta h1 and h2 about same size, h2 claw long (longer than the last 3 segments) and slightly serrated.

Third Thoracic Leg (T3). Three segmented (Fig. 4G) with almost equally-long and smooth d1 and d2 setae and long dp seta on 1st segment. Second and 3rd segments with slightly-plumosed “e” and “f” setae, respectively. Seta “e” about 3 times longer than seta “f.” Terminal segment with h1–3 setae, h1 seta very short, h2 hook-like and h3 seta-like extending about half way of 3rd segment.

Uropod. Ending with 2 claws and 2 setae (Fig. 5A); anterior claw very strong and curved; posterior seta thinner about 2/3 of anterior claw, slightly longer than smooth, anterior seta. Uropodal attachment (Fig. 5B) with a curved dorsal branch.

Zenker Organ. 30 whorls ending with sperm canal (Fig. 5C).

Hemipenis. Medium in size (Fig. 5D). Lobe a (medial lobe of lateral shield) trapezoid, dorsal lobe of lateral shield (d) with pointing end. Lobe b (ventral lobe of medial shield) rounded, slightly longer than lobe a. Lobe c (medial lobe of ventral shield) slightly rounded. Bursa copulatrix (e) small. Transverse fold (f) about at mid-length.

Description (Female). Carapace similar in shape of male (Fig. 2A, E–F, M). L 1.34–1.70 mm; H 0.56–0.68 mm; W 0.45 mm (n = 4). H/L 0.42; W/L 0.30. G-claws (length ratio $G1 \approx G2 \approx GM > G3 > Gm$) present on A2 (Fig. 3C). Setae z1–3 thin, slightly exceeding half of terminal claws. T1 (Fig. 4E) normally developed, similar size with male T1, endopod with 2 short and 1 long h1–3 setae ($h2 > h1 > h3$). Seta h2 twice L of h1. All smooth. Endite with 10 + 3 apical setae. Genital part rounded and without appendages (Fig. 5E). All other soft parts similar to that of male.

Accompanying Taxa. *Cyclocypris dalyana*, *Cypridopsis* cf. *elongata*, and *Pseudocandona* cf. *stagnalis* (each included in Appendix 1); *Ilyocypris* sp. and *Limnocythere* sp. (not included in Appendix 1).

Remarks. Table 1 compares characters between *Dolerocypris reyesi* sp. nov. and *D. ikeyai* because these 2 species are the only 2 of the genus bearing very short (reduced) natatory (swimming) setae on A2. Other species are known with long swimming setae reaching or extending terminal claws of A2.

The genus *Dolerocypris* comprises 7 species (*D. fasciata*, *D. ikeyai*, *D. marina*, *D. opesta*, *D. sinensis*, *D. sisetensis*, *D. tenuis*) with 2 subspecies (*D. f. fasciata*, *D. f. nipponensis*) (Meisch et al., 2019). Savatentalinton and Sutta-

of MxI palp with 5 setae (4 long, smooth, apical setae and 1 short seta). First palp 3 times the length of 2nd palp. Second (terminal) segment rectangular with 5 claw-like, smooth, unequally-long setae.

Rake-Like Organ. Six to 7 teeth (Fig. 4B).

First Thoracic Leg (T1). Prehensile palps slightly asymmetrical ending with hooked-like fingers modified into clasping organs (Fig. 4C–D). Right palp stronger and more robust than left. Left palp thinner, longer, and proximally curved more tightly than right. Fingers ending with a strong, triangular part. Endite (masticatory process) with 2 groups of anterior setae, small antero-ventral group with 4 short setae, large group with ca. 10 smooth and plumosed setae. Two unequally-long a setae; medium-sized d and b setae present; c seta absent. Vibratory plate with 6 (5 long, 1 short) medium-sized setae.

Second Thoracic Leg (T2). Five segmented (Fig. 4F) with medium-sized plumosed d1 and d2 setae ($d1 > d2$) on 1st segment. Second, 3rd and 4th segments with e, f, 2g setae plumosed, respectively. Longest g seta 2 times longer than terminal segment, small seta

Table 1. Comparison of *Dolerocypris ikeyai* and *Dolerocypris reyesi* n. sp. (Smith and Kamiya, 2006).

Characters		<i>Dolerocypris ikeyai</i> ^a	<i>Dolerocypris reyesi</i> n. sp.
A1 segments		7 segmented	7 segmented
A1 Rome organ		short, stout	shorter
A1 Ya seta		L ca. last 4 segments	L ca. last 3 segments
A1 1st segment		a-lobe well developed	not a lobe-like
A2		5? or 4 (cf. Fig 4e with male)	4 segmented
A2 sw setae		reduced, not reaching middle L of penultimate segment, 5 very short setae and 1 seta	total of 6 very short setae, longest is not 2 x of others.
A2 Aesthetasc Y		twice length of other 5, on anterior-most side	
A2 z-setae		2 segmented, smooth	3 segments, shorter, blunt
A2 z-setae		3-thin setae in female and A2 female, z1-3 approximately 3/4 length of claws G1 to G3. Gm 80% length of GM. GM reaches to end of claws G1–G3. In males: z1-2 claw like, z3 short thin, z4 absent.	z1 claw like, z2 thin long (ca. z1) seta like, z3 short blunt claw like
A2 t-setae		t1-4 present	t2-4 present, t1 reduced/absent
A2 Aesthetasc y1-3		only y2-3 present	only y3 present, others not seen
T1 a-d setae		2a short, b, d present, c missing, 4 vibratory setae?, h3 plumosed	6 vibratory setae plumosed, c absent, 14 anterior setae, female has a spine, h1-3 setae smooth
T1 prehensile palps		2 segmented, hook-like, 1 thin spine	a setae longer setosed, b, d setae plumose and thick, 2 (one smaller) spines
T2		5 segmented	5 segmented
T2 d-setae		d1-2 short, similar in size, smooth	d1-2 setae plumose, d1>d2
T2 e-g setae		f > e > 2g (1 shorter)	similar, but plumose
Cleaning leg T3		3 segmented	3 segmented
T3 d-setae		all 3 smooth	similar
T3 e-g setae		e-f smooth	similar, e-f setae plumose
T3 terminal segment		pincer like	pincer like, h2 segmented, h1 short
Md S-setae		S1 plumosed, Seta s2	S1 plumose, s2 ca 3/4 of S1
Terminal segment		approximately 3/4 (1/4 must be?) of length of s1 cone-shape.	similar, all setae smooth
Md- alpha, beta, gamma setate		Final segment with 3 stout setae and 3 thinner, shorter setae	Alpha seta short ca 1/3 of S1 seta and slender. Beta seta short, broad and hirsute; Gamma seta stout and hirsute.
Md setal group		Alpha short and slender. Beta seta short, broad and hirsute; Gamma seta stout and hirsute.	
Md-palp vibratory setae		3+1 (all smooth)	3 thin and smooth and equal in size+1 (plumosed)
Md-coxa		NA	8-9 well developed plumosed setae
Mxl terminal segment		Coxa well developed	Coxa well developed
Mxl palp		with endite sporting (5 in figure) robust teeth.	with endite sporting (6) robust teeth.
		elongated	slightly elongated
		1st segment with 4 apical setae on outer corner, and 1 shorter, subapical seta. Final segment with 1 stout, claw-like seta and 5 shorter, thinner setae.	1st segment with 4 apical setae and 1 long (ca. equal size) subapical seta. Final segment with 2 stout, claw-like seta and 4 shorter setae.
		First endite with 2 hirsute, subapical setae on inner edge perpendicular to endite, and 1 smooth seta on inner edge at base of endite.	All setae smooth. First endite with 2 smooth, subapical setae, and 2 subequal smooth setae on inner edge at base of endite, plus ca. 10 short setae. Second endite with two claw-like and 4 thin setae. Third segment with 2 smooth bristles and 4 equally long smooth setae.

Table 1. Comparison of *Dolerocypris ikeyai* and *Dolerocypris reyesi* n. sp. (Smith and Kamiya, 2006).

Characters	<i>Dolerocypris ikeyai</i> ^a	<i>Dolerocypris reyesi</i> n. sp.
Mxl-bristle	2 smooth bristles	2 slightly segmented, smooth
Hemipenis	Ventral lobe of medial shield protruding slightly beyond other lobes. Medial lobe of ventral shield angular. Medial lobe of lateral shield trapezoid, dorsal lobe of lateral shield elongate and rounded.	lobes slightly different (see the description in the text)
Zenker Organ	with 21 rosettes.	with 29-31 rosettes.
Carapace size	L=1030–1200 mm, H= 410– 500 mm.	L =1640 mm, H=501 mm, W =450 mm
Carapace	RV overlapping LV	LV overlapping RV
Carapace shape	Carapace elongate, sub-crescent lateral outline, ventral margin almost straight.	Elongate, crescent, anterior part of the carapace more tightly curved, and the apex of the curve lower down.
Carapace internal	Inner lamella very wide both anteriorly and posteriorly.	anterior inner lamella wider than posterior
Carapace inner list	a prominent inner list on the anterior of the right valve absent	LV posteriorly weak or anteriorly absent
Surface	smooth	long strait lines better seen in SEM
Muscle scars	6 adductor scars (4 small scars with 2 slightly larger scars above) and 2 mandibular scars.	similar
Dorsal scars	NA	5-6 long scars
Left valve internal view	smooth	ventrally with two medium-sized teeth

^a This study.

jit (2016) provided detailed information about worldwide distribution of the genus while they pointed out that taxonomic problems occur with the classification of *D. marina* due to missing descriptions of the uropodal attachment by Hartmann (1965). This part is necessary for identification due to occurrence of Triebel’s loop that is used to characterize whether or not a species belongs to subfamilies Cypricerinae or Dolerocypridinae (see Nagler et al., 2014). The attachment is simple in Dolerocypridinae but the loop is present in the former. Due to lack of this important detail, the taxonomic status of *D. marina* is not clear in the genus.

Savatenalinton and Suttajit (2016) divided the species of the genus into South American and Eurasian groups. The first group includes *D. opesta* and *D. tenius* while the second group consists of 4 species (*D. fasciata*, *D. ikeyai*, *D. sinensis*, *D. sisaketensis*). It is clear that *D. fasciata* and *D. sinensis* are more frequently known in different countries than other species (e.g., Hartmann, 1964). However, some reports are also doubtful; for instance, Brehm (1923) underlining the lack of color bands on the carapace indicated that the species from Kwanhsien (Sichuan, China) belonged to *D. sinensis* or *D. fasciata*. Since Hartmann’s list in 1964, geographic distributions of both species have been encountered from several other parts of the world (e.g., Meisch, 2000; Smith and Kamiya, 2006; Akdemir and Külköylüoğlu, 2014). In NA, *D. sinensis* representing the genus was first recognized from stormwater management ponds in the Red Run watershed (Owings Mills, Maryland) (Gray et al., 2010). Therefore, *D. reyesi* sp. nov. is the second species of the genus in NA. The oldest fossil species of the genus were known from the Late Cretaceous from Brazil (Almeida and Carmo, 2013), while Palaeartic species are reported from early Oligocene (Carbonnel and Ritzkowski, 1969), Miocene (Janz, 1997; Pipík and Bodergat, 2004), Pliocene-Pleistocene (Tunoğlu et al., 2012) and Holocene (Griffiths et al., 1993).

As pointed out above, except for *D. ikeyai*, occurrence of short natatory setae on A2 distinguishes the new species from other species (Table 1). Furthermore, another critical difference between the 2 species is seen in the carapace dimensions. Accordingly, Meisch (2000, p. 359) stated that “LV overlaps RV ventrally. RV exceeds LV in length” for the general description of the genus. In *D. ikeyai*, RV overlaps LV anteriorly and posteriorly (Smith and Kamiya, 2006). Most recently, Savatenalinton and Suttajit (2016) reported a new species, *D. sisaketensis*, collected from rice fields and streams of 2 districts in Thailand and that the species exhibited RV overlapping LV anteriorly and posteriorly. In contrast, LV overlaps RV anteriorly and posteriorly in *Dolerocypris reyesi* sp. nov. Such a difference may be considered variation among the species of the genus while other characters correspond to the description of the genus.

Habitat. The species was found in shallow pools in a 20 m long rock-shelter. The water originated from small springs above the rock-shelter that flowed through the karstic rock-shelter and into a shallow canyon.

Suborder Cypridocopina Baird, 1845

Superfamily Darwinuloidea Brady and Robertson, 1885

Family Darwinulidae Brady and Norman, 1889

Genus *Microdarwinula* Danielopol, 1968

Microdarwinula zimmeri (Menzel, 1916) (*in* Menzel, 1917)

Appendix 3A

Material Examined. Three females and 1 juvenile from Spring 22-337 (Appendix 2, site 44), a karst spring at Fort Cavazos, Bell County, Texas, USA, lat 31.2001485 N, long 97.5721002 W, collected April 6, 2010, by James Reddell and Marcelino Reyes; 9 females examined from Asellid Spring (F 22-213) (Appendix 2, site 3), Fort Cavazos, Bell County, Texas, USA, lat 31.2102550 N, long 97.5853629 W, collected July 30, 2007, by Charles Pekins, James Reddell, and Marcelino Reyes.

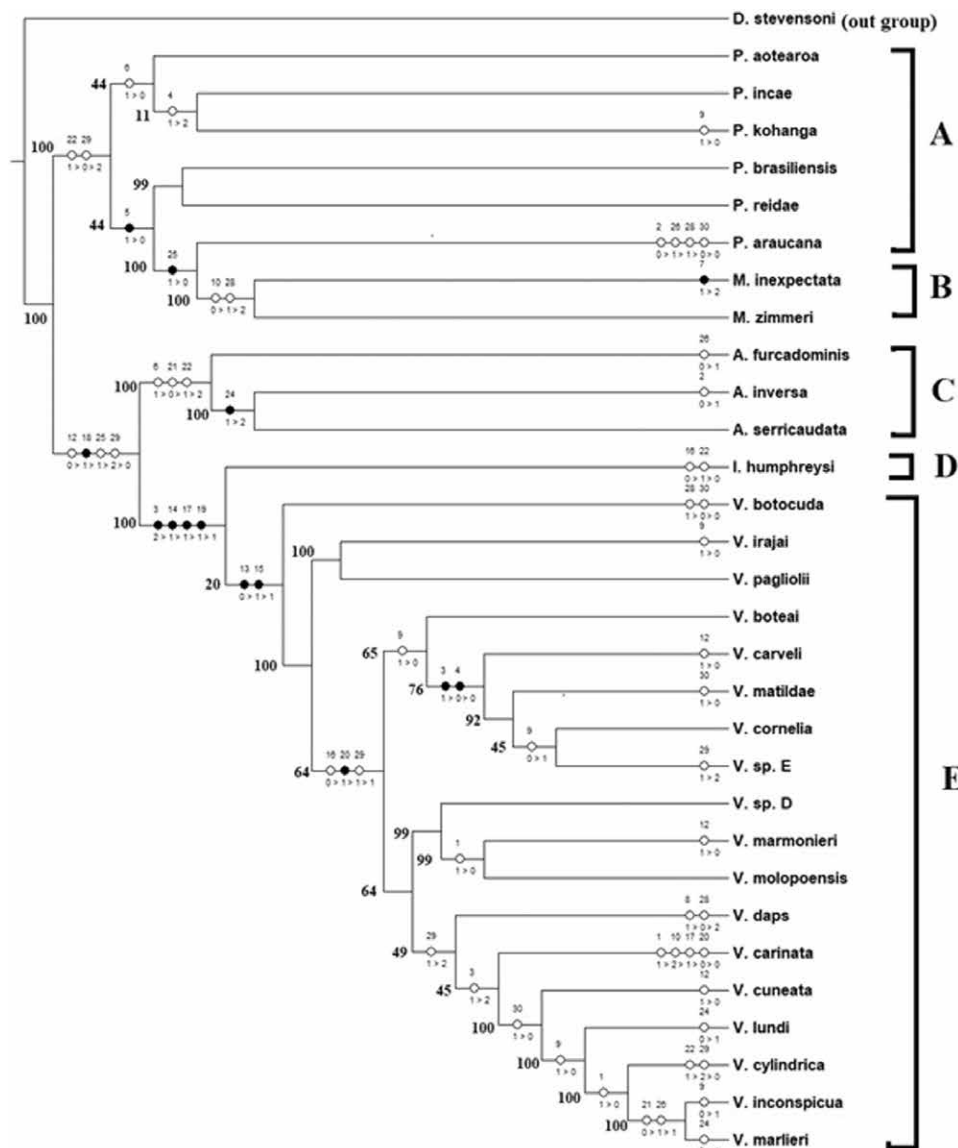


Figure 6. Strict consensus tree of phylogenetic analysis in WinClada-NONA software with 31 taxa and 30 characters. Species without clear morphological descriptions are not shown. Pleisiomorphic and apomorphic characters are coded as 0 and 1, respectively. Note nonhomoplasious changes (black circles) and homoplasious changes (white circles) with character number (number above the circles), character state (number below the circles), and character state transformation between each character state (>). The key to the character numbers and character states is Appendix 4. Bold numbers indicate percent values.

Remarks (*Microdarwinula zimmeri* and *Microdarwinula brevis*). When Danielopol (1968) described the genus represented by *M. zimmeri*, living individuals of the species were listed from Equatorial Africa (Tanzania), Asia (Sunda Islands), and Europe (Romania). Schäfer (2005, 2008) reported fossil specimens of *M. brevis* and *M. aff. zimmeri* from early Miocene and late Oligocene deposits in Germany, respectively. Danielopol (1968) underlined that the fossil species *D. brevis* described from the Miocene deposits from Germany by Straub (1952) was similar, and now it is a synonym. He also underlined that his reports from Romania were possibly relicts of the Tertiary. Martens et al. (1997) listed the species from 1 Holocene locality in France and 8 Quaternary localities in Germany. Including these previous records, live individuals of the species are known from Indonesia, Africa, Madagascar, and Cuba (Martens et al., 1997). Taylor (1992) reported an undescribed species from 2 springs located in south-central Ontario (Canada) as *Microdarwinula* sp. This taxon has not been officially described at species level as *M. zimmeri* (similarly, see the remarks for *Penthesilenula aotearoa* and *P. brasiliensis* below). However, the taxon was accounted *M. zimmeri* later in some studies (e.g., Martens et al., 1997; Rossetti and Martens, 1998; Pinto et al., 2005; Smith and Delorme,

2010) without a clear explanation and description of the species, despite the fact that Taylor (1992) used only carapaces for the description and did not use internal morphology of the specimens. This makes it difficult to accept that her specimens belong to *M. zimmeri* because characters in carapace (e.g., presence of internal teeth, elongate shape of carapace) and soft body parts (e.g., absence of y1 aesthetasc in A2) are important for the species description and separation it from its only conspecific *M. inexpectata* reported from Brazil by Pinto et al. (2005).

Remarks (*Microdarwinula zimmeri* and *Microdarwinula inexpectata*). In a recent study using 30 morphological characters with 23 taxa (21 species and 2 unnamed taxa in *Vestalenula*), Rossetti et al. (2011) illustrated phylogenetic clustering results where 2 species (*M. zimmeri* and *M. inexpectata*) were also compared. Among the characters, 27 of 30 were the same while 3 characters (shape of valve in lateral view (Appendices 4 and 5, character no. 1), L/H ratio (Appendices 4 and 5, character no. 9), and hinge type (Appendices 4 and 5, character no. 7)) were used to distinguish the species. However, Rossetti et al. (2011) did not use absence/presence of y1 aesthetasc on A2 as a different character despite the fact that the character was among the 3 “differential diagnostic” characters (Pinto et al., 2005) and was even used a taxonomic key to separate the 2 species (Karanovic, 2012). Furthermore, comparing the character states used by Rossetti et al. (2011), *M. zimmeri* and *M. inexpectata* seem to display more common characters. For example, character states for lateral view of carapace were given as rounded (state 2) in *M. zimmeri* and sub-squarish or rounded (state 1 or 2) in *M. inexpectata*. However, in the original description of *M. inexpectata*, carapace was described as ovoid shaped in lateral view (or relatively rounded) (Pinto et al., 2005). Similarly, there is an overlap in the ratio of the 2 species; for instance, *M. zimmeri* L/H ratio was between 1.8–2.2 (state 1) when it was less than 1.8 (state 2) in *M. inexpectata*. If one compares the ratio from the original description of Pinto et al. (2005), L/H ratio can be found higher than 1.8 (up to 1.85, if not more). We underline that using these 2 character states might not be enough to portray differences between the 2 species; therefore, results of cluster analyses of Rossetti et al. (2011) seem to be confusing. Along with a few other slight differences, Pinto et al. (2005) underlined that valve morphology can be ambiguous when comparing the lineages (i.e., *M. zimmeri* and *M. inexpectata*). Rossetti et al. (2011) pointed out similar view that identification of species or genus can be incorrect when valve morphology is exclusively used in the Darwinulidae. This is because valve morphology can be affected by ecological conditions more than soft body parts, which are especially more conservative in the family. Thus, overall, one may consider that *M. inexpectata* may be a synonym to *M. zimmeri* due to plastic valve morphology under different aquatic conditions. Van Doninck et al. (2003) mentioned the presence of *M. zimmeri* from 8 different localities including NA, but they did not show these localities in their map of NA (their Fig. 2) and also did not provide information about sampling sites. Smith and Delorme (2010) provided left and right valves of *M. zimmeri* from interstitial fen sediments of Mantua Bog (Ohio) but did not clarify whether the species had soft body parts during sampling. Thus, live individuals of *M. zimmeri* reported in the present study can be counted as the 2nd (if not the 1st) record of the species from NA and the US.

Family Darwinulidae Brady and Norman, 1889

Genus *Vestalenula* Rossetti and Martens, 1998

Vestalenula marmonieri Rossetti and Martens, 1999

Appendix 3B

Material Examined. Twenty-one females from Cute Chick Spring (Appendix 2, site 14), Fort Cavazos, Bell County, Texas, USA, lat 31.1944975 N; long 97.5875553 W, collected on March 31, 2010, by James Reddell and Marcelino Reyes.

Remarks. In general, the genus *Vestalenula* can be separated from its close relative *Penthesilenula* (see below) based on the presence/absence of a postero-ventral keel on the RV (no keel in *Penthesilenula*), the presence of an antero-ventral internal tooth on the LV in *Vestalenula*, presence of 1 dorsal seta on the 1st segment of A1 in *Vestalenula*, and the presence of 1 seta and 1 spine in the A2 exopod in *Vestalenula* (2 setae and 1 spine present in *Penthesilenula*) (Rossetti and Martens, 1998; Rossetti et al., 2011). Based on the keel length on the RV, the genus may include 2 groups: it is short in the *V. boteai* group but elongate in the *V. danielopoli* group. However, taxonomic separation of these groups can be questionable because the keel length or even the keel itself may not be clearly seen in some cases (Karanovic, 2012). Also, as noted above for *M. zimmeri*, using such carapace morphology requires great attention (Pinto et al., 2005; Rossetti et al., 2011) because carapace morphology (referring to phenotypic plasticity) is more flexible to environmental changes than soft body parts. In our specimens, the keel on RV is short but not rounded, suggesting that they belong to the *V. boteai* group.

According to earlier studies (Rossetti and Martens, 1999; Smith and Delorme, 2010), the genus is not known from NA. Indeed, its distribution has up to now seemed to be limited in the north of the New Caledonia Island (a tributary of the River Diahot) (Rossetti and Martens, 1999) and Australasian (Yonderup Lake and Eil Spring in Australia) (Martens and Rossetti, 2002, and references therein). Unlike *Darwinula* (and now *Penthesilenula*, this study) and 1 species of *Alicenula* from Florida (Keyser, 1975), different species of the genus *Vestalenula* have been unofficially known from USA (e.g., Külköylüoğlu, 1999), and they are already known from Europe (Radovan Kyška-Pipík, Slovak Academy of

Sciences, personal communication). However, most recently, Külköylüoğlu et al. (2021) reported occurrences of the 2 species of the genus (*V. cuneata*, *V. cylindrica*) from several aquatic sites in Texas indicating that rare occurrences of several genera of the family (or none so far) can also be related to lack of studies in the USA (and NA). Thus, this report of *V. marmonieri* is the first official report of the species from NA. Similar to the other 3 species of the family stated here (*P. aotearoa*, *P. brasiliensis*, *M. zimmeri*), finding *V. marmonieri* from Texas is considerably important for expanding its geographical distribution further in NA. Artheau (2007) proposed that the wide range of geographical distribution of the Holocene species of the genus was probably related to various ways of adapting to the conditions in subterranean habitats. Thus, this supports independent colonization of these habitats since the Tertiary and/or earlier times. The reporting of species of the genus from the Neogene in Italy, Crete, and Serbia (Ligos et al., 2009) and Turkey (Tuncer, 2020) and from the Quaternary in Portugal (Minati et al., 2008) and Siberia (Konovalova, 2019) exhibited strong supportive evidence that even the fossil species of the genus are widely distributed worldwide. Our specimens were collected from Cute Chick Spring, which emerges from small holes in the floor below the entrance to Cute Chick Cave. Including the previous studies mentioned herein, our finding suggests that *V. marmonieri* can be found from lakes, springs and shallow hyporheic (possibly interstitial) aquatic habitats.

Family Darwinulidae Brady and Norman, 1889

Genus *Penthesilenula* Rossetti and Martens, 1998

Penthesilenula incae Delachaux, 1928

Appendix 3C

Material Studied. 16 females from Asellid Spring (F 22-213) (Appendix 2, site 3), Fort Cavazos, Bell County, Texas, USA, lat 31.2102550 N, long 97.5853629 W, collected on July 30, 2007, by Charles Pekins, James Reddell, and Marcelino Reyes.

Remarks. According to previous studies (Delachaux, 1928; Rossetti et al., 1996; Rossetti and Martens, 1998; Pinto et al., 2004), *P. incae* is so far known from Lake Huaron (Peru) and Laguna Guaqui (Bolivia). This is the first record of its occurrence in North America. Although the species, under the genus *Darwinula*, was first described from a lake in Peru by Delachaux (1928), line drawings and details in the carapace were not clearly expressed (Rossetti and Martens, 1998). Therefore, it was redescribed under the genus *Penthesilenula* by Rossetti et al. (1996). The genus *Penthesilenula* includes 2 groups: *P. incae* and *P. africana*. They are basically separated by the presence of a caudal and/or a postero-ventral internal tooth on the valves. In the *P. incae* group, an internal caudal tooth is present on the LV and a postero-ventral tooth is absent, while in the *P. africana* group a caudal tooth is absent and a postero-ventral tooth is present (Rossetti and Martens, 1998). Our specimens seem slightly smaller in length (ca. 750 mm) than the reports in the literature, ranging from 772 mm (Rossetti et al., 1996) to 870 mm (Delachaux, 1928). Pinto et al. (2004) underlined that different populations of the genus (e.g., *P. brasiliensis*) exhibited highly-variable carapace morphology while soft body parts displayed robust characters. These authors also discussed the situation from an evolutionary perspective, indicating that the taxonomic explanation of such variabilities in carapace morphology was not simply due to environmental factors (e.g., water temperature) that play an effective role on valve size more than valve shape. This is correct when there are indeed overlapping ranges between the length and height ratios among the species of the genus. There is not much known about its ecology because of its rare occurrences from only a couple of sample sites.

Penthesilenula aotearoa Rossetti et al., 1998

Appendix 3D

Material Studied. 46 females from Spring 22-337 (Appendix 2, site 44), Fort Cavazos, Bell County, Texas, USA, lat 31.2001485 N, long 97.5721002 W, collected on April 6, 2010, by James Reddell and Marcelino Reyes.

Remarks. Under the genus *Darwinula*, the species was first described from a small swamp flowing into the Karori Stream (New Zealand) (Rossetti et al., 1998). However, the same species from the same locality was removed to another genus *Penthesilenula* in the same year by Rossetti and Martens (1998). The second report of the species was obtained from a swamp, spring, and wet moss sites in São Paulo, Brazil (Pinto et al., 2004). Thus, this is the first report of the genus and the species *P. aotearoa* from NA. Similar to *P. incae* mentioned above, this species belongs to the *P. incae* group. The species was collected from a spring site coming out of a 15 cm diameter hole in talus and flowing into a canyon (Reddell and Reyes, 2010).

PHYLOGENY

WinClada-NONA analyses with 31 taxa and 30 characters exhibited a majority tree (Length = 89, consistency index = 0.43, retention index = 0.77) in more than 100 equally-parsimonious trees (Fig. 6, Appendices 4 and 5). The species used here are mainly separated into 6 clustering groups of genera comprising *Darwinula* (outgroup), *Penthesilenula* (Group A), *Microdarwinula* (Group B), *Alicenula* (Group C), *Isabenula* (Group D), and *Vestalenula* (Group E), while *Penthesilenula* and *Vestalenula* included 2 subgroups each (see the Discussion for details). These results are found consistent before and after exclusion of 6 taxa/species (Fig. 6).

DISCUSSION

New Records of Species

Other than the species remarked upon above, *Typhlocypris* cf. *prespica* and 3 other taxa (*Cypridopsis* cf. *herpeticata*, *Pseudocandona* cf. *parallela*, *P.* cf. *pratensis*) collected from Texas and 1 (*Physocypris exquisita*) collected from Mexico are newly reported in this study. Due to a lack of individuals, these taxa are not discussed here. Moreover, the other species not discussed here are already known from Texas (Appendix 1). Finding the species stated above as new records from Texas, the US, and NA is important for at least 2 reasons. First, geographical distribution of these species has now been expanded into the Northern Hemisphere. Considering the fact that Texas is near the border of the neotropical region, this finding is not surprising, but it portrays strong support for the historical correlation between the continents (i.e., North and South America). Second, distribution of some species (e.g., *Penthesilenula aotearoa*) is probably much wider than currently known. It is true that such a scarcity of reporting the species from a few regions implies a lack of studies. The lack of studies is the problem for species and higher taxonomic levels. This is important because reporting scarcity of the species can be associated with inadequate information about species description (Rossetti and Martens, 1998). Although we fail to provide water quality measurements, distribution and occurrence of several species appear to be correlated with conditions in the waters they inhabit. This may be the case for *P. Aotearoa* that seems to inhabit slow- (or not) flowing water bodies related to springs. However, this needs to be confirmed in future studies due to the lack of ecological data.

The Family Darwinulidae

As remarked above, comparing the levels of plasticity on carapace structure and soft body parts, it may be true that carapace shape and size of some species of Darwinulidae are more prone to environmental factors than soft body parts. Indeed, such a relationship was also illustrated in several other ostracod species (e.g., Keyser and Walter, 2004; Keyser, 2005). For example, Minati et al. (2008) found certain differences in carapace morphology of *Vestalenula cylindrica* in some European countries (Austria and Slovakia versus Portugal and southern France), suggesting that such differences could be the indication of micro-evolutionary changes. If so, using characters such as carapace size and/or occurrence or size of tooth and keel should require utmost attention and probably be avoided in taxonomic keys. Similar problems can be seen with soft body parts in 2 possible ways: (1) damage of soft body parts during dissection may bring about additional difficulty for species identification, and (2) even soft body parts can be changed due to environmental factors as was shown in the hemipenis of *Heterocypris incongruens* by Yavuzatmaca and Külköylüoğlu (2019). Thus, our study gives the utmost attention to species description when using these characters.

The Genus *Vestalenula*

According to Meisch et al. (2019), most recently, the family Darwinulidae covers 6 genera (*Alicenula*, *Darwinula*, *Isabenula*, *Microdarwinula*, *Penthesilenula*, and *Vestalenula*). This supports the view of previous studies (e.g., Martens et al., 2005; Rossetti et al., 2011). Results of our phylogenetic analyses (Fig. 6) are generally in agreement with this view, while clustering relationships of 2 genera (*Microdarwinula* and *Isabenula*) deserve discussion (but see the remarks above). Among these 6 genera, it is not surprising that they consist of subgroups. For example, this is the case in *Vestalenula* (Fig. 6). Artheau (2007) underlined that *Vestalenula* bears 2 subgroups as the *V. danielopoli* group (keel on the LV elongated) and the *V. boteai* group (keel short). However, as the author clearly stated, the second group (Fig. 6 in Artheau, 2007) included both species with short and long keel. Accordingly, Artheau implied that this character (even if it is considered as a plesiomorphic one) may not be enough to conclude that the *V. danielopoli* group as an ancestor has historical priority over the *V. boteai* group. Thus, one may consider that length of keel may not indicate clear separation among these groups since such a character on the carapace appears to exhibit more plasticity than considered before as suggested by Smith et al. (2015) for the species *V. cylindrica* (cf. Rossetti and Martens, 1999). Moreover, it is known that carapace structure seems to be more vulnerable to environmental changes than soft body parts. This view was also shown in other ostracods. For example, Finston (2000) compared morphological and molecular (allozyme) characters of species of *Mytilocypris*, a common genus in saline aquatic bodies of Australia, and found serious incompatibility between them. According to Finston, one of the explanations of such incongruence was due to the plasticity of some characters such as carapace size, shape, and allometry. If so, separation of a new species into one of those subgroups based on this character(s) without soft body parts (e.g., the species *V. flexuosa* described without soft body parts by Rossetti and Martens (1999)) can confuse distinguishing the species/taxa from other members of the groups.

The Genus *Penthesilenula*

This issue is also similar for the genus *Penthesilenula* in which there are 2 subgroups: the *P. incae* group (LV without postero-ventral tooth and with caudal tooth) and the *P. africana* group (LV with postero-ventral tooth and without caudal tooth). Pinto et al. (2005) described a new species *P. reidae* from waters in bromeliad pouches in Brazil. The species

was placed into the *P. africana* group. The authors specifically pinpointed that the species was closely related to the *P. brasiliensis* lineage since the soft body parts of *P. reidae* were almost identical with a few slight differences (Pinto et al., 2005). Based on these perspectives, one may consider that both species may be synonyms. This is probably the reason that these 2 species were found in the same lineage of Rossetti et al. (2011).

The Genus *Microdarwinula*

In terms of *Microdarwinula*, our clustering analysis showed that it was clustered closer to the genus *Penthesilenula*. This clustering relationship was earlier suggested by Martens et al. (2005) and Rossetti et al. (2011). In our case, we found 2 species of *Microdarwinula* (*M. inexpectata*, *M. zimmeri*) clustered in the same subgroup with *P. araucana*. Martens et al. (2005) did not include *P. araucana* in their analyses due to uncertainty about morphological characters of it (see also other species in their study). Unlike these studies, we accounted for the species in our analyses. Inclusion of this species (and other species not used in previous studies) was due to new information about them. However, this does not change the general view that *Microdarwinula* appears to be closely related to *Penthesilenula*. The separation of 2 species of the genus *Microdarwinula* with *P. araucana* was based on the presence of a postero-internal tooth in the LV of the *Microdarwinula* species (Fig. 6).

The Genus *Isabenula*

Another genus that is worth discussion herein is *Isabenula*. It is represented by 1 species (*I. humphreysi*) in our analyses. The genus was first proposed as a new one by Rossetti et al. (2011). Ever since its introduction, the genus has been only known with its type species. It was clustered between the 2 genera *Alicenula* and *Vestalenula*. The species *I. humphreysi* was separated from the genus *Vestalenula* with 4 nonhomoplastic characters (characters 3, 14, 17, 19 in Fig. 6). Both genera are clearly separated from other genera discussed above (but see the remarks). Except for some slight differences, *Vestalenula* showed subgroups nested within the genus as reported similarly in the previous studies discussed above. Our finding different results can be related to 3 possibilities: (1) we used more taxa/species than previously used in the literature because we included new reports not used in those earlier studies; (2) we used different character states in our analyses (see Appendices 4 and 5); and (3) we used a different clustering program. It is important to underline that—as our results imply—using more characters and/or different character states along with additional species can change clustering relationships among the genera (Külköylüoğlu et al., 2019). Nevertheless, our results with slight differences discussed above confirm the previous findings.

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APPENDIX

Appendix 1.																															
Taxa/Site no	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
<i>Cavernocypris reddelli</i>	+	+			+	+	+	+		+		+	+	+		+	+	+	+	+	+					+	+				
<i>Cavernocypris wardi</i>																															
<i>Chlamydotheca texasiensis</i>															+													+			
<i>Cyclocypris dalyana</i>					+		+			+											+		+								
<i>Cypridopsis cf. elongata</i>																							+			+					
<i>Cypridopsis cf. herpestica</i>									+																						
<i>Cypridopsis vidua</i>			+							+						+								+	+						
<i>Darwinula stevensoni</i>			+								+		+										+	+	+						
<i>Dolerocypris reyesi</i> n.sp.																							+								
<i>Herpetocypris intermedia</i>																								+	+						
<i>Heterocypris incongruens</i>																													+	+	
<i>Ilyocypris gibba</i>																															
<i>Microdarwinula zimmeri</i>			+																												
<i>Penthesilenula aotearoa</i>																															
<i>Penthesilenula incae</i>			+																												
<i>Physocypris denticulata</i>																															
<i>Physocypris exquisita</i>																															
<i>Physocypris gibbera</i>																															
<i>Pseudocandona cf. albicans</i>				+												+													+		
<i>Pseudocandona jeanneli</i>											+																				
<i>Pseudocandona cf. parallela</i>																															
<i>Pseudocandona cf. pratensis</i>	+																				+						+				
<i>Pseudocandona semicognita</i>																															
<i>Pseudocandona stagnalis</i>									+			+											+			+					
<i>Schornikovdona bellensis</i>																															
<i>Typhlocypris cf. prespica</i>																															
<i>Vestalenula marmonieri</i>														+																	

Appendix 1. (Continued)

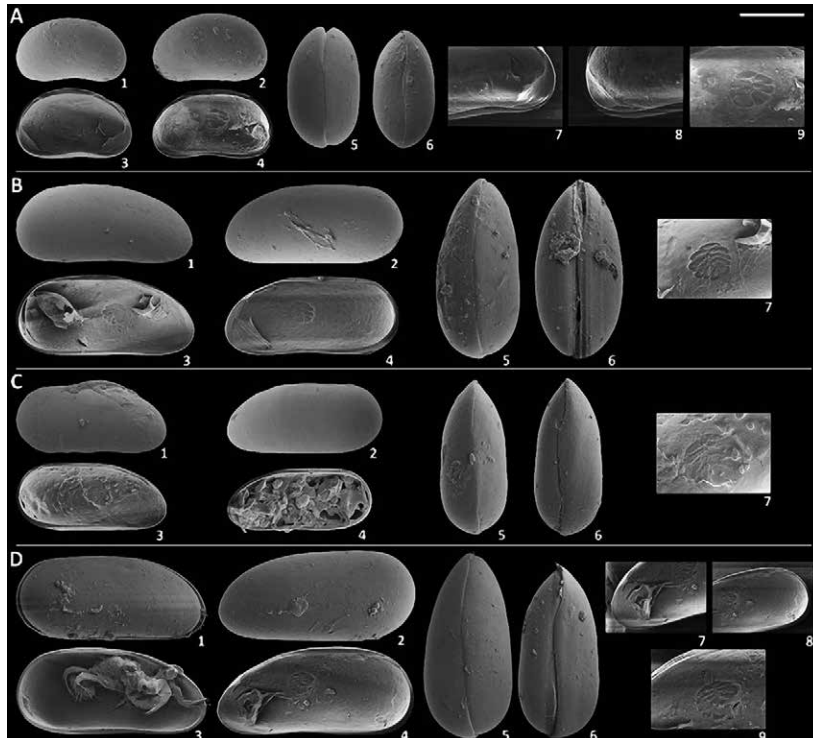
Taxa	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62		
<i>Cavernocypris reddelli</i>										+		+		+	+	+	+	+	+		+					+					+		
<i>Cavernocypris wardi</i>													+																				
<i>Chlamydotheca texasiensis</i>																																	
<i>Cyclocypris dalyana</i>													+	+	+							+								+		+	
<i>Cypridopsis cf. elongata</i>																																	+
<i>Cypridopsis cf. herpestica</i>																						+											
<i>Cypridopsis vidua</i>								+												+	+					+						+	
<i>Darwinula stevensoni</i>						+	+										+				+												
<i>Dolerocypris reyesi</i> n. sp.																																	
<i>Herpetocypris intermedia</i>																																	
<i>Heterocypris incongruens</i>																											+						
<i>Ilyocypris gibba</i>																												+					
<i>Microdarwinula zimmeri</i>																		+															
<i>Penthesilenula aotearoa</i>																		+															
<i>Penthesilenula incae</i>																																	
<i>Physocypris denticulata</i>	+	+																															
<i>Physocypris exquisita</i>										+																							
<i>Physocypris gibbera</i>					+																												
<i>Pseudocandona cf. albicans</i>						+	+								+							+											
<i>Pseudocandona jeanneli</i>																																	
<i>Pseudocandona cf. parallela</i>																	+																
<i>Pseudocandona cf. pratensis</i>																																	
<i>Pseudocandona semicognita</i>												+						+		+	+												
<i>Pseudocandona stagnalis</i>									+																					+			
<i>Schornikovdona bellensis</i>			+																														
<i>Typhlocypris cf. prespica</i>																															+		
<i>Vestalenula marmonieri</i>																																	

Appendix 2.

Site No	Site name	County	Date	Cat. No.	Coordinates
1	1913 Rockshelter Spring (F 36-9), Fort Hood	Bell	01.04.2010	72.558	31.1078392; -97.5519471
2	Amphitheater Spring (F 20-84), Fort Hood	Bell	25.07.2007	58.381	31.2352512; -97.5745017
3	Asellid Spring (F 22-213), Fort Hood	Bell	30.07.2007	58.413	31.2102550; -97.5853629
4	Banzai Mud Dauber Cave, Camp Bullis	Bexar	04.06.2007	56.606	29.6485451; -98.5316278
5	Bill's Spring. Fort Hood.	Bell	05.04.2010	71.219	31.1900247; -97.5406016
6	Bill's Spring. Fort Hood.	Bell	30.03.2010	71.233	31.1900247; -97.5406016
7	Bill's Spring. Fort Hood.	Bell	13.04.2010	72.577	31.1900247; -97.5406016
8	BLORA Shelter Spring (F BLORA-4), Fort Hood	Bell	14.03.2010	70.910	31.1380789; -97.5598995
9	BLORA Shelter Spring (F BLORA-4), Fort Hood	Bell	02.10.2007	61.545	31.1380789; -97.5598995
10	Bullhead Spring, (F 23-371) Fort Hood	Bell	05.04.2010	70.949	31.1902920; -97.5434939
11	Camp Bullis Cave No:1, Camp Bullis	Comal	20.12.2000	36.871	29.7471700; -98.6110970
12	Copperhead Spring Cave (F 35-1), Fort Hood	Bell	25.07.2007	58.099	31.1264089; -97.6045419
13	Copperhead Spring Cave (F 35-1), Fort Hood	Bell	02.10.2007	61.533	31.1264089; -97.6045419
14	Cute Chick Spring, Fort Hood	Bell	31.03.2010	71.214	31.1944975; -97.5875553
15	Dandridge Spring Cave	Val Verde	22.05.2002	33.324	29.8041843; -101.0059391
16	Dripping Harvestman Spring (F 22-84), Fort Hood	Bell	30.07.2007	61.557	31.2080329; -97.5818377
17	El Sapo Cave, Fort Hood.	Bell	30.03.2010	71.008	31.1671955; -97.5314778
18	Faucet Spring, Fort Hood	Bell	17.03.2010	70.938	31.1453204; -97.5644893
19	Faucet Spring, Fort Hood	Bell	14.03.2010	70.990	31.1453204; -97.5644893
20	Fern Spring (F 22-290), Fort Hood	Bell	06.04.2010	71.194	31.1947765; -97.5709110
21	Geocache Cave (F 33-16), Fort Hood	Bell	30.07.2007	58.404	31.1564841; -97.6268805
22	Geocache Cave (F 33-16), Fort Hood.	Bell	30.07.2007	58.409	31.1564841; -97.6268805
23	Hidden Rockshelter (F 35-11), Fort Hood	Bell	16.03.2010	72.550	31.1330307; -97.5987080
24	Homestead Spring No.1, Fort Hood	Bell	13.05.2012	80.274	31.1224868; -97.5808046
25	Homestead Spring No.1, Fort Hood	Bell	30.04.2012	80.303	31.1224868; -97.5808046
26	Homestead Spring No.2, Fort Hood	Bell	13.05.2012	80.290	31.1225769; -97.5807928
27	Jelly Bean Spring (8A-1), Camp Bullis	Bexar	17.04.2001	36.863	29.6445270; -98.5924620
28	La Cantera Cave No. 2, Larson's Pit, 5mi.	Bexar	26.09.1995	31.436	29.5910300; -98.6103600
29	Little Hunt Spring	Hays	06.10.2016	Zara 9403	30.1240600; -97.8630200
30	Mixmaster Cave Fort Hood	Coryell	05.11.1998	31.635	31.2206066; -97.6163974
31	Mixmaster Cave Fort Hood	Coryell	05.11.1998	31.637	31.2206066; -97.6163974
32	Mormon Spring No. 3	Travis	23.01.2017		30.3131901; -97.7747410
33	Mormon Spring No. 3	Travis	07.02.2017		30.3131901; -97.7747410
34	Nolan Creek Cave (F 33-2), Fort Hood	Bell	12.05.2010	72.538	31.1516532; -97.6349607
35	PC Spring	Williamson	27.09.1999	55.445	30.4798590; -97.7419449
36	Perch Jerk Spring, Fort Hood	Bell	21.05.2012	80.250	31.1677431; -97.6481702
37	Perch Jerk Spring, Fort Hood	Bell	27.05.2012	80.261	31.1677431; -97.6481702
38	Phantom Lake Spring Cave	Jeff Davis	08.04.1995	55.443	30.9349390; -103.8496738
39	Pipe Spring (F 22-106), Fort Hood	Bell	02.10.2007	61.549	31.1889335; -97.5614702
40	Pozo La Pilita, Rancho Azufrosa, Tamaulipas, Mexico	**	05.01.2003	35.565	22.9940667; -98.1586689

Appendix 2. (Continued)

41	Rimstone Dam Rockshelter Lower Spring, Fort Hood	Bell	16.04.2012	80.242	31.1907026; -97.5599322
42	Shoal Creek. Spring No.4.	Travis	27.02.2017	Zara-9497	30.2963600; -97.7487430
43	Spicewood Creek Pipe Spring (F 115-140), Fort Hood	Bell	11.03.2010	70.984	31.1741240; -97.5409924
44	Spring (22-337), Fort Hood	Bell	16.04.2012	80.232	31.2001485; -97.5721002
45	Spring (F 23-378), Fort Hood.	Bell	05.04.2010	71.022	31.1901247; -97.5414711
46	Spring (F BLORA-17), Fort Hood	Bell	17.03.2010	70.924	31.1414030; -97.5707997
47	Spring (F BLORA-17), Fort Hood	Bell	14.03.2010	70.999	31.1414030; -97.5707997
48	Spring 22-293, Fort Hood	Bell	06.04.2010	72.592	31.2001485; -97.5721002
49	Spring 35-14, Fort Hood	Bell	20.10.2010	72.824	31.1258286; -97.5968003
50	Spring 4C-18 (middle), Camp Bullis	Bexar	18.04.2001	36.833	29.6827330; -98.5733940
51	Spring 4C-18 (south), Camp Bullis	Bexar	18.04.2001	36.855	29.6827240; -98.5733840
52	Spring 4C-27, Camp Bullis	Bexar	18.04.2001	36.826	29.6884370; -98.5679330
53	Spring 4C-27, Camp Bullis	Bexar	31.05.2007	56.674	29.6884370; -98.5679330
54	Spring 7-49, Camp Bullis	Bexar	14.04.2001	36.955	29.6769940; -98.6148921
55	Spring 7-51, Camp Bullis	Bexar	17.04.2001	36.862	29.6693700; -98.6063330
56	Spring 7-51, Camp Bullis	Bexar	04.06.2007	56.705	29.6693700; -98.6063330
57	Spring 8A-1, Camp Bullis.	Bexar	31.05.2007	56.719	29.6445270; -98.5924620
58	Spring 9-154, Camp Bullis	Bexar	31.05.2007	56.741	29.6674497; -98.5503310
59	Spring 9-156, Camp Bullis	Bexar	31.05.2007	56.672	29.6726491; -98.5558460
60	Stampede Creek Upper Spring (F 53-32), Fort Hood	Coryell	06.04.2010	75.275	31.3072276; -97.8290450
61	Stealth Cave, Camp Bullis	Bexar	20.12.2000	72.733	29.6606570; -98.5992170
62	Waterfall Spring, Fort Hood	Bell	16.04.2012	80.218	31.1953142; -97.5633040



Appendix 3.

Appendix 4.

No Characters with character states

-
- 1 Lateral view: sloping (0), sub-squarish (1) or rounded (2)
 - 2 L/R (0) or R/L (1) overlap
 - 3 Postero-ventral keel on RV: absent (0), short (1) or long (2)
 - 4 Ventro-frontal internal tooth in LV: long (0), short (1) or absent (2)
 - 5 Posterior internal tooth (mostly in LV) in ventral position: present (0) or absent (1)
 - 6 Posterior internal tooth (mostly in LV) in caudal position: present (0) or absent (1)
 - 7 Hinge: adont (0), with simple large cardinal teeth (1) or subdivided large cardinal teeth (2)
 - 8 Size: >0.6 mm (0) or ≤0.6 mm (1)
 - 9 Le/H ratio: >2.2 (0), between 1.8 and 2.2 (1) or < 1.8
 - 10 Brooding space: externally visible (0) or not (1)
 - 11 Position of Cms: towards the front (0) or central (1)
 - 12 Muscle scars: average number of scars >8 (0) or ≤8 (1)
 - 13 A1, first segment: with 2 (0) or 1 (1) dorsal seta(e)
 - 14 A1, "exopodite": with 3 (0) or 2 (1) setae
 - 15 A1, 2nd segment: with (0) or without (1) dorso-apical seta
 - 16 A1, third segment: with (0) or without (1) ventro-apical seta
 - 17 A1, fourth segment: with (0) or without (1) ventro-apical seta
 - 18 A1, fourth segment: with 2 (s2 s3) (0) or 1 (1) large dorsal seta(e)
 - 19 A2, exopodite: with 2 (0) or 1 (1) seta(e) (+spine)
 - 20 A2, first segment of endopodite: with 2 (0) or 1 (1) ventro-apical seta(e)
 - 21 Md-palp, penultimate segment: seta z long (0) or short (1)
 - 22 Md-palp, penultimate segment: seta y long (0), short (1) or absent (2)
 - 23 Md-palp, last segment: «poil stevensoni» present (0) or absent (1)
 - 24 Md-palp, last segment: number of apical claws: 5 (0), 4 (1) or 3 (2)
 - 25 Md-palp, last segment: seta a claw-like (0), spine-like (1), absent (2)
 - 26 Md-palp, last segment: seta c present (0) or absent (1)
 - 27 T1, penultimate palp segment: number of seta(e): 2 (0) or 1 (1)
 - 28 CR: number of seta(e): 2 (0), 1 (1) or absent (2)
 - 29 P-abd: with projections (0), smooth (1) or absent (2)
 - 30 Caudal seta: present (0) or absent (1)
-

Appendix 5.

Morphological Characteristic																															
Taxa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	
<i>A. furcabadominis</i>	0	0	2	1	1	0	0	1	0	0	0	1	0	0	0	0	0	1	0	0	1	2	1	1	2	1	1	1	0	1	
<i>A. inversa</i>	0	1	2	1	1	0	0	1	0	0	0	1	0	0	0	0	0	1	0	0	1	2	1	2	2	0	1	1	0	1	
<i>A. serricaudata</i>	0	0	2	1	1	0	0	*	0	0	0	1	0	0	0	0	0	1	0	0	1	2	1	2	2	0	1	1	0	1	
<i>D. stvensoni</i>	0	1	2	2	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	2	1	1	
<i>I. humphreysi</i>	1	0	1	1	1	1	0	1	1	0	0	1	0	1	0	1	1	1	1	0	0	0	1	0	2	0	1	1	0	1	
<i>M. inexpectata</i>	*	0	2	1	0	1	2	1	1	1	1	1	0	0	0	1	0	0	0	0	0	0	1	0	0	0	1	2	2	1	
<i>M. zimneri</i>	2	0	2	1	0	1	1	1	*	1	1	1	0	0	0	1	0	0	0	0	0	0	1	0	0	0	1	2	2	1	
<i>P. aotearoa</i>	1	0	2	1	1	0	0	*	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	1	2	1	
<i>P. araucana</i>	1	1	?	?	?	?	?	1	1	0	?	?	?	?	?	?	?	?	0	0	0	0	1	0	0	1	?	0	2	0	
<i>P. brasiliensis</i>	1	0	2	*	0	1	0	1	*	0	0	*	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	*	2	1	
<i>P. incae</i>	1	0	2	2	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	1	2	1	
<i>P. kohanga</i>	*	0	2	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	1	2	1	
<i>P. malayica</i>	1	0	?	?	?	?	?	1	1	0	0	1	?	?	0	?	1	1	0	0	0	0	1	1	?	1	?	0	0	0	
<i>P. reidae</i>	*	0	2	1	0	1	0	1	*	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	1	2	1	
<i>P. setosa</i>	*	0	?	?	?	?	?	1	1	?	0	0	?	?	?	?	?	?	0	0	0	0	1	0	1	0	?	?	2	?	
<i>P. sphanga</i>	1	0	?	?	?	?	?	1	2	1	0?	0	?	?	?	?	?	?	0	0	0	0	?	1	0	0	?	?	2	?	
<i>V. boteai</i>	1	0	1	1	1	1	0	1	0	0	0	1	1	1	1	1	1	1	1	1	0	1	1	0	2	0	1	1	1	1	
<i>V. botocuda</i>	1	0	1	1	1	1	0	1	1	0	0	1	1	1	1	0	1	1	1	0	0	1	1	0	2	0	1	0	0	0	
<i>V. carinata</i>	2	0	2	2	1	1	0	1	1	1	0	1	1	1	1	0	1	1	0	0	1	*	0	2	0	1	1	2	1		
<i>V. carveli</i>	1	0	0	0	1	1	0	1	0	0	0	0	1	1	1	1	1	1	1	1	0	1	1	0	2	0	1	1	1	1	
<i>V. cornelia</i>	1	0	0	0	1	1	0	1	1	0	0	*	1	1	1	1	1	1	1	1	0	1	1	0	2	0	1	1	1	1	
<i>V. cuneata</i>	1	0	?	?	?	?	?	1	1	0	0	0	1	1	1	1	1	1	1	1	0	1	1	0	2	0	1	1	2	0	
<i>V. cylindrica</i>	0	0	2	2	1	1	?	1	1	0	0	0	1	1	1	1	1	1	1	1	0	2	1?	0	2	0	1	2	0	0	
<i>V. danielopoli</i>	1	0	0	0	1	1	0	1	1	0	0	1	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	
<i>V. daps</i>	1	0	1	?	?	?	?	0	1	0	?	?	1	1	1	1	1	1	1	1	0	1	1	0	2	0	1	2	2	1	
<i>V. flexuosa</i>	0	0	1	1	1	1	1	1	1	0	0	1	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	
<i>V. inconspicua</i>	1	0	?	?	?	?	?	1	1	0	0	1	?	?	?	?	?	?	?	1	1	1	1	1	0	1	1	?	2	?	?
<i>V. irajai</i>	1	0	1	1	1	1	0	1	0	0	0	1	1	1	1	0	1	1	1	0	0	1	1	0	2	0	1	1	0	1	
<i>V. lundii</i>	1	0	?	?	1	1	0	1	0	0	?	?	1	1	1	1	1	1	1	1	0	1	1	1	1	0	1	*	2	*	
<i>V. marlieri</i>	0	0	?	?	?	?	0	1	0	0	?	?	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	*	2	*
<i>V. marmonieri</i>	0	0	1	1	1	1	0	1	1	0	0	0	1	1	1	1	1	1	1	1	0	1	1	0	2	0	1	1	1	1	
<i>V. matildae</i>	1	0	0	0	1	1	0	1	0	0	0	*	1	1	1	1	1	1	1	1	0	1	1	0	2	0	1	1	1	0	
<i>V. molopoensis</i>	0	0	1	1	1	1	0	1	1	0	0	1	1	1	1	1	1	1	1	1	0	1	1	0	2	0	1	1	1	1	
<i>V. pagliolii</i>	*	0	1	1	1	1	0	1	1	0	0	*	1	1	1	0	1	1	1	0	0	1	1	0	2	0	1	1	0	1	
V. sp. D	1	0	1	1	1	1	0	1	1	0	0	?	1	1	1	1	1	1	1	1	0	1	1	0	2	0	1	1	1	1	
V. sp. E	1	0	0	0	1	1	0	1	1	0	0	1	1	1	1	1	1	1	1	1	0	1	1	0	2	0	1	1	2	1	

IRON OXIDE CRUSTS IN 2 HYPOGENE CAVES IN GREECE

Georgios Lazaridis^{1,c}, Lambrini Papadopoulou¹, Vasilios Melfos¹, and Panagiotis Voudouris²

Abstract

Iron oxide deposits in the form of crusts are examined in the field and in the laboratory with scanning electron microscopy. Samples came from two caves developed in different geological settings but assumed by previous studies to be of hydrothermal-hypogene origin. The relation of iron oxide crusts to cave wall and ceiling morphology created by the cave's primary dissolution (speleogenesis), as well as to the formation of other speleothems, is investigated. Scanning electron microscope (SEM) elemental analysis showed a high iron content (20–78 % by weight) consistent with iron oxide minerals such as goethite, hematite, magnetite, etc. Three distinct types of iron oxide crusts were identified from the four samples that were analyzed: (1) high iron content crusts with some porosity formed on the host rock surface and discontinuities, (2) high iron content (but lower than type 1) crusts, including limestone clasts and pores originating from dissolved clasts, and (3) high iron content, significantly-porous crusts that are formed by a network of filaments that indicate microbial activity. The formation of these deposits in relation to other speleothems and to dissolution events is discussed.

INTRODUCTION

Hypogenic cave formation is a special category of speleogenesis experiencing an increase in interest as more of these caves are identified globally (Klimchouk et al., 2017). These caves differ from those in classical karst that are developed by epigene (or hypergene, as defined by Dublyansky, 2014) speleogenesis because they are formed by water recharge from lower hydrostratigraphic units and sources disconnected from the adjacent surface (Klimchouk, 2017). In many of these caves, iron oxide and iron hydroxide deposits have been reported, which are in many cases the final products of sulfide oxidation (Onac and Forti, 2011). Examples of hydrothermal caves with deposits of iron oxides and hydroxides include: Iboussières Cave in France that contains spongy, microbial, ferruginous material that forms black tubes as they are deposited in vertical wall channels (Audra 2017a), Sima de la Higuera Cave in Spain, where iron-manganese coatings are found (Gázquez et al., 2012a), the inactive sulfuric-acid-speleogenetic Rhar Medjraha cave in Algeria with iron oxides having a spongy texture and in association with microbial mats (Coiffait and Quinif, 1978, cited in Audra, 2017b), the El-Balayza Caves in Egypt where iron and manganese oxides are associated with feeders (Mostafa, 2012, cited in Audra, 2017b), a number of caves in southwest Sardinia, Italy, where at least in one cave crust formation is subaerial by water films (De Waele et al., 2017); Cave of the Winds and Glenwood Caverns in Colorado, USA, where a bacteria has been reported to have deposited iron oxide/hydroxide (Luiszer, 1994; Maslyn et al., 2017); natural cavities of the karstic iron ore deposit of Warda in northern Jordan, where the authors assume a speleogenetic process for the ore deposit based on the bacterial oxidation of methane (Al-Malabeh et al., 2008), Mbobu in South Africa, where goethite speleothems on the ceiling are formed by dripping water (Martini, 2017), caves of the Apennines Mountains, where goethite coatings are related to the presence of mineralized water (Galdenzi and Menichetti, 2017), the Buda thermal karst of Hungary, where iron hydroxide precipitates dominated by goethite are associated with cave rafts, indicating hypogene cave discharge areas (Mádl-Szőnyi et al., 2017), and caves in Germany, where siderite weathering results in carbonic acid speleogenesis and goethite deposits (Kempe, 1971; Kempe et al., 2017; and the references therein). This list is not exhaustive, and ferromanganese deposits are also known from hypergene caves (e.g. Onac et al., 1997).

Iron oxide crusts are found also in two Greek caves, Maronia Cave and Mavros Vrachos Quarry (MVQ) Cave (Fig. 1), which are considered to be related to hydrothermal speleogenesis of hypogene origin. These crusts were sampled and investigated, and their structure and composition are described and discussed according to other known cases and formation processes. Formation of other speleothems and dissolution processes are considered in order to describe their relationship to the iron oxide crusts.

CAVE DESCRIPTIONS AND SAMPLING

Maronia Cave is the largest known cave in Thrace and has been well studied from the perspective of geology, archaeology and biology (e.g., Lazaridis, 2005; Melfos et al., 2005; Pavlides et al., 2005; Sylaiou et al., 2009; Panti, 2015). It is formed in limestones of the Eocene with nummulites. The limestone unit is relatively thin and unconformably deposited on green schists above a basal Eocene conglomerate and sandstones. The nummulitic limestones mark the beginning of the

¹ School of Geology, Aristotle University of Thessaloniki, GR-54124, Thessaloniki, Greece

² School of Geology and Geoenvironment, National and Kapodistrian University of Athens, GR-15780, Zographou, Athens, Greece

^c Corresponding author: geolaz@geo.auth.gr

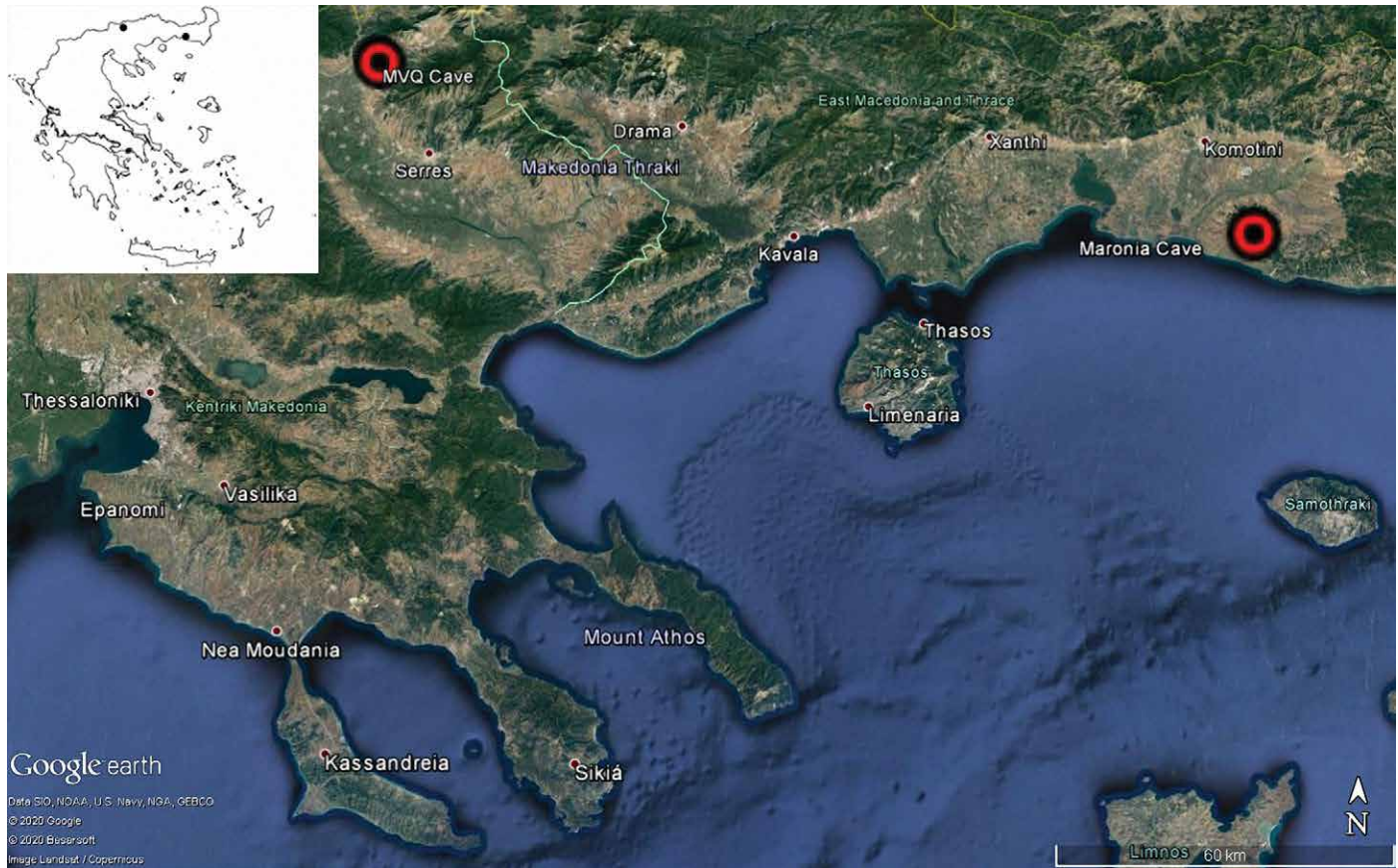


Figure 1. Geographic location of Greek caves where iron crusts were sampled and investigated. Map from Google Earth. Western red dot, MVQ Cave. Eastern red dot, Maronia Cave. Inset, location within Greece.

Tertiary stratigraphy in the Tertiary basins of Thrace. They are slightly inclined to the west. Both the sedimentary and metamorphic rocks were intruded and overlain by Oligocene subvolcanic and volcanic rocks represented by altered andesites alternating with tuffs (Kouris, 1980; Papadopoulos, 1982; Melfos, 1995; Pavlides et al., 2005). Maronia Cave (Fig. 2) covers about 1 ha and is well decorated with several speleothem types (Lazaridis, 2005; Melfos et al., 2005). Its small-scale dissolution morphology is considered to resemble that of hypogene caves (Vaxevanopoulos and Melfos, 2010), but most forms referred to, such as cupolas, are polygenetic. Our observations of the speleothems verify that there is a significant degree



Figure 2. Ground plan of Maronia Cave with sampling sites Sp55, Sp56, and Sp57 (based on Petrocheilou, 1984).

of biocorrosion (Bruxelles et al., 2016; Audra et al., 2016; Audra et al., 2017; Cailhol et al., 2019) due to the large bat populations that could be responsible for the formation of cupolas and related micro-scale forms. So, only large- and meso-scale morphology of the cave and other geochemical data could be used to investigate its speleogenesis.

During our research in the cave for possible presence of hydrothermal calcite deposits, iron oxide crusts were

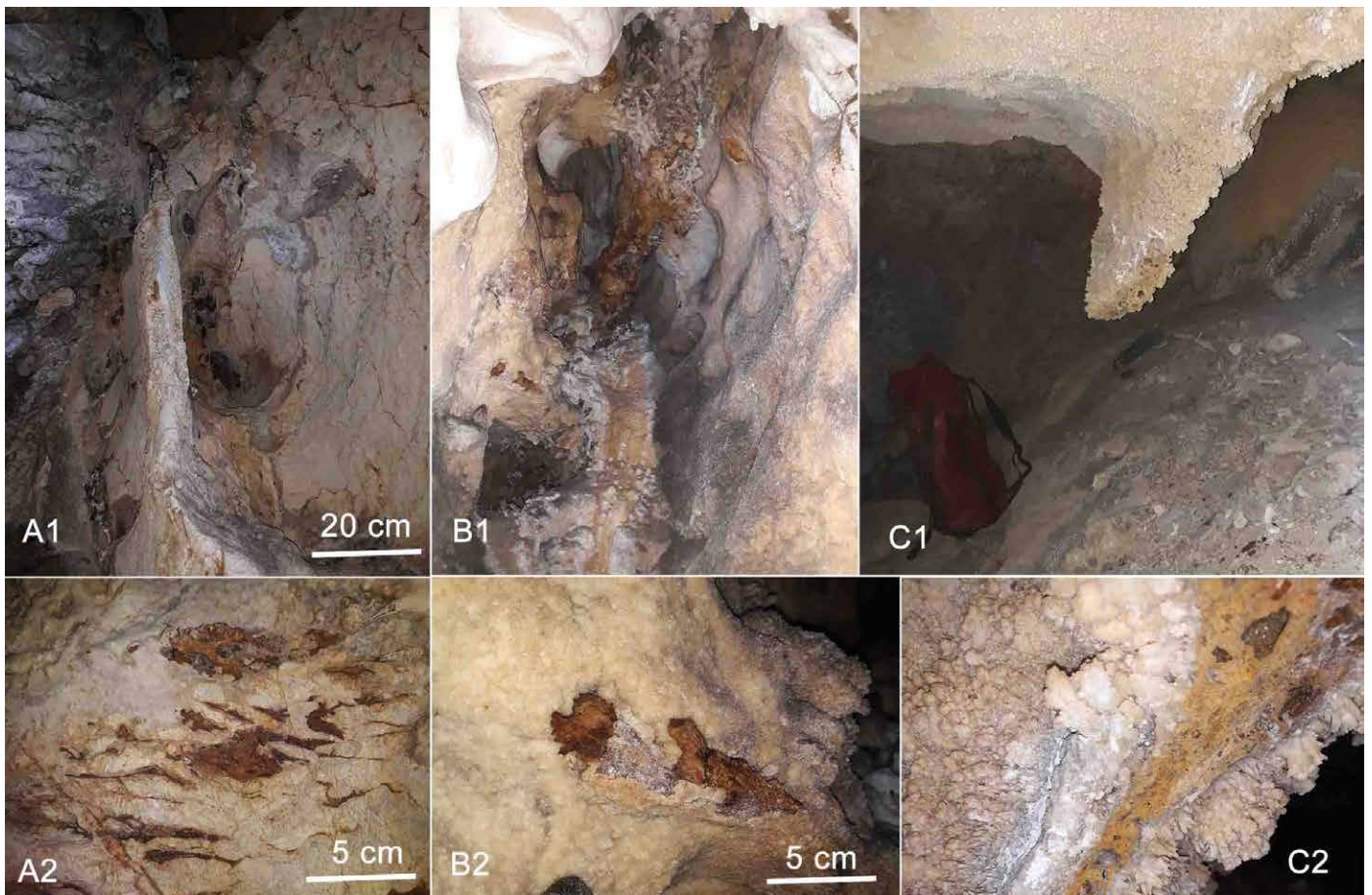


Figure 3. Photographs of iron oxide crust occurrences in Maronia Cave. (A1, A2) Sampling site Sp55. (B1, B2) Sampling site Sp56. (C1, C2) Sampling site Sp57 in a pendant covered by carbonate coralloids, crusts, and coatings. Fig. A2, B2, and C2 each show the crust in greater detail.

found. We chose three locations for sampling that were spread along the north half of the cave (Figs. 2 and 3). The first two samples come from cupola-shaped areas of the cave ceiling and from the sidewalls of these sites. The third sample was collected from a pendant that is covered with coralloids. Below the coralloids there is a massive iron oxide crust.

MVQ Cave (Fig. 4) was discovered by quarrying at the Mavros Vrachos hill about 220 m MSL in the southwestern part of the northeast-southwest striking Krousovitis Basin in the Serres region, northern Greece. This basin is filled with Neogene and Quaternary sediments (Karistineos, 1984) overlying mainly gneisses and marbles that belong to the Rhodope Massif of the Hellenides and granitoid intrusions of Tertiary age (e.g., granites, granodiorites, etc.). Travertine deposits occur in several locations in the basin.

In the entrance area of MVQ Cave are two vertical passages, divided by a conical breccia deposit, forming in total a breccia pipe morphology. The southern passage is about 8 m in depth and choked by autochthonous sediments. The northern passage is about 20 m deep and connects to the main cave chamber. A 40 m long passage starts vertically from the east side of the chamber. It is inclined and stops at a small lake 56 m below the entrance elevation. This passage extends underwater for more than 10 m where two almost-vertical passages are developed more deeply. Another vertical passage starts at the southern part of the main chamber and is connected to small horizontal and vertical passages about 40–50 m below the entrance level. The deepest parts are narrow and inaccessible.

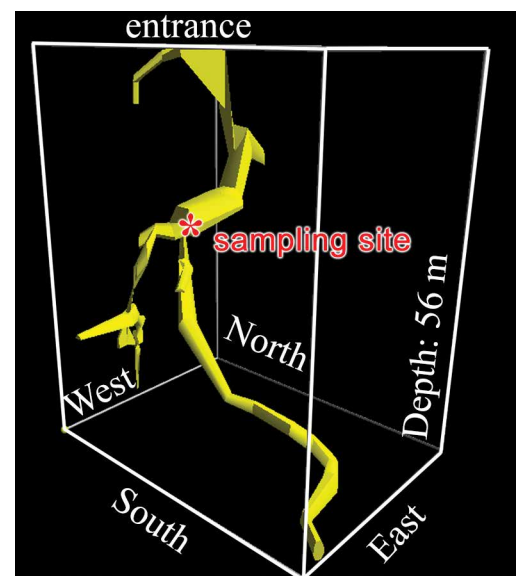


Figure 4. Block diagram of Mavros Vrachos Quarry Cave. Sampling site is depicted.

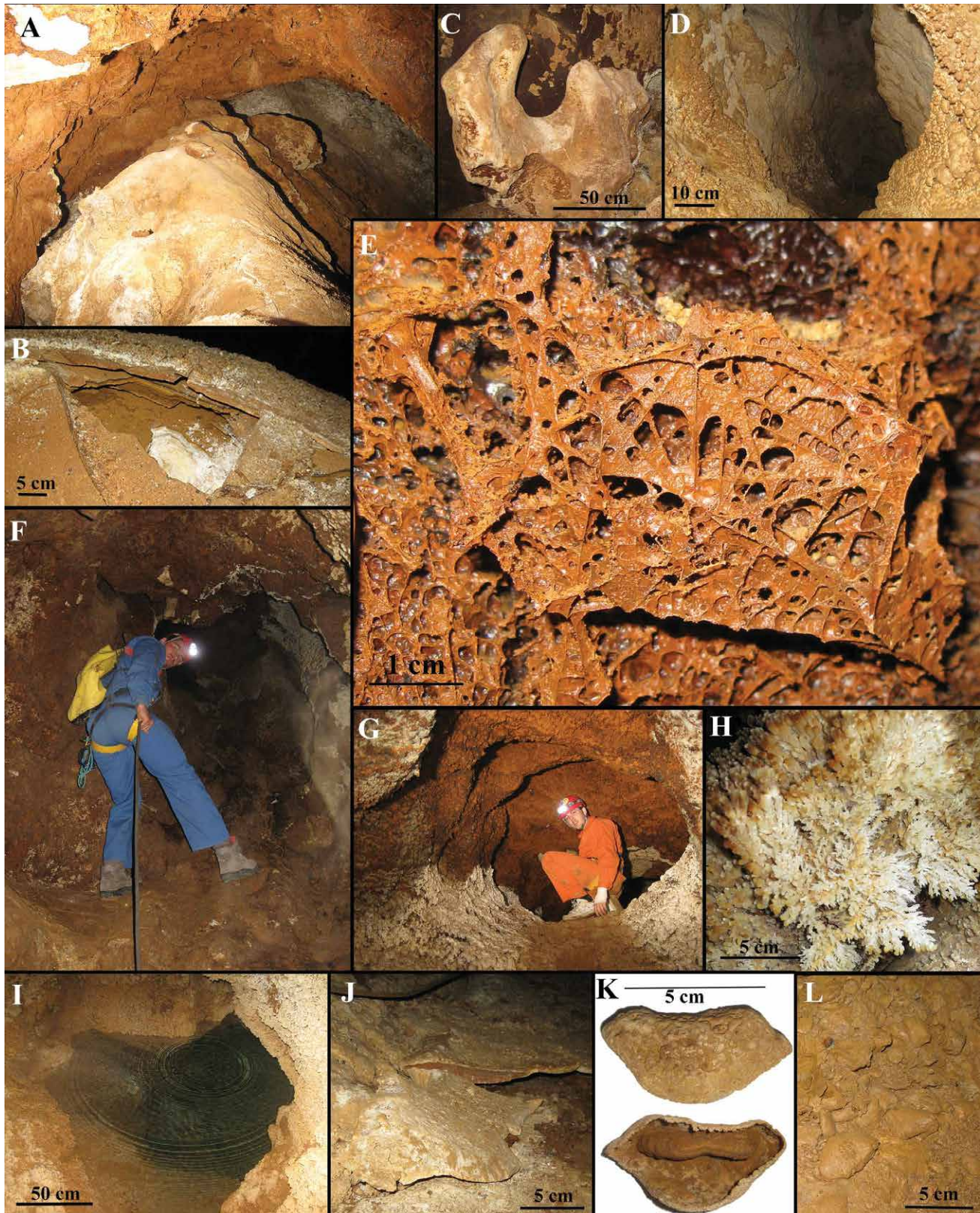


Figure 5. Photographs inside Mavros Vrachos Quarry Cave. (A) The main chamber covered by the iron oxide crust and view of the sedimentary ridge along its axis. (B) Details of broken layers in the ridge of the main chamber. (C) Dissolution remnant on the floor of the southern passage. (D) Feeder conduit at the south part of the cave. (E) Detail of the spongework structure of the iron oxide crust. (F) The vertical passage that connects the eastern conduit to the main chamber. (G) Part of the eastern conduit with symmetrical cross-section covered by the iron oxide crust and frostwork-coralloid alternations at the lower half of the passage. (H) Detail of the frostwork and coralloids. (I) The lake that is formed at the deepest part of the cave, 56 m below the entrance elevation. (J) Mudstones in proximity to the lake. (K, L) Detail of cave caps formed on mud nodules and in situ, respectively. Scale bars differ for each photograph.



Figure 6. Macroscopic appearance of the examined iron oxide crusts from Maronia Cave. (Sp55, Sp 56, Sp57) Sample from the corresponding sites shown in Fig. 2. (Limestone clasts) Detail from the sample from site Sp57. Lower left scale bar applies to the samples from sites Sp55 and Sp56. Upper right scale bar applies to the sample from site Sp57 (except the limestone clasts detail).

An iron oxide crust a few centimeters thick (5–10 cm) covers most of the cave interior, and a few other speleothems are found, such as calcite spar consisting of a few millimeter-long crystals, carbonate crusts and aragonite and calcite frostwork alternating with globular coralloids, cave powder (Hill and Forti, 1997), and some mud nodules partially covered by a calcite layer resembling cave caps (Fig. 5J–L). The calcite spar is widespread within the cave in small pockets of the iron oxide crust. A ridge that consists of successive carbonate crusts (in at least the uppermost 10 cm) forms the cave floor along the long axis of the main chamber. This is the second most common type of speleothem in the cave due to its extent, after the iron oxide crusts. Its layers are not horizontally deposited, but instead, they follow the inclination of the ridge surface. Above the ridge there are no stalactites or any dripping sites. In general, dripping speleothems are almost absent. The alternation of frostwork and coralloids occur in the central chamber and the east passage, where it is delimited at the lowest parts of the passage. Cave powder and cave caps are found at the small lake in the deepest part of the cave and are related to its surface and its area of seasonal fluctuations. One sample of the iron oxide crust was collected from the main chamber of the cave for further investigation.

METHODS

Morphological and chemical investigation of iron oxide crust samples were carried out at the Scanning Microscope Laboratory, Aristotle University of Thessaloniki, using a JEOL Ltd. JSM840A SEM equipped with an energy-dispersive spectrometer with a 20 kV accelerating

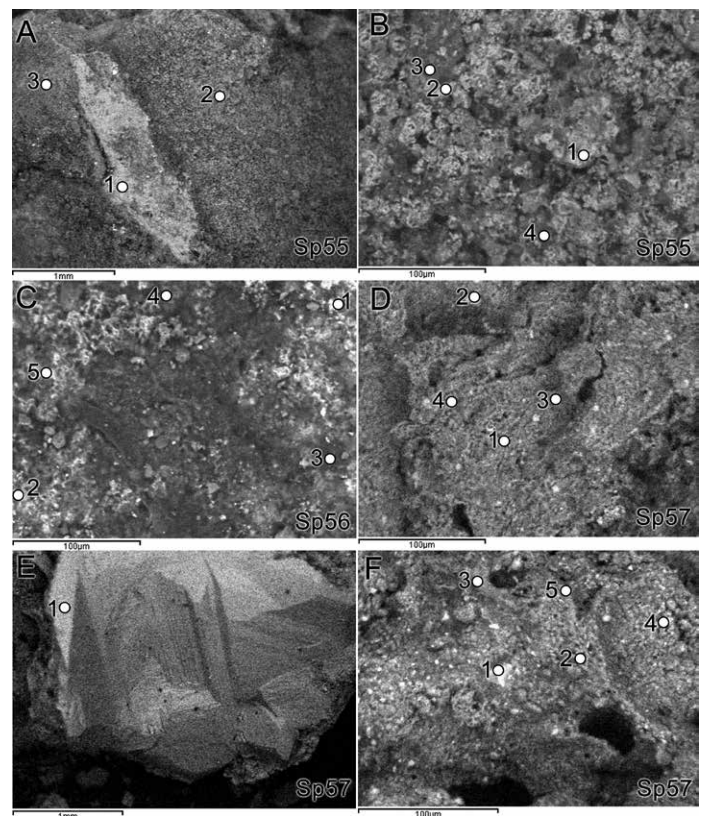


Figure 7. Backscattered SEM images of iron oxide crust samples from Maronia Cave. Numbered white circles correspond to the elemental composition sites provided in Table 1. (A, B) Sample Sp55 where mainly iron oxides and iron hydroxides are observed. (C) Sample Sp56. (C1) Iron oxides and iron hydroxides. (C2–C5) Aluminosilicates. (D, F) Sample Sp57 with iron oxides, iron hydroxides, and aluminosilicates. (E) Sample Sp57 with calcite. Scale bars correspond to the images above each bar.

Table 1. Elemental composition of the studied sample sites from Maronia Cave from Energy Dispersive Spectroscopy in weight percent (see Fig. 7).

Element	Sample Sp55						
	A1	A2	A3	B1	B2	B3	B4
Al	5.29	1.02
Si	...	3.81	2.66	17.74	...
Ca	...	3.38	1.83	18.02	2.09	12.07	2.85
Mn	21.16
Fe	77.73	67.73	75.74	29.13	60.02	35.10	74.63
As	9.27
O	22.27	25.09	22.43	26.41	24.94	35.08	22.52
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00

Element	Sample Sp56				
	C1	C2	C3	C4	C5
Mg	2.97
Al	...	2.97	4.98	4.23	2.88
Si	...	3.98	20.17	13.97	2.09
P	5.02
S	1.92
Ca	3.16	...	1.64
Fe	77.73	66.75	20.78	48.28	64.41
As
O	22.27	26.30	43.97	33.52	26.01
Total	100.00	100.00	100.00	100.00	100.00

Element	Sample Sp57									
	D1	D2	D3	D4	E1	F1	F2	F3	F4	F5
Mg	1.88	0.30	2.46
Al	1.17	3.77	...	8.94	...	5.36	14.01	8.33	14.96	12.72
Si	2.78	5.62	19.74	28.95	...	8.88	20.27	36.26	23.58	31.77
K	0.86	1.29	0.86	0.74
Ti	9.45
P	...	3.08
Ca	...	7.48	...	4.49	71.47	0.02	...
Fe	68.97	48.84	44.91	11.58	...	51.92	10.40	3.99	15.73	5.52
O	25.20	30.91	35.35	46.04	28.53	31.38	45.02	50.13	44.86	49.25
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

voltage and a 0.4 mA probe current. Pure cobalt was used as an optimization element. For SEM observations, the samples were coated with carbon (to an average thickness of 20 nm) using a vacuum evaporator JEOL-4X. Energy-dispersive X-ray spectroscopy analysis allows the chemical determination of phases with a size as small as $1 \times 1 \mu\text{m}$. In total, three representative sub-samples from the samples from sites Sp55, Sp56, and Sp57 in Maronia Cave were investigated as well as 1 sub-sample from the MVQ Cave sample.

RESULTS

In Maronia Cave, the first 2 samples are macroscopically similar (Fig. 6), with Sp55 and Sp56 consisting of massive porous areas and crusts. Both samples show dark red to brown color while sample Sp57 is more massive, porous, and lighter in color. Upon close examination of the external part of the sample that is covered with coralloids, its pores are

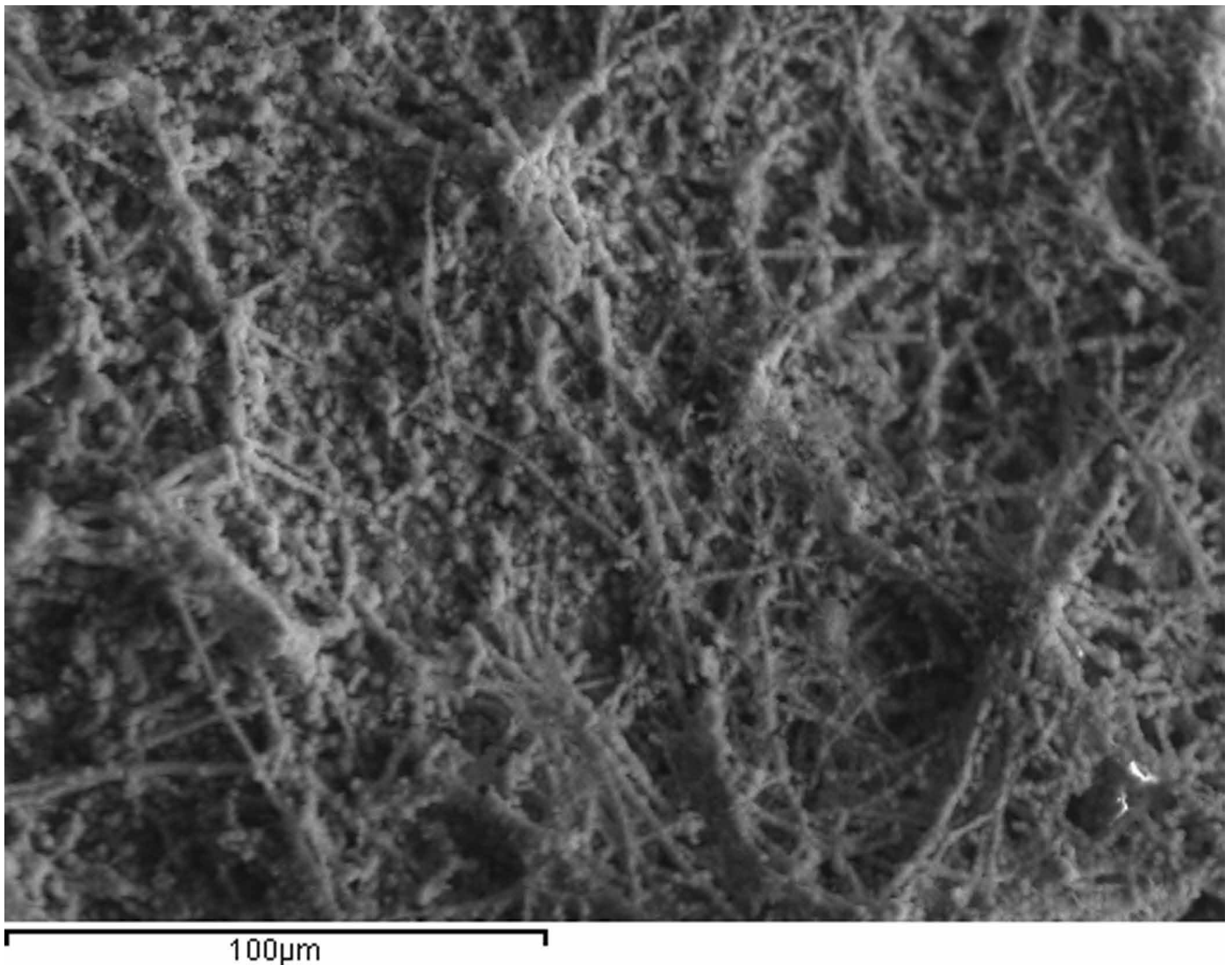


Figure 8. Backscattered SEM image from the iron oxide crust in Mavros Vrachos Quarry Cave where a network of tiny filaments is observed. Scale bar shown below image.

found to be lined with small euhedral calcite crystals. Some small fragments of limestone, in the shape and size of the pores, are found in areas where large pores are missing.

With the SEM (Fig. 7), sample Sp55 appears to consist of micrometer-sized crystals and aggregates, and the surface exhibits pores that are about 10 μm in size. The maximum iron content is 78 % by weight, which is consistent for iron oxide minerals such as goethite, hematite, and magnetite. In one subsampling point, manganese replaces the iron content. Other elements found are aluminum, silicon, calcium and arsenic. Similarly, sample Sp56 also has a high iron content of about 78 %. Places with a lower content of iron display higher amounts of silica and other elements such as aluminum, magnesium, calcium, phosphorous, and sulfur. It also consists of small grains (about 15 μm in diameter) and massive areas. The third sample, Sp57, displays lower iron content that reaches 69 %, where calcium carbonate and aluminosilicate minerals make up a small percent of the crust. Apart from iron, calcium, silicon, and aluminum, other elements found in this sample include magnesium, potassium, phosphorous, and titanium. Although macroscopically it appears more porous, it looks relatively massive in the SEM. Grains of aluminosilicates are only a few micrometers in diameter, whereas calcite crystals are few millimeters in diameter. The results of chemical analyses for these samples are summarized in Table 1.

The sample from MVQ Cave (Fig. 8) is much more porous than the samples from Maronia Cave and very fragile. Its color is more or less similar to the other iron oxide crusts along the cave's length or depth. The analyzed sample consists mainly of iron, which reaches 96 % by weight (Table 2). Its structure observed in the SEM consists of tiny filaments that are oriented at every direction forming a network structure. The filaments consist of spherical aggregates with 1–5 μm radii. These spherical aggregates consist of needle-like crystallites that are radially arranged.

Table 2. Mean values and range of elements in weight % found in the Fe-oxide crust from Mavros Vrachos Quarry Cave.

Element	Mean	Min	Max
O	27.33	2.96	36.70
Na	0.99	0.99	0.99
Mg	0.53	0.49	0.58
Al	1.29	0.32	2.15
Si	3.48	0.53	4.54
Ca	0.35	0.22	0.62
Fe	67.43	56.35	96.19
Total	100.00	N.A.	N.A.

from dissolution of trace elements in the vadose parts of caves (Spilde et al., 2006). Although ferromanganese deposits in the caves of the Guadalupe Mountains (USA) have been considered the residue of dissolution during speleogenesis (Davis, 2000; Polyak and Provencio, 2001) or thought to be formed by condensation corrosion (Queen, 1994), the enrichment of iron and manganese oxides remains problematic. However, it could be explained by a microbe-driven process modeled by a number of studies (Northup et al., 2000, 2003; Boston et al., 2001; Spilde et al., 2005, 2006).

The iron oxide crusts in Maronia Cave form two different types: one type covers sidewalls and the other is found as a pendant-like ceiling protrusion. The first type displays higher iron content and fills small discontinuities and voids on

DISCUSSION

Both caves sampled in this study have been included in the list of hypogene or possibly hypogene caves in Greece (Lazaridis, 2017). Various occurrences of iron oxides and iron hydroxides in hypogene caves under a number of conditions are summarized in Table 3. Both subaerial and subaqueous conditions have been found to be responsible for the formation of these deposits. Manganese-rich deposits may be associated with the iron oxides. The spongy porous appearance might be associated with microbial activity. In general, iron and manganese are mobilized from mineralizations in the host rock (Gázquez et al., 2011, 2012a, 2012b) or

Table 3. Hypogene caves with Fe-oxides and hydroxides and their main characteristics.

Cave/Country	Type of Deposit	Associated with	Conditions	Reference
Mavros Vrachos Quarry Cave, Greece	Spongy texture with microbial filaments	Symmetrical passages, feeders, cupolas	Hypogene/ hydrothermal speleogenesis, subaqueous formation	Present work
Maronia Cave, Greece	Porous crust	Pendant forms, discontinuities, cupolas	Possibly Hypogene speleogenesis	Present work
Iboussières Cave, France	Spongy microbial ferruginous material	Vertical wall channel "black tubes"	Hypogene speleogenesis	Audra et al., 2017
Rhar Medjraba, Algeria	Spongy texture	Microbial mats	Hypogene/ sulfuric acid speleogenesis	Coiait and Quinif, 1978
El-Balayza Caves, Egypt	Iron oxides	Manganese oxides and feeder morphology	Hypogene speleogenesis	Mostafa, 2012
Cave in southwestern Sardinia, Italy	Iron oxides	...	Subaerial formation by water films	De Waele et al., 2017
Cave of the Winds and Glenwood Caverns, Colorado, USA	Spongy texture with bacteria filaments or small uniform grains (1–2 µm)	Iron oxide mammillaries of inorganic origin/ breccias/ calcite	Hypogene speleogenesis/ subaqueous formation	Luiszer, 1994; Maslyn et al., 2017
Lechuquilla Cave, New Mexico, USA	Rusticles: "subaqueous streamers of iron mineral sheaths of iron-oxidizing bacteria"	Calcite crust	"reduced iron-rich fluid was trickling from above into standing water"	Davis, 2000
Mbobu Mkulu, South. Africa	Goethite speleothems on the ceiling	...	Dripping water/ subaerial formation	Martini, 2017
Caves of the Apennines Mountains, Italy	Goethite coatings	Presence of mineralized water	Hypogene speleogenesis	Galdenzi and Manichetti, 2017
Caves of the Buda thermal karst, Hungary	Iron hydroxide precipitates dominated by goethite	Cave rafts and discharge areas	Hypogene/ hydrothermal speleogenesis/ subaqueous formation	Mádl-Szonyi et al., 2017
Sima de la Higuera Cave, Spain	Ferromanganese oxyhydroxides	Botryoidal aggregates/ hydrothermal calcite veins/ related to boxwork pattern	Hypogene speleogenesis/ multiphase process of formation	Gázquez et al., 2012a
Germany	Goethite deposits	Siderite weathering	Hypogene speleogenesis	Kempe, 1971; Kempe et al., 2017
Warda, Northern Jordan	Karstic iron ore deposit	Calcite veins	Hypogene speleogenesis	Al-Malabeh et al., 2008

the walls and ceiling. By the term discontinuity, we mean any type of break in the rock, such as bedding planes, joints, faults, etc. These crusts cover the host rock in cupolas, indicating that the crust formed at or soon after the termination of speleogenesis. Regarding the second type, its extent and relation to the dissolution forms of the bedrock cannot be clearly observed due to speleothem coverage. It can be assumed that the presence of limestone clasts in the deposit indicates an early karstification filling of a discontinuity. Since the bedding planes are of low angle, a tectonic discontinuity can be suggested. This deposit was later exposed from dissolution that removed most of the limestone clasts, reshaped the passage and left the iron-rich filling as an undissolved remnant.

In MVQ Cave the deposition of the various carbonate speleothems is related to distinct conditions. The calcite spar seems to be related to the formation conditions of the iron oxide crust. It is contemporaneous or follows its deposition since it appears to either be covered by or to cover the iron oxide deposits. The formation of the carbonate crusts and the ridge in the central chamber is enigmatic. Any relation to dripping water or flowing water is excluded because of its location and shape, respectively. So, most probably it is related to subaqueous formation conditions, and as it can be supposed from its distance from the sidewalls at the west side and around the passages, its shape is affected by rising water inputs in the chamber. This assumption is worthy of further investigation. The deposition of frostwork is related to subaerial capillary films, and for its growth there are several prerequisites concerning the cave environment, the bedrock and the rate and chemistry of the seeping water (Hill and Forti, 1997, and references therein). It is common for frostwork to alternate with coralloids (popcorn) due to admixtures in solution or to changes in speed of crystallization. Frostwork and coralloids from MVQ Cave have been developed on the iron oxides, which have high porosity (a sponge-work pattern), fine filamentous textures, and the resistance of the goethite to solution by capillary films. Due to these characteristics, they are ideal bedrock (according to the conditions considered by Coudray and Cabral, 1978a, 1978b) for the formation of frostwork and coralloids. Coralloids from MVQ Cave consist of calcite and aragonite. The deposition of aragonite is preferable under evaporative conditions, which, along with the presence of air flow, is favorable for the growth of frostwork. However, the existence of air flow inside MVQ Cave is not strong, and it is questionable if it existed before the artificial entrance. Finally, the cave powder and the cave caps are related to the conditions that are presently found in the deepest part of the cave associated with stagnant water. Speleothems that are related to seeping water (e.g., stalactites) are almost completely absent indicating a restricted entrance and recharge from surface above the cave and/or an effective sealant above the cave.

The iron oxide crust in MVQ Cave appears to have been formed subaqueously due to its presence in the whole known extent of the cave and its similar thickness throughout the cave. It is also formed inside all the dissolution forms of small-scale morphology that are indicative of the presence of rising water conditions, suggesting a probable deposition during the latest stages of speleogenesis. SEM observation shows that its fragile structure resembles structures formed by microorganisms (Fig. 8).

CONCLUSIONS

The four samples examined here can be considered as three different types of Maronia and MVQ Cave iron oxide crusts: high iron content crust with some porosity and formation on host rock surface and discontinuities; high iron content but lower than the first type, that include limestone clasts and pores originating from dissolved clasts; and a high iron content, significantly porous crust that is formed by a network of filaments that indicate microbial activity. The 1st type is considered co- or post-dissolutional since it covers cupolas. The second type, which developed secondary porosity due to dissolution of clasts, seems to have undergone a phase of dissolution that also formed the passage in a way that left behind the insoluble iron crust that was filling some early void. The third type is subaqueous and most apparently phreatic due to its extent and isotropic deposition. It is the dominant speleothem in MVQ Cave and a small number of other speleothems are either related to its presence and capillary water flow or to the presence of stagnant water. All-in-all, vadose speleothems are almost absent in this cave, indicating a delimited vadose input and reinforcing the phreatic origin of the crusts. Oxidation might be related to microbial activity in this case, whereas for the first 2 types no evidence of such activity is observed. This type is worthy of study in further detail to investigate the conditions under which the cave and the crust were formed.

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