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A NEW STYGOBIOTIC SPECIES OF *DIACYCLOPS* KIEFER, 1927 (COPEPODA, CYCLOPIDAE) FROM CAVES IN NEW MEXICO, UNITED STATES

BY

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ABSTRACT

A new species of the freshwater cyclopine copepod genus *Diacyclops* is described from a North American cave system in SE New Mexico, U.S.A. The new species, *D. nikolasarburni* n. sp., differs from its congeners by its having of a unique combination of characters including: 17-segmented antennules, three-segmented rami in legs 1-4, a basic 2333 spinal formula with a highly variable pattern, naked inner margin of caudal rami, but dorsal surface with spinules, subequal terminal spines of leg 4 endopod, and inner spine of fifth leg about as long as bearing segment. This species has also a variable length/width ratio of the caudal ramus (4.6-6.0, average 5.0); the lateral caudal seta is inserted on the distal one-quarter of ramus. The 2333 spinal formula was dominant (55%) in the population but other patterns were also present. The new species belongs to Group 1 species of *Diacyclops* (sensu Reid & Strayer, 1994). The local population of this stygobiotic species probably resulted from radiation-stranding processes of epigean forms of widely distributed *Diacyclops* and is probably endemic to this cave system.

RESUMEN

Se describe una nueva especie de copépodo ciclopoide de aguas continentales del género *Diacyclops* proveniente de un sistema de cuevas en el sureste de Nuevo México, Estados Unidos. La nueva especie, *D. nikolasarburni* n. sp. difiere de sus congéneres por tener una combinación única de caracteres que incluyen: anténulas con 17 segmentos, patas natatorias 1-4 con ramas de tres segmentos, formula espinaria básica de 2333 con un patrón altamente variable, margen interno de las ramas caudales desnudo, pero superficie dorsal con espínulas, espinas terminales del tercer segmento endopodal de la cuarta pata subiguales, espina interna de la quinta pata aproximadamente del mismo largo que el segmento. Esta especie tiene una variable proporción largo/ancho de las ramas caudales (4.6-6.0, promedio: 5.0); la seta lateral de la rama caudal se inserta en el 1/4 distal de la rama. La fórmula espinaria 2333 es dominante (55%) en la población pero otros patrones también estuvieron

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presentes. La nueva especie pertenece al Grupo 1 de especies de *Diacyclops* (sensu Reid & Strayer, 1994). La población local de esta especie estigobiótica es resultado, probablemente, del proceso de radiación de formas epigeas ampliamente distribuidas de *Diacyclops* y es posiblemente endémica de este sistema de cavernas.

INTRODUCTION

The genus *Diacyclops* Kiefer, 1927 is one of the most diverse and successful among the freshwater cyclopoid copepods. It contains more than 100 nominal species (Stoch, 2001; Boxshall & Halsey, 2004; Dussart & Defaye, 2006) from many different epigean freshwater environments but they are also frequently recorded from groundwater and caves (Pesce, 1994; Fiers et al., 1996; Reid, 2004). This genus appears to be most diverse and well known in the Palearctic region but is also widespread and speciose in the Nearctic region (Dussart & Defaye, 2006; Boxshall & Defaye, 2008). Its knowledge in the Americas is still developing, but it is clear that it is most diverse in North America than in the other subcontinents; up to 28 nominal species of *Diacyclops* have been recorded from this region (Yeatman, 1959; Reid, 1998; Reid & Williamson, 2010). Six species have been recorded in Mexico, Central America, and the insular Caribbean (Reid, 1990; Fiers et al., 1996, 2000; Suárez-Morales & Reid, 1998) and only two in South America (Reid, 1985; Rocha & Botelho, 1998; Locascio & Menu-Marque, 2001). Members of this genus have frequently been found in North American caves and groundwater environments (Reid, 2004; Pipan & Culver, 2005; Elliot, 2007). Overall, the knowledge of the copepod diversity related to this kind of habitats is still lagging and certainly deserves further taxonomic research in order to discover endemic or cryptic species with restricted distributional ranges (Fiers et al., 1996, 2000).

The bi-national ecoregion known as the Chihuahuan Desert (CHD) includes states of northern Mexico and southern United States (parts of Texas and New Mexico). The CHD comprises many arid and semi-arid areas with different hydrological basins and a wide variety of limnological conditions. It has been deemed as a high priority for conservation among the hydrological basins of North America. Recent taxonomic surveys of the cyclopoid copepod fauna from different aquatic environments of arid and semiarid areas of northern Mexico related or adjacent to the CHD has resulted in the discovery of several new species (Mercado-Salas et al., 2006, 2009; Mercado-Salas & Suárez-Morales, 2009; Suárez-Morales & Walsh, 2009). Most of the records of *Diacyclops* from semi-arid and arid environments of the southern United States are from open waters, only a few are from groundwater or cave environments (Reid & Williamson, 2010). From a collection of cyclopoid copepods from caves in SE New Mexico, within the CHD system, a new species of *Diacyclops* is described and illustrated based on male and female specimens. These caves are part of the Rustler Formation, dating to the Permian period. The formations yields moderate to large quantities of fresh to brackish groundwater, primarily from solutions openings in its upper section (Boghici & Van Broekhoven, 2001; Powers et al., 2006). The significance of the discovery of the new species is discussed in terms of the currently known diversity and distributional patterns of the genus in this kind of environments.

MATERIAL AND METHODS

Zooplankton samples were collected in November and December 2011 in pools located in two unnamed caves designated by the U.S. Bureau of Land Management as: BLM-NM-250-127 (Cave A) and BLM-NM-250-309 (Cave B) in SE New Mexico, which are part of the Burton Flats Karst area (fig. 1). In both cases copepods were collected from isolated pools at the innermost zone of the caves. Samples were obtained using standard plankton nets or a hand net hauled near the shoreline of the water bodies. The biological material was then fixed and preserved in 95% ethanol. Several male and female *Diacyclops* were collected during this survey. A close examination of these specimens was performed in the laboratory and distinct morphs were observed, thus motivating a deeper analysis. Specimens were prepared, dissected and examined following Reid & Williamson (2001) and Reid (2003). Dissected specimens/appendages were mounted in semi-permanent slides with glycerine sealed with Entellan®, a commercial, fast-drying mounting medium and sealant. Drawings were prepared at 1000× magnification with the aid of a camera lucida mounted on a standard Olympus CX31 compound microscope. Some specimens were prepared for SEM examination with a JEOL LV 5900 microscope in the University of Aguascalientes, Mexico. The process included dehydration of specimens in progressively higher ethanol solutions (60, 70, 80, 96, 100%), drying and gold coating following standard methods. This hitherto unknown species was described and illustrated following the current standards for the taxonomic study of the genus (Fiers et al., 2000; Stoch, 2001). The type specimens were deposited in the National Museum of Natural History, Smithsonian Institution (USNM, Washington, DC, U.S.A.) and in the collection of zooplankton held at El Colegio de la Frontera Sur (ECO-CH-Z), in Chetumal, Mexico. Original samples remain in facilities of Zara Environmental (Manchaca, TX, U.S.A.).

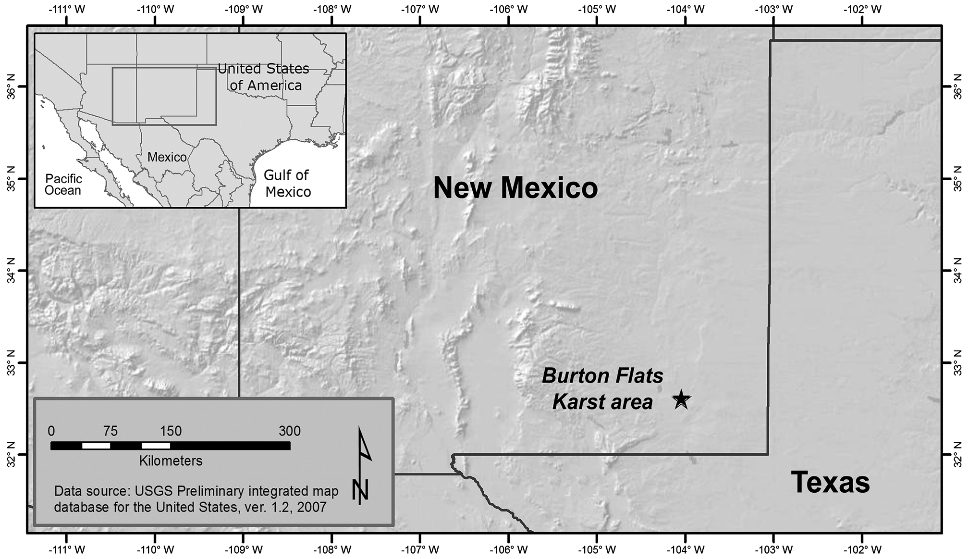

Fig. 1. Map of SE New Mexico, U.S.A., showing the location of the caves where the new species of *Diacyclops* was collected.

RESULTS

Taxonomy

Order CYCLOPOIDA Rafinesque, 1815
Family CYCLOPIDAE Rafinesque, 1815
Subfamily CYCLOPINAE Rafinesque, 1815
Genus *Diacyclops* Kiefer, 1927
Diacyclops nikolasarburni n. sp. (figs. 2-8)

Material examined.— Holotype. Adult female, dissected, mounted in glycerine sealed with Entellan (USNM-1196521), Cave A, Burton Flats karst, SE New Mexico (32°36′N 104°02′W), United States. coll. November 11, 2011 by W. Larsen. Paratypes. Five adult females, undissected, one from Cave A, same date and collector (USNM-1196522), four from Cave B, same geographic coordinates, same date and collector; specimens ethanol-preserved, vial (USNM-1196523 (3 specimens), 1196524 (1 specimen)); 9 adult females, undissected, same locality, date, and collector, ethanol-preserved, vial (ECO-CHZ-08962); 5 adult females from Cave B, same date and collector, vial (ECO-CHZ-08975); 6 adult females from Cave B, dissected, slides mounted in glycerine sealed with Entellan, coll. November 7, 2011 (ECO-CHZ-08976). Other material examined included +75 undissected adult and juvenile specimens, deposited in first author's laboratory at El Colegio de la Frontera Sur, Chetumal, Mexico.

Type locality.— Cave A (32°36′N 104°02′W), SE New Mexico, United States (fig. 1). This cave has two entrances, a walk-in access, which leads to a short passage that opens up into a large collapse chamber with a skylight overhead. Floodwater that enters the walk-in access crosses under this skylight and enters the continuation of the cave into the dark zone. Biomonitoring Zone 1 begins at this

TABLE I
Armature of swimming legs 1-4 (spines in Roman numerals, setae in Arabic numerals) of *Diacyclops nikolasarburni* n. sp.

Leg	Coxa	Basis	Endopodite	Exopodite
Leg 1	0-1	1-I	0-; 0-0; 0-2	I-0; I-0; 0,3
Leg 2	0-1	1-0	1-0; 2-0; 3,1,I,1	I-1; I
Leg 3	0-1	1-0	1-0; 2-0; 3,1,I,1	I-1; I-1; IV,1,4
Leg 4	0-1	1-0	1-0; 2-0; 2,II,1	I-1; I-1; III,1,4

Sequence follows external to internal positions. See tables II and III for variant patterns in both caves.

passage, which immediately tees. The right route, following survey stations, goes down a short climb and doubles back under itself in a low crawl, the beginning of Zone 2. It opens up into a chamber, where two passages take off. The left passage, marked as Zone 3, is a multi-level breakdown chamber that connects through small holes back to the skylight entrance area. The right passage drops down and cuts back underneath itself, dropping down to a low crawl, the beginning of Zone 4. This zone goes down a 2 m climb to a sump pool, where the specimens were collected from. This water is clear and deep, with a green hue and a surface sheen of petroleum.

Description.— Female. Length range of Cave A specimens ($n = 14$) 0.90-1.02 mm, average 0.97 mm; Cave B specimens ($n = 16$) 0.95-1.27 mm, average: 1.07 mm (table I). Body (fig. 2B) robust in dorsal view, lateral margins of pedigers 3-5 produced posteriorly. Posterior margins of pedigers 3 and 4 smooth in all specimens examined (fig. 7C), with shallow pattern of reticulate striations (fig. 7D, E) on the surface. Genital double somite (fig. 2A-C) broadest at anterior half, tapering posteriorly. Seminal receptacle (fig. 2D) with posterior half weakly expanded, anterior margin produced, arms short. Hyaline fringes of urosomites crenulated (fig. 7F).

Anal somite with posterior margin ornamented with row of spinules along ventral margin and reaching 1/3 around dorsal margin; anal operculum broad, short (in Cave A), to subquadrate, relatively long (in some Cave B females); anal sinus smooth. Caudal ramus (fig. 2D, table I) length/width ratio variable, ranging between 4.1 and 5.3 (average 4.8, $n = 14$) in Cave A and between 4.0 and 6.1 (average 5.0, $n = 16$) in Cave B specimens. Dorsal and ventral surface of caudal rami with spinules arranged irregularly (fig. 8F). Inner margin smooth in most specimens, some specimens with few short, slender hairs. Lateral seta inserted at posterior 0.7-0.75 of way back along outer margin of ramus. Innermost terminal seta longest; dorsal seta about half the length of ramus (0.44-0.56 of ramus length). All caudal setae biserially setulated.

Antennule (fig. 2F) 17-segmented, reaching midlength of pediger 3. Surface of segments smooth except for short comb of 7-8 spinules on first segment. Armature

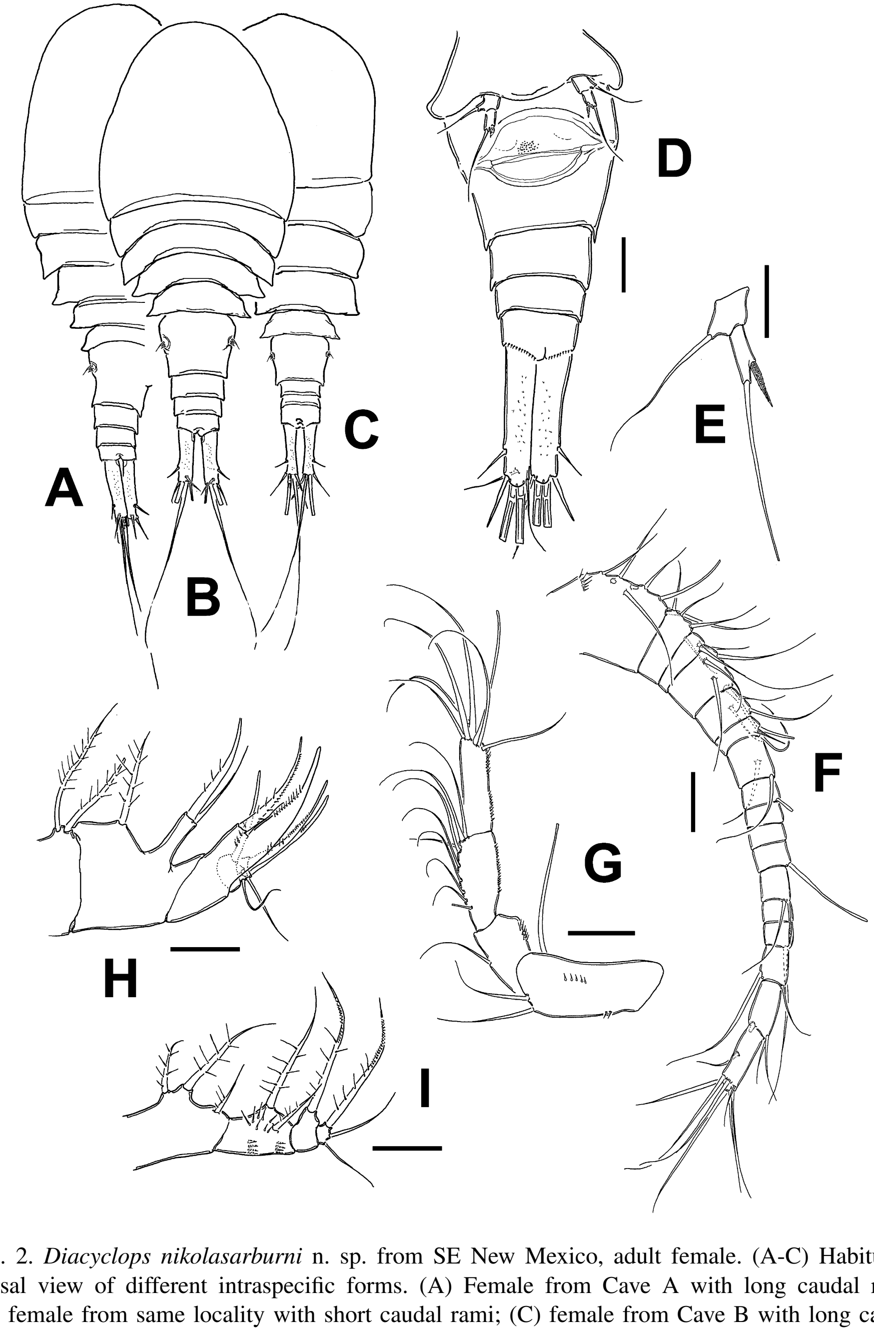

Fig. 2. *Diacyclops nikolasarburni* n. sp. from SE New Mexico, adult female. (A-C) Habitus in dorsal view of different intraspecific forms. (A) Female from Cave A with long caudal rami; (B) female from same locality with short caudal rami; (C) female from Cave B with long caudal rami. (D-I) Female holotype from Cave A. (D) urosome with fifth pedigerous and genital somites, ventral view; (E) fifth leg; (F) antennule; (G) antenna; (H) maxilla; (I) maxilliped. Scale bars: A-C = 200 μm; D-I = 50 μm.

of antennule segments (Arabic numerals) with number of setae, spines (spn), and aesthetascs (aes) in parentheses: 1(8), 2(4), 3(2), 4(6), 5(4), 6(1 + spn), 7(2), 8(1), 9(1), 10(0), 11(1), 12(1 + aes), 13(0), 14(1), 15(2), 16(2 + aes), 17(7 + aes). Spine on segment 6 slender, reaching about midlength of succeeding segment. Segments 16 and 17 smooth, without hyaline membrane. Antennules of specimens from Cave B with same structure and armature (figs. 5A and 8A).

Antenna (fig. 2G) 4-segmented, basipodal segment with exopodal seta; segment armed with several groups of spinules on both frontal and caudal surfaces. Ornamentation pattern equal in specimens with short and long caudal rami and from both localities (figs. 3H, I, 5C and 8B). Endopodal segment 1 with medial patch of spinules; setation patterns of endopodal segments 1-3 being 1, 9 and 7 setae, respectively, in all specimens examined.

Labrum (figs. 3B and 7A, B) with 10-12 teeth between rounded lateral corners; ventral surface bearing two rows of 3-5 short setules. Mandible palp with one short and two long setae; gnathal blade (fig. 3C) with 8-10 teeth plus uniserially pinnate dorsal seta. Maxillule with typical morphology, surface of basipod and palp smooth in specimens from both sites (figs. 3D, E, 5D and 8C, D). Maxilla with same structure in specimens from both pools and with short and long caudal rami (figs. 2H, 3F, 5E and 8E); appendage with short precoxal endite bearing 2 long pinnate setae. Claw with single row of teeth covering middle of inner margin, endopod 2-segmented. Maxilliped (figs. 3G and 5F) 4-segmented, with 3, 2, 1 and 3 pinnate setae on syncoxa, basis and two endopodal segments, respectively. Second segment with two rows of spinules on caudal surface; frontal surface ornamented with two loose rows of short setae, identical in all specimens examined.

Legs 1-4 (figs. 4A-F and 6A-E) with three-segmented endopodal and exopodal rami; regular setation pattern as in table I. Spine formula 2333, shown by 57 specimens from both caves (55% of those present in the sample), 15 specimens (14%) with a 2444 formula, 7 (6.5%) with 3444. Variant patterns with one or two supernumerary spines on one or more legs (i.e., 2343, 2344, 2443, 3333, and left-right ramal asymmetries) observed in 18 specimens (17%). These spinal formulae and variant patterns were indistinctly present in more than 100 specimens examined from both caves (tables II and III). Setal formula (number of setae on terminal exopodal segments of legs 1-4) 4444 in all cases, but some specimens with supernumerary seta in one ramus. Intercoxal sclerites of legs 1-3 with distal and medial groups of hairs; leg 4 sclerite naked except for some specimens from Cave B showing a medial row with 6-7 elements.

Leg 1 coxal sclerite with group of hairs on each side of sclerite. Coxa with row of spinules on outer distal margin; insertion point of inner coxal seta naked

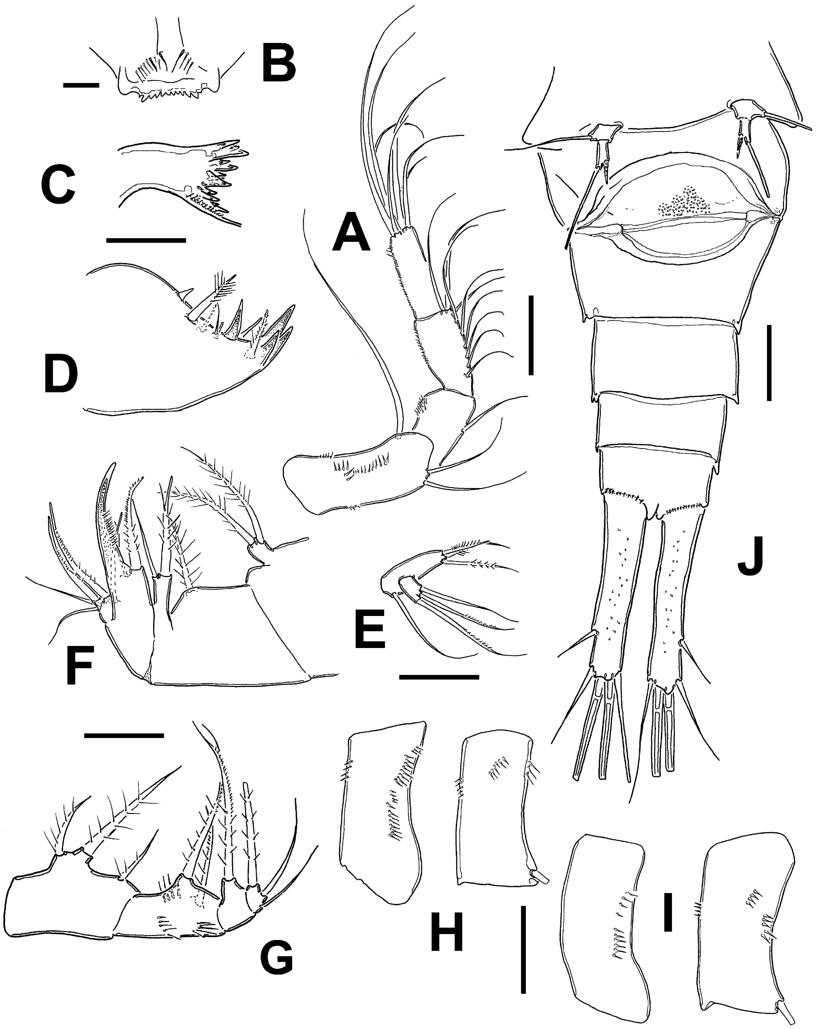

Fig. 3. *Diacyclops nikolasarburni* n. sp. from SE New Mexico, holotype adult female from Skylight cave, New Mexico. (A) Antenna; (B) labrum; (C) mandible blade; (D) maxillule (part); (E) maxillular palp; (F) maxilla; (G) maxilliped; (H) ornamentation of antennary basis (left side caudal view, right side frontal view), specimen with short caudal rami; (I) same of specimens with long caudal rami; (J) urosome, ventral view. Scale bars: A-I = 50 μm, J = 100 μm.

(figs. 4A and 6A). Some specimens with supernumerary spines on third exopodal segment (fig. 4B) or one seta less on third endopodal segment (fig. 6B).

Leg 2 with endopodal and exopodal rami longer than in leg 1, coxal sclerite with group of short hairs on each side of sclerite. Coxa with smooth outer margin; insertion point of inner coxal seta naked. Basipod with short outer basipodal seta, inner margin ornamented with row of setules (fig. 4C). Leg 3 as leg 2 except

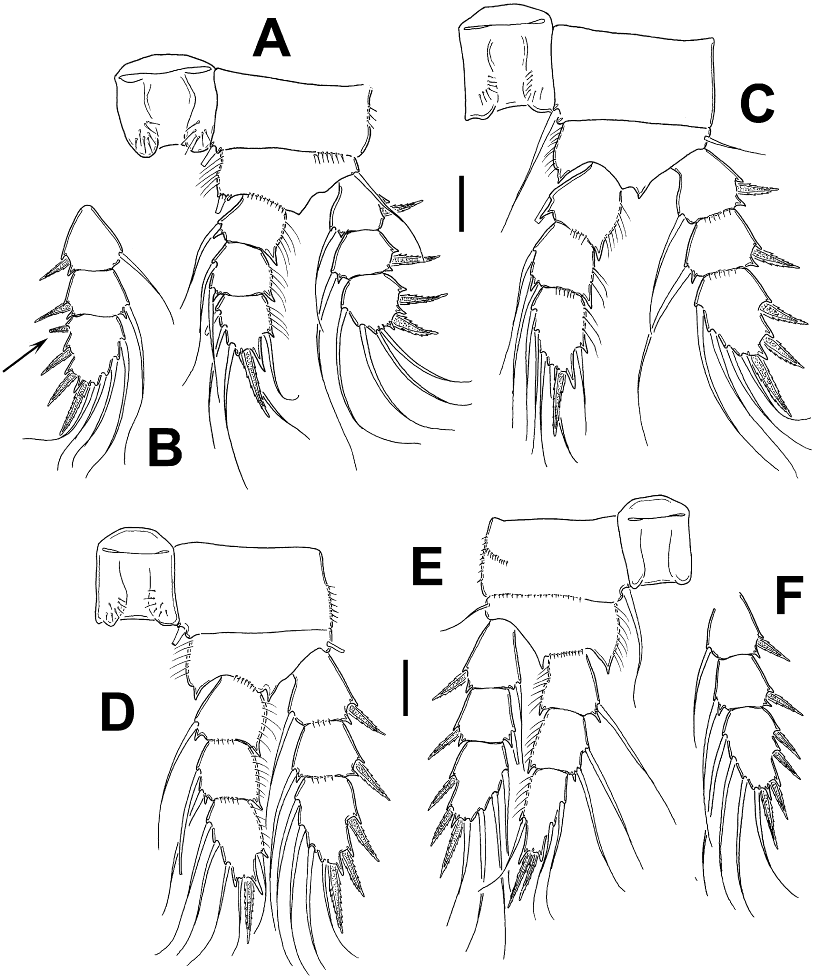

Fig. 4. *Diacyclops nikolasarburni* n. sp. from SE New Mexico, holotype adult female. (A) First leg with coxal plate; (B) exopod of first leg of specimen with variant spinal pattern (3 spines plus bud of supernumerary spine); (C) second leg with coxal plate; (D) third leg with coxal plate; (E) fourth leg; (F) exopod of fourth leg of specimen with variant pattern (4 spines). Scale bars: A-D = 50 μm; E, F = 100 μm.

for outer margin of coxa ornamented with row of short setules and insertion of endopodal rami with row of short spinules (fig. 4D).

Leg 4 (fig. 4E, F) with inner coxal seta; caudal surface of coxa furnished with row of spinules along outer margin and short transverse median row of spinules; distal margin with row of spinules. Outer margin of basipod with short outer basipodal seta. Group of minute spinules at insertion of endopodal ramus. Endopodal segment 3 narrow, about 3.4-times longer than broad in most specimens, 2.4 in some others. Same segment armed with 2 inner and 1 outer

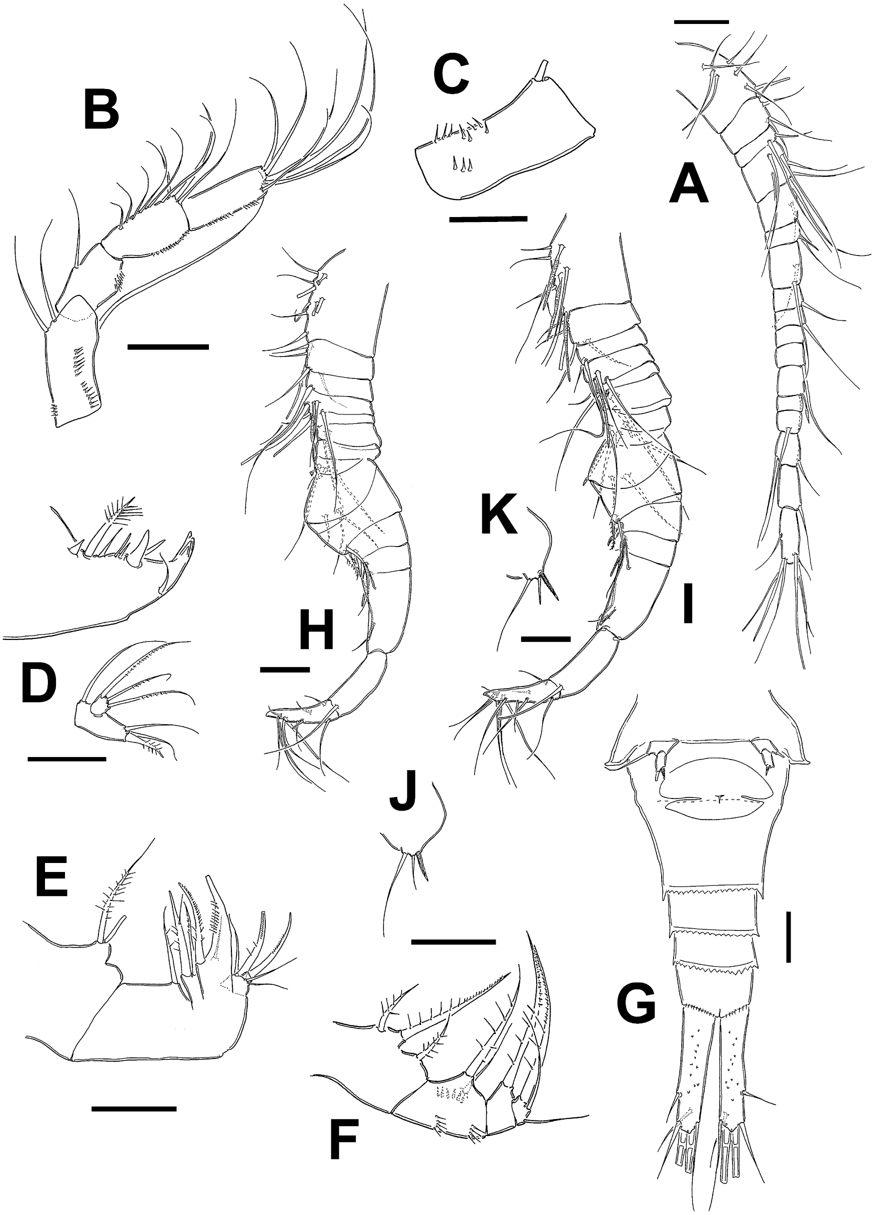

Fig. 5. *Diacyclops nikolasarburni* n. sp. from SE New Mexico, adult female from Selenite cave. (A) Antennule; (B) antenna; (C) antennary ornamentation, caudal view; (D) maxillule and maxillular palp; (E) maxilla; (F) maxilliped; (G) urosome, ventral view; (H) male antennule, specimen with 3444 spinal formula; (I) same of specimen with 2333 spinal formula; (J) sixth leg of 3444 specimen; (K) sixth leg of 2333 specimen. Scale bars: A-F, H-K = 50 μm, G = 100 μm.

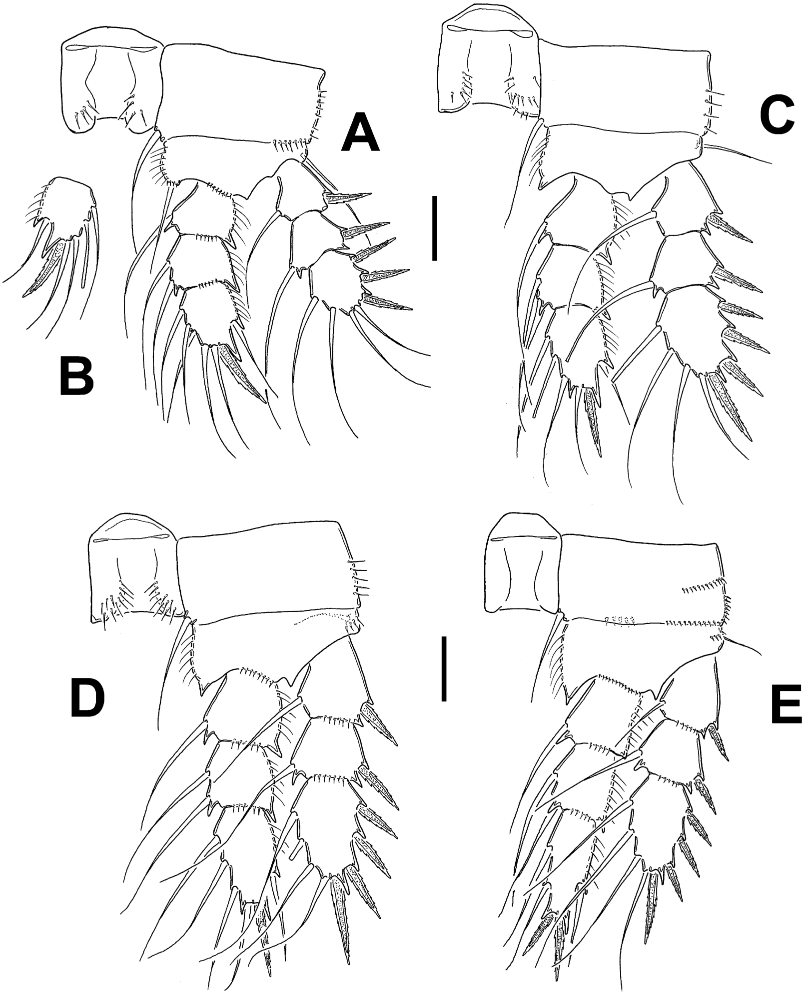

Fig. 6. *Diacyclops nikolasarburni* n. sp. from SE New Mexico, adult female from Selenite cave. (A) First leg with coxal plate; (B) third endopodal segment of first leg, specimen with variant setation pattern (one seta less); (C) second leg with coxal plate; (D) third leg with coxal plate; (E) fourth leg with coxal plate. Scale bars = 50 μm.

setae and 2 terminal spines, inner spine shorter than outer spine (inner/outer spine length = 0.73-0.78%).

Leg 5 (fig. 2E) with inner terminal spine serrate, as long as or slightly longer than distal segment.

Male.— Length of Cave A specimen ($n = 1$) 0.94 mm, of Cave B specimens ($n = 2$) 0.85-0.95 mm. Length/width ratio of caudal ramus 4.3-5.3 (average: 4.8), setation pattern as in female. Antennule (fig. 5H, I) geniculate, 16-segmented, segments 1, 4, 9, 13, 15 and 16 + 17 with 3, 1, 1, 1 and 1 short aesthetascs,

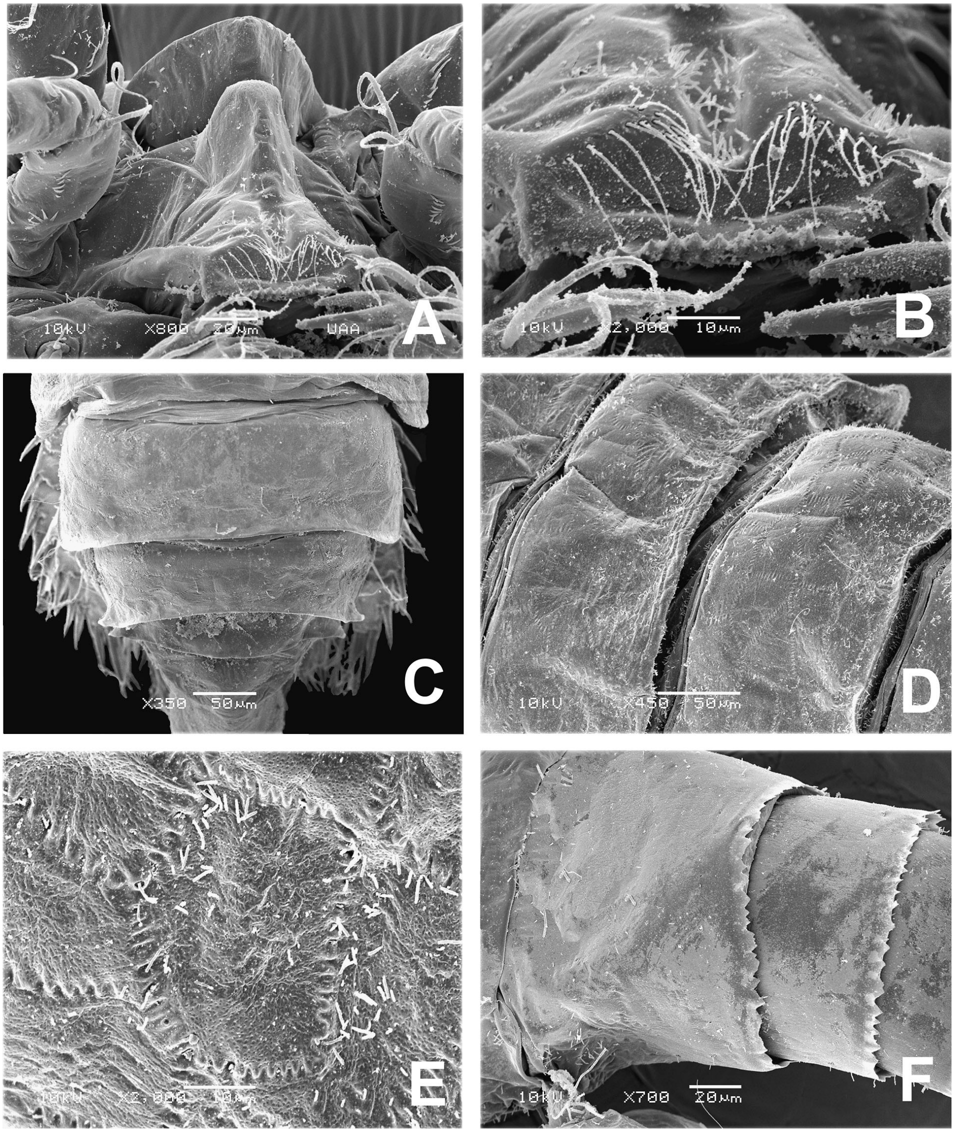

Fig. 7. *Diacyclops nikolasarburni* n. sp. from SE New Mexico, adult female from Cave A. (A) Rostrum, ventral view; (B) labrum, ventral view; (C) thoracic somites showing pointed shape of posterolateral margins, dorsal view; (D) detail of dorsal surface of thoracic somites showing cuticular ornamentation pattern; (E) detail of cuticular reticulation on surface of thoracic somites; (F) detail of crenulation along posterior margin of urosomites.

respectively. Antennules equally structured and armed in specimens from both sites. Antenna as in female except spinules pattern on basipodite somewhat simpler, and endopodite segment 2 with 8 setae.

Labrum, mouthparts, and legs 1-4 as in female.

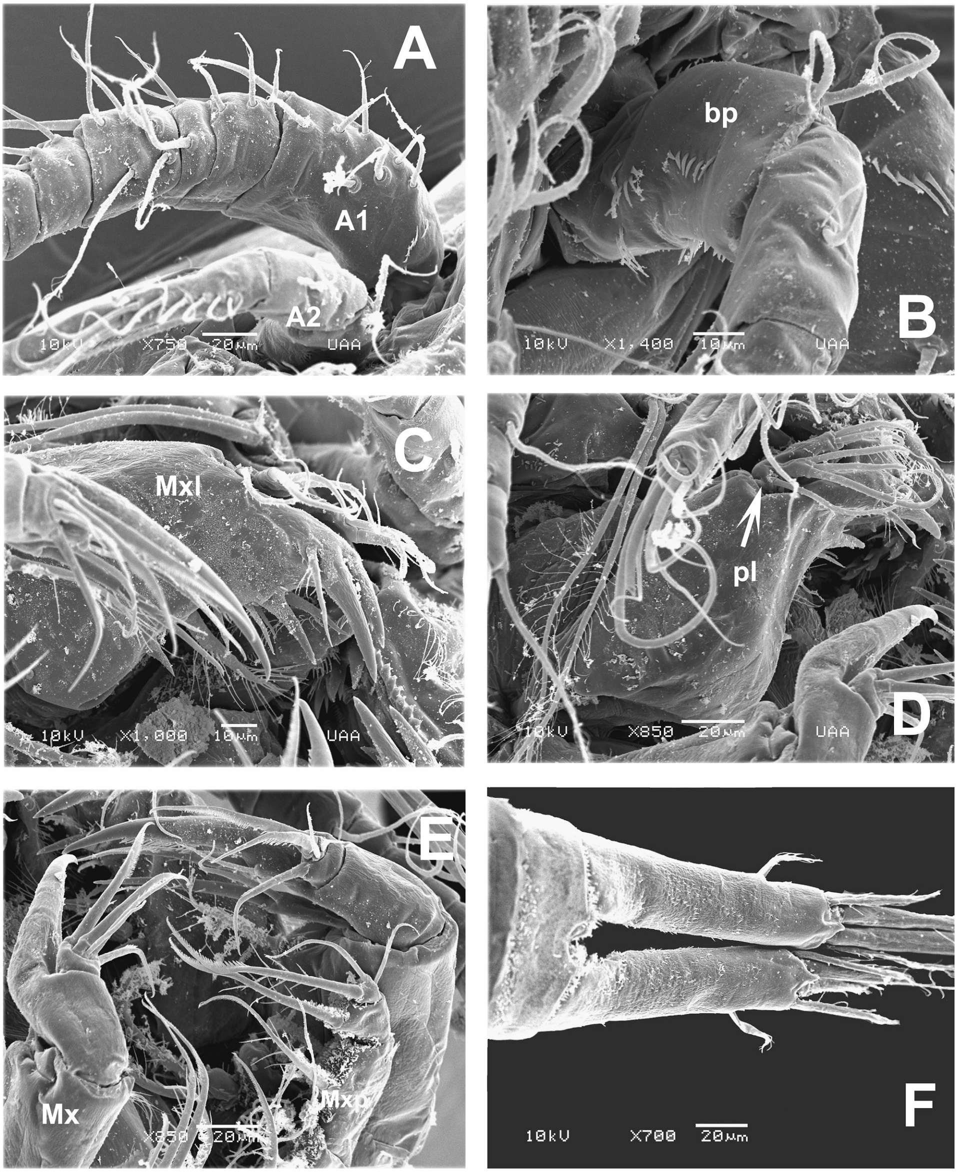

Fig. 8. *Diacyclops nikolasarburni* n. sp. from SE New Mexico, adult female from Cave A. (A) Basal segments of antennule and antenna, ventral view; (B) antennal basis (bp) showing spinulation pattern, ventral view; (C) maxillule (Mxl); (D) maxillular palp (pl); (E) maxilla (Mx) and maxilliped (Mxp), ventral view; (F) caudal rami showing spinulation on dorsal surface.

Leg 6 (fig. 5J, K) represented by flat, rounded plate bearing medial spine and two setae. Identical in specimens from both caves.

TABLE II

Spinal formula (number of spines on third exopodal segment of legs 1-4) of specimens ($n = 43$) of *Diacyclops nikolasarburni* n. sp. collected in Cave A, New Mexico

1	2333	2333	♀	23	2333	2333	♀
2	2333	2333	♀	24	2444	2444	♂
3	2444	2444	♀	25	3444	3444	♂
4	2333	2333	♀	26	2333	2333	♂
5	2343	2333	copp.	27	–	–	damaged
6	2333	2333	copp.	28	2333	2333	copp.
7	2333	2333	♂	29	3444	3444	♂
8	2333	2333	♀	30	2333	2333	copp.
9	2334	2333	copp.	31	2333	2333	♀
10	2444	2444	♀	32	2333	2343	copp.
11	2444	2444	♀	33	–	–	damaged
12	2333	2333	copp.	34	2333	2343	♀
13	3444	3444	♀	35	–	–	damaged
14	33	33	copp.	36	2333	2333	copp.
15	2233	2233	♂	37	2444	2444	♀
16	2333	2333	♀	38	2333	2333	♀
17	2333	2333	♀	39	3444	3444	♀
18	2333	2333	♂	40	2333	2333	♀
19	2333	2333	♂	41	3444	3444	♀
20	2333	2333	♂	42	3444	3444	♂
21	2333	2333	♀	43	3444	3444	copp.
22	2333	2333	copp.				

copp. = copepodites.

Etymology.— The species is named in memory of the late Nikolas Arburn, a young cave explorer from this area of New Mexico, who dedicated his life to the exploration and discovery of cave systems in this arid region.

Remarks.— A first examination of the specimens from these caves in SE New Mexico showed that some of them have a relatively longer caudal rami, thus suggesting that more than one species could be present in these sites. Another source of information to explore possible interspecific variation was the spine formula, but it has different patterns irrespective of the relative length of the caudal rami or the locality (table IV). Our taxonomical analysis/approach followed Stoch's (2001) criteria, by which traditionally relevant characters for the genus (number of antennulary segments, segmentation of swimming legs, spine formulae) are useful only in the separation of species groups, because others (length of caudal rami and caudal setae) are highly variable even within the same population. Given the high incidence of cryptic species in *Diacyclops*, and as recommended by Fiers et al. (2000) and Stoch (2001), micro-morphological characters (i.e., spinulation and armature of antennal basis, setation of mandible palp and maxilliped, shape of leg 4 basis, ornamentation of legs 1-4 coxal

TABLE III

Spinal formula (spines on third exopodal segment, right and left rami of legs 1-4) and variability of specimens ($n = 60$) of *Diacyclops nikolasarburni* n. sp. found in Cave B, New Mexico

1	2444	2444	♀	32	2333	2333	♀
2	2444	2444	♀	33	2344	2344	♀
3	2333	2333	♀	34	3433	2433	♀ copp.
4	2444	2444	♀	35	3433	2433	♀ copp.
5	2334	2333	♀	36	2333	2333	
6	2333	2333	♀	37	3433	2433	
7	2343	2333	♀	38	2333	2333	♀
8	2333	2333	♀	39	2333	2333	copp.
9	2333	2333	♀	40	2333	2333	♀
10	2333	2333	♀	41	2333	2333	♀
11	2333	2333	♀	42	2333	2343	♀
12	2444	2444	♀	43	2344	2344	♀
13	2333	2334	♀	44	3333	2333	♀
14	2444	2444	♀	45	2333	2333	♂
15	2333	2333	♀	46	2333	2333	copp.
16	2444	2444	♀	47	2444	2444	♀
17	2333	2333	♀	48	3333	2333	♀
18	2333	2333	♀	49	2333	2333	copp.
19	2333	2333	♂ copp.	50	2333	2333	♀
20	2333	2333	♀	51	2333	2333	♀
21	2333	2343	♀	52	2333	2333	copp.
22	2333	2333	♀	53	2343	2343	♂
23	2333	2333	♀	54	3343	2333	♀
24	2333	2333	♀	55	2444	2444	♂
25	2333	2333	♀	56	2333	2333	♂
26	2433	2333	♀	57	2333	2333	♀
27	2333	2333	♀	58	2333	2333	♀
28	2333	2333	♀	59	2333	2333	♀
29	2443	2443	♀	60	2333	2333	♀
30	2444	2444	♀				
31	2333	2333	♀				

copp. = copepodites.

sclerite, length of male antennule aesthetascs) were evaluated in attempting to find interspecific differences in the specimens of the population examined. The exploration of these microcharacters in specimens of *D. nikolasarburni* n. sp. with long or short caudal rami and with spine formulae of 2333 or 3444 revealed that they are quite consistent in the entire population, thus indicating that a single species is present in the samples examined despite the high variability of the primary morphological and morphometric characters traditionally used to separate species of *Diacyclops*.

The new species belongs to Group I of *Diacyclops* (sensu Reid & Strayer, 1994), related to the *bicuspidatus* group (Pesce, 1994), which contains all species with

TABLE IV

Total body length (TL), length of caudal rami (CR) and length/width ratio of caudal rami of specimens of *Diacyclops nikolasarburni* n. sp. with different spinal formulae (SF, right/left leg)

SF	TL (mm)	CR (mm)	CR L/W ratio
Cave A: variants of basic pattern 2333			
2443/2444	1.04	0.10	5.31
2443/2444	1.13	0.12	4.47
2444/2444	1.13	0.11	5.42
2444/2444	1.06	0.12	4.66
2444/2444	1.14	0.14	6.10
2344/2444	1.11	0.13	5.66
2444/2444	1.00	0.13	4.36
2444/2444	0.95	0.13 (male)	4.27
2343/2434	1.14	0.15	5.66
Average	1.07	0.12	5.10
Cave B: variants of basic pattern 2333			
2333/2333	1.08	0.13	4.50
2333/2333	1.15	0.12	3.80
2333/2333	1.01	0.12	6.10
2343/2333	1.19	0.14	6.20
2343/2333	0.98	0.11	4.20
2333/2333	1.27	0.15	5.70
2333/2333	1.14	0.12	5.50
2333/2333	1.03	0.13	4.00
2333/2333	0.86	0.11 (male)	4.80
Average	1.08	0.12	4.90
Cave A: pattern 3444 and variants			
2444/2444	0.90	0.12	5.07
3444/3444	0.92	0.12	4.30
2444/2444	0.77	0.10	5.00
2444/2444	0.92	0.11	5.10
3444/3444	1.01	0.12	5.06
Average	0.90	0.11	4.90
Cave A: basic pattern 2333 and variants			
2333/2343	1.09	0.12	4.10
2333/2333	0.99	0.13	4.86
2333/2333	0.98	0.13	4.30
2333/2333	1.02	0.13	4.55
2333/2333	0.99	0.13	5.31
2333/2333	0.94	0.11 (male)	5.37
2333/2333	0.95	0.14	4.70
2333/2333	0.90	0.12	4.10
2333/2333	0.94	0.12	4.30
Average	0.97	0.12	4.60

Data from each of the two localities surveyed.

3-segmented endopods and exopods in swimming legs 1-4. Currently, the group includes more than 40 species. Many of these species diverge from the new species by the number of antennular segments; for instance, members of the "*crassicaudis*" subgroup can be separated by their having of 12-segmented antennules (Pesce & Maggi, 1982; Reid, 1992). Following Reid & Williamson's (2010) key to the species of the genus, we reach couplet 20, in which the combination of characters presented (i.e., caudal ramus 5-7 times longer than broad and lateral caudal seta inserted at 2/3 of ramus vs. 4-5 and 3/4, respectively) does not entirely apply to our specimens, with caudal rami 5 times longer than broad (a 4.3-6.2 variation range) and the lateral caudal seta inserted at 3/4 of ramus. Comparisons were made to include a group of the four remaining species in the key: *D. bicuspidatus* s. str. (Claus, 1857), *D. navus* (Herrick, 1882), *D. thomasi* (Forbes, 1882) and *D. lewisi* Reid, 2004. The former three species are closely related, mainly epigean forms (Reid et al., 1989), whereas the latter is a cave-dwelling species (Reid, 2004).

The new species differs from *D. navus* by the length of the subdistal inner spine of the fifth leg; in *D. navus* this spine is as long as the outer terminal seta and about twice as long as the second segment (Reid, 1989), whereas the spine is much shorter in the new species. The second antennary segment has 8 setae in *D. navus*, vs. 9 in *D. nikolasarburni* n. sp. In *D. navus* the basipodal segment of the maxilliped has three rows of spinules on the caudal surface and one on the frontal surface (Reid et al., 1989, their fig. 3b), whereas the new species has two caudal and two frontal rows of spinules on the same segment. The coxal sclerites of legs 1-4 are naked in *D. navus* (cf. Reid et al., 1989, their figs. 3 and 4) vs. an ornamented pattern in legs 1-3 shown by the new species. The terminal spines of the third endopodal segment of leg 4 are subequal in *D. navus* (cf. Reid, 1989, their fig. 4b) and in *D. nikolasarburni* n. sp., the outer spine is consistently longer than the inner one. The length of the dorsal caudal seta differs between these two species, it is relatively longer (as long as ramus) in *D. navus* (cf. Reid, 1989, their fig. 1e) and about half as long as ramus in the new species.

The new species differs from the cosmopolitan *D. bicuspidatus* s. str. by having a relatively shorter inner subapical spine of the fifth leg; the spine is as long as the segment whereas it is 1.3-1.6-times longer than the corresponding segment in *D. bicuspidatus* (cf. Rylov, 1967; Dussart & Defaye, 2001, their fig. L106). In the new species the subapical spine of the fifth leg is clearly shorter than the apical seta (4.0 times as long as spine) whereas this ratio is 2.5-3.1 in *D. bicuspidatus* (cf. Sars, 1913; Rylov, 1967). In addition, the outer/inner ratio of the apical spines of the third endopodal segment of fourth leg differ in both species: 1.16 in the new species vs. 1.4-1.5 in *D. bicuspidatus* (cf. Sars, 1913; Rylov, 1963; Dussart & Defaye, 2001). In *D. nikolasarburni* n. sp. the length/width ratio of the caudal rami varies between 4.6 and 6.1 (average 5.1), whereas in *D. bicuspidatus* values range between 6 and 7.5 (Sars, 1913; Rylov, 1967, his fig. 53; Dussart & Defaye, 2001). The position of the lateral caudal seta differs in these two species; in *D. bicuspidatus* s. str. it is inserted between 61-65% of way back along ramus (Sars, 1913; Rylov, 1967; Dussart & Defaye, 2001), whereas in the new species, it is consistently located at a more distal position (71-75%) both in specimens with short and long caudal rami.

The length of inner and outer spines of leg 4 endopod allowed us to distinguish the new species from *D. thomasi*; in this species the outer apical spine is twice as long as the inner spine, the latter being curved outward. Neither of these characters is present in *D. nikolasarburni* n. sp.

Diacyclops nikolasarburni n. sp. differs from *D. lewisi* in several characters. In the latter species, the subdistal inner spine of the fifth leg is more than 3 times as long as the bearing segment and it has a row of spines at the insertion point (Reid, 2004, his fig. 11E); in the new species the subdistal spine is as long as the segment and the insertion margin is smooth. In *D. lewisi* the third endopodal segment of leg 4 is narrow and the outer terminal spine is almost as long as the segment, whereas in *D. nikolasarburni* the segment is broader and the inner terminal spine is relatively shorter, about half the length of the segment. The ornamentation of the second segment of the maxilliped shows two rows of spinules on the frontal surface in the new species vs. a single row in *D. lewisi*. The coxal ornamentation is different in these species; *D. lewisi* has two rows of strong spinules (Reid, 2004, fig. 14B) which are absent in the new species. In general, all leg segments are broader and shorter in the new species than in *D. lewisi*, which has slenderer segments. The male sixth leg of *D. lewisi* resembles that of the new species, but in the former the plate is ornamented with a diagonal row of spines, whereas this structure is smooth in *D. nikolasarburni* n. sp.

The new species shows some affinities with another cave-dwelling congener, *D. salisae*: 17-segmented antennules, 3-segmented endopods and exopods of legs 1-4, the proportions of the caudal rami, the ornamentation of the antennary basis, the length of the inner spine of the fifth leg, and the structure and armature of the third endopodal segment of leg 4 and its terminal spines (see Reid, 2004). These species differ in the ornamentation of the coxal plates, the spine formula (3444 in *D. salisae*, 2333 in the new species), the ornamentation of the second and third segments of the maxilliped, the former with a naked caudal surface in *D. salisae* and with two rows of spines in the new species and the latter with a row of spinules in *D. salisae* which is absent in *D. nikolasarburni* n. sp. In addition, *D. salisae* has transverse rows of spinules on the inner margin of the caudal ramus, whereas the inner surface of the caudal rami is smooth in the new species.

So, among the North American species, *D. nikolasarburni* n. sp. is morphologically close to the complex of *D. bicuspidatus* (see Reid & Williamson, 2010).

Overall, the new species from New Mexico can be distinguished from all other North American members of *Diacyclops* by a unique combination of characters: 17-segmented antennules, antennal exopodal seta present, its spine (2333 or variant) and setal (4444) formulae, the length/width proportion of its caudal rami (usually 4.6-5.1), the position of the lateral caudal seta (at 70-75% of way back along ramus), the outer apical spine of the fourth leg third endopodal segment only slightly longer than the inner spine, the relative length of its fifth leg inner spine, and the spinulation pattern of the antennal basis.

Variability.— The variable spine formula of the swimming legs 1-4 is an interesting character of the new species; it has a basic 2333 formula with different variant patterns (see tables I-III). *Diacyclops nikolasarburni* n. sp. appears to be a highly variable species, with different local morphs (see fig. 2A). Some specimens have relatively long caudal rami, with a length/width ratio 5.5-6.1, whereas others had a clearly lower range (4.1-4.8), irrespective of the spine formula or body size (table IV). They are identical in all other taxonomically relevant structures including the spinulation pattern of the antennal basis, the ornamentation and armature of all mouthparts, the ornamentation and structure of leg 4, and the structure and armature of leg 5, with an inner spine as long as the bearing segment. The size of the females examined ranges between 0.77 to 1.27 mm; however, the average total length of specimens from Cave A (0.93 mm) is only slightly lower than in Cave B (1.07 mm).

DISCUSSION

The Rustler Formation, the geological unit in which this speleological survey was carried out, has some economic significance as a source of sulfur and groundwater but also because it is close to a waste isolation pilot plant (Siegel et al., 1991; Powers et al., 2006). The invertebrate fauna already described in the area is largely limited to fossil species (Croft, 1978; Powers et al., 2006). The aquatic invertebrate fauna in this region has received little attention, with exception of some sites like Carlsbad caverns where some species of branchiopods, rotifers and other invertebrates have been studied (Barr & Reddell, 1967).

Copepods are very successful colonizers of groundwaters and their local diversity may equal or exceed that of their epigean relatives (Stoch, 1995). Barr & Reddell (1967) reported the occurrence of epigean species of *Eucyclops* and *Acanthocyclops* in the adjacent Carlsbad Caverns. Hence, karstic aquifers usually harbour both epigean and hypogean species (Rouch & Danielopol, 1997; Bruno et al., 2003). In the Rustler formation aquifer recharge takes place by flow from deeper aquifers and percolation of surface water (Powers et al., 2006); thus, it was expected that this mixed pattern (i.e., epigean + hypogean forms) would be observed in the copepod fauna of these caves. *Diacyclops nikolasarburni* was the only copepod species in these caves, not accompanied by typical generalist freshwater cyclopoid species, thus showing the physiographic isolation and exclusive environmental conditions of these caves in SE New Mexico.

Earlier epigean populations of *D. bicuspidatus* or related forms would have colonized the area and invaded the subterranean waters. There is recent evidence of cryptic speciation in the *D. bicuspidatus* complex (Monchenko, 2000); also, the variability range of *D. bicuspidatus* referred to by Karanovic & Krajicek (2012) does not cover the morphological differences found in the new species. The new species is similar to *D. bicuspidatus* but it also resembles other well-defined stygobiotic species. Furthermore, as observed by Monchenko (2000), epigean populations of *D. bicuspidatus* from the same region but living in different ecological conditions fail to successfully interbreed; thus, it is expected that the physiographic isolation and peculiar habitat conditions of the stygobiont *D. nikolasarburni* would lead to an even sharper reproductive isolation and morphological divergence.

Several members of the *D. bicuspidatus* species group are cave-dwelling or groundwater forms (Pesce, 1994; Reid, 2004). Like other true cave-dwelling copepods found in the region (Suárez-Morales & Iliffe, 2005), the new species is presumed to have a restricted distributional range and is probably endemic to this system. It was not expected to find morphological modifications or reductions in these cave-dwelling *Diacyclops* as most stygobiont species of the genus are quite similar to their epigean relatives (see Reid & Strayer, 1994; Galassi, 2001). It is important to re-examine the copepod diversity of the Nearctic and Neotropical regions in which the habitat diversity provides favourable conditions for local speciation processes (Fiers et al., 1996; Suárez-Morales et al., 2004).

This work allows an increase of the number of species of *Diacyclops* known from North America. Records of *Diacyclops* from North American caves and groundwaters currently comprise 11 species: *Diacyclops jeanneli* Chappuis, 1929, *D. crassicaudis brachycercus* (Kiefer, 1929), *D. yeatmani* Reid, 1988, *D. languidoides* (Lilljeborg, 1901), *D. conversus* Reid, 2004, *D. sororum* Reid, 1992, *D. indianensis* Reid, 2004, *D. lewisi* Reid, 2004, *D. salisae* Reid, 2004, *D. clandestinus* (Reid et al., 1991; Reid, 2004; Elliott, 2007; Lewis & Reid, 2007) and *D. nikolasarburni* sp. nov.

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