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Zeldia spannata sp. n. (Nematoda: Cephalobidae) from the Mojave Desert, California

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Summary: *Zeldia spannata* sp. n. is described from the Mojave Desert, California. The new species shares with congeners a single guard process in each primary axil and asymmetrical triangular-shaped lips; it differs from them by the presence of elongate spanner-shaped, deeply bifurcate probolae (*vs* typically low, rounded and bicornuate). In addition, the new species has five lateral incisures (*vs* three in most other species of the genus), areolated lateral fields and tessellate cuticle (*vs* non-tessellate). Unlike some other *Zeldia* this new species lacks punctations. With respect to the bifurcate probolae, *Z. spannata* sp. n. resembles *Chiloplacus* or *Stegelleta*. Although the new species is reminiscent of some *Nothacrobeles* with respect to overall asymmetry, triangular shape of the lips, and the presence of tines on lips, maximum parsimony and maximum likelihood analyses of the ITS1, 5.8S and ITS2 of rRNA gene fragments from 13 taxa do not allow resolution of relationships between *Zeldia* species and several other cephalobid clades. The morphological characters of the new species are the basis for a broader, emended definition of the genus *Zeldia*, and suggest novel implications for evolution of Cephalobidae.

Key-words: Cephalobidae, Mojave Desert, morphology, new species, SEM, taxonomy, *Zeldia*.

Resumen.- Se describe *Zeldia spannata* sp. n. del desierto de Mojave, California. La nueva especie comparte con sus congéneres un único proceso guarda en cada axila primaria, así como los labios asimétricos y triangulares; pero difiere de ellas en la presencia de probolas con forma de llave inglesa alargada y provistas de profundas bifurcaciones, en tanto que en las otras especies de *Zeldia* las probolas son típicamente bajas, redondeadas y bicorneadas. Además, *Z. spannata* sp. n. tiene cinco incisuras laterales (*vs* tres en la mayor parte de las otras especies), campos laterales areolados, y cutícula teselada (*vs* no teselada). A diferencia de algunas otras especies del género, la nueva especie carece de líneas de puntos. En lo que se refiere a la presencia de probolas bifurcadas, se asemeja a *Chiloplacus* o *Stegelleta*. Aunque la nueva especie es reminiscente de algunas pertenecientes al género *Nothacrobeles* si atendemos a la simetría general, la forma triangular de los labios y la presencia de púas en los mismos, los análisis de parsimonia máxima y probabilidad máxima de los fragmentos ITS1, 5.8S y ITS2 de rRNA de 13 táxones no permiten resolver las relaciones de parentesco entre especies de *Zeldia* y otros varios clados de cefalóbidos. Los rasgos morfológicos de la nueva especie son la base para una diagnosis corregida y más amplia del género *Zeldia*, al tiempo que sugieren implicaciones novedosas para la evolución de familia Cephalobidae.

Palabras clave: Cephalobidae, Desierto de Mojave, microscopía electrónica de barrido, morfología, nueva especie, taxonomía, *Zeldia*.

INTRODUCTION

Cephalobidae Filipjev, 1934 are microbial-feeding, ubiquitous terrestrial nematodes including representatives ranging from moist tropical habitats to the deserts of Africa and the USA as well as the driest soils of Antarctica. Taxonomy within Cephalobidae is problematic; many genera are poorly defined. Discoveries of new species frequently blur the boundaries of accepted genera and molecular resolution casts doubt on the monophyly of many extant genera and families (De Ley, 1997; Nadler *et al.* submitted). *Zeldia* Thorne, 1937 is particularly problematic, having been defined by some characters later demonstrated to be unreliable (Yeates, 1967; Allen & Noffsinger, 1972; Rashid *et al.*, 1984)

The taxonomic boundaries of *Zeldia* are not clearly defined since it includes some characters also shared by other genera including *Chiloplacus* Thorne, 1937 and *Nothacrobeles* Allen & Noffsinger, 1971 (Abolafia & Peña-Santiago, 2003). *Zeldia* spp.are characterized by a single sclerotized guard process at each primary axil, paired asymmetrical triangular lips with or without tines, low rounded or bicornuate labial probolae, three (rarely four) lateral lines and ventrally arcuate conoid to ventrally sigmoid-conoid acute tails in females (Allen & Noffsinger, 1972). Although there are fourteen species described, *Zeldia punctata* (Thorne, 1925) Thorne, 1937 is the most widespread and most studied.

Herein we describe *Zeldia spannata* sp. n. with new characters that broaden the diagnosis of *Zeldia* and will be important considerations in conjunction with molecular data for understanding phylogeny as a basis for future revision of Cephalobidae. The new species, Z. *spannata* sp. n., is named in recognition of its high spanner-shaped labial probolae, a spanner being a bifid wrench-like tool.

MATERIALS AND METHODS

Nematodes were extracted from soil using a combination of sieving and modified Baermann funnel techniques. The isolate was maintained on 2% water agar with 0.05 µl / ml cholesterol in Petri-dishes at room temperature (18-20° C) and designated as culture strain JB 140.

For morphometric studies, nematodes suspended in a small drop of water in a vial were fixed by quickly adding 3-4 ml formaldehyde-acetic acid fixative (4:1) at 96-100° C (Seinhorst, 1966). The fixed nematodes were then processed to anhydrous glycerin as described by Seinhorst (1959) and mounted in glycerin on glass slides for examination. The morphometric characters were measured as defined by De Ley *et al.* (1999) and Rashid *et al.* (1989).

For SEM, specimens were prepared as previously described by De Ley *et al* (1999) and Baldwin *et al.* (2001). Nematodes fixed in 5% aqueous formalin solution for a minimum of 24 h were rinsed in several changes of 0.1M phosphate buffer (pH 7) and post-fixed overnight in 4% aqueous osmium tetroxide solution. Post-fixed specimens were rinsed in several changes of cold 0.1M phosphate buffer within 15 minutes and dehydrated through a series of 20-100% absolute ethanol for 35-40 minutes followed by two 30 minutes changes in 100% ethanol. The specimens were then dried in a Tousimis Autosamdri®-815 critical point drier (Rockville, MD, USA) and kept in the incubator overnight at 30-40° C. The dried specimens were then mounted on double-sticking copper-adhesive tape attached to aluminum stubs. Stubs with mounted nematodes were coated for 3 minutes with a 25 nm layer of gold palladium in Hummer® V sputter coater (Alexandria, VA, USA). The specimens were observed with an XL 30-FEG Phillips® 35 scanning electron microscope (Eindhoven, the Netherlands) at 10kV.

To infer phylogenetic context of the new species, DNA sequence data were obtained from thirteen nematode samples (Fig. 3). Several specimens of each sample were transferred to an Eppendorf tube containing 16 ml ddH_2O, 2 ml 10X PCR buffer and 2 ml proteinase K (600 mg / ml) (Promega, Benelux, the Netherlands) and homogenized for several seconds using an ultrasonicator. The tubes were incubated at 65°C (1 h) and then at 95°C (15 minutes). Protocols for PCR, cloning and automated sequencing used in this study are as described by Tanha Maafi *et al.* (2003). The forward TW81 (3'-GTTTCCGTAGGTGAACCTGC - 5') and reverse AB28 (3'-ATATGCTTAAGTTCAGCGGGT - 5') primers were used for amplification and sequencing of the ITS1-5.8S-ITS2 fragment. Original sequences reported here have been deposited in GenBank (accession numbers given in Fig. 3).

Two alignments (entire and culled) were generated for phylogenetic analyses. The entire alignment was obtained after eleven sequences of ingroup and two outgroup taxa were aligned using ClustalX 1.64 with default parameters for gap openings and gap extensions and manually edited using GenDoc 2.5 software. The culled alignment was obtained from the entire alignment after manually removing ambigiously-aligned fragments.

Maximum parsimony (MP) and maximum likelihood (ML) analyses were performed on both alignments using PAUP* 4b4a (Swofford, 2003). *Cervidellus* sp. and *Acrobeles complexus* Thorne, 1925 were the outgroup taxa as inferred from the detailed analyses of the near-complete 28S gene dataset (Nadler *et al.*, submitted). Gaps were coded as missing data and molecular characters were assessed as unordered and equally weighted. For ML, all necessary parameters were estimated from the data

using ModelTest based on Akaike Information Criterion (Posada & Crandall, 1998). Robustness of the clades was assessed by bootstrap analysis yielding a bootstrap percentage (BS) for each node estimated from 1000 and 100 replicates for MP and ML analyses, respectively.

Zeldia spannata sp. n.
(Figs 1-3)

Measurements: See Table I.

Females: Body cylindrical narrowing at extremities, almost straight in most heat-relaxed specimens, less commonly sigmoid or slightly curved ventrally near anus; head slightly offset. Cuticle 2.5-2.9 µm thick at mid-body. Annuli prominent, 2.8-3.4 µm wide at widest part of body. Cuticle lacking punctations. Tessellation includes shallow irregular discontinuous longitudinal lines. Five prominent deep lateral incisures define four longitudinal alae. Outer incisures are crenate and begin at 13-14 annuli (from anterior end). Innermost three incisures begin at the 25-26th annulus. All incisures terminate at or near tail tip. Areolation continuous with transverse striae of annuli. Lip region 9-13 µm wide, almost equal to stoma length; includes three labial probolae (one dorsal, two subventral) and three pairs of lips (cephalic probolae). Labial probola elongate (7-9 µm), deep single level bifurcation beginning at about half-length and lacking tines. Termini of prongs with rounded tips curving toward one another and resulting in overall spanner-shape. Labial probolae clearly demarcated from radial ridge by a distinct stem having flattened, thin outer membranous margins and thick inner section. Radial and tangential ridges prominent. Cephalic probolae asymmetrical triangular flap-like lips. Anterior margin of each lip fringed by four triangular, attenuated and anteriorly projecting tines. Tines adjacent to each primary axil with broad base and elongate relative to other tines. Single triangular, sclerotized guard process in each primary axil extends anteriorly about one third length of adjacent tines. Base of guard process clearly demarcated from anterior most annulus. Labial, cephalic papillae and amphid openings typical for Cephalobidae. Stoma typical of Cephalobidae; triradiate with distinct cuticular thickenings (rhabdia), length about equal to lip region width. One short tooth-like cuticular process associated with the adoral surface of each labial probola. Pharynx differentiated into corpus, isthmus and basal bulb at 5:1:1 ratio. Corpus cylindrical, lumen distinctly zig-zag to wavy. Basal bulb with well-developed grinder pharyngeal-intestinal valve distinct and hemispheroid. Intestinal lumen broad (about 50 % of body width) from anterior end to rectum. Rectum arcuate anteriorly and rectal glands obscure; anus an arcuate transverse slit. Tail about two anal body widths long with conoid, rounded terminus, occasionally slightly curved ventrally; terminus cuticular tip hyaline. Phasmid openings variable, typically less than half tail length. Excretory pore near anterior end of the isthmus at 34-35th annulus. Deirid conspicuous in the lateral field near level of anterior end of basal bulb about 12-13 annuli posterior to excretory pore. Reproductive system monodelphic and prodelphic, reflexed at junction of uterus and oviduct. Vulva prominent. Vagina with thick cuticular lining and oriented anteriad at about 45° to body axis. Uterus highly thickened near vagina, set off by deep constriction from thin-walled more elongate anterior region. Offset spermatheca at point of flexure and junction with oviduct. Spermatheca variable in length and shape, typically occurring as cluster of cells with irregular shape and more rarely clearly defined, narrow and elongate with distinct cells and nuclei. Spermatheca typically associated with a large prominent pseudocoelomocyte. Oviduct short, with 4-6 pairs of apparent cells and increases in width toward junction with uterus. Ovary long (extends far posterior to post-vulva sac) and sometimes with post-vulval S- shaped flexure; oocytes in a single row. Postvulval sac (PVS) variable in length, 0.9-2.0 times as long as vulval body width.

Males: Unknown.

Diagnosis and relationships: Although *Zeldia spannata* sp. n. shares with other members of the genus a single guard process and paired asymmetrical triangular lips, it is readily distinguishable from all other species of the genus by the elongate spanner-shaped labial probolae (*vs* low, rounded or bicornuate probolae), the five prominent lateral lines (*vs* three or rarely four often faint lines), areolation of the lateral field, and tessellation of the body wall cuticle. Unlike many other species of the genus including *Zeldia punctata*, the body wall cuticle lacks punctations.

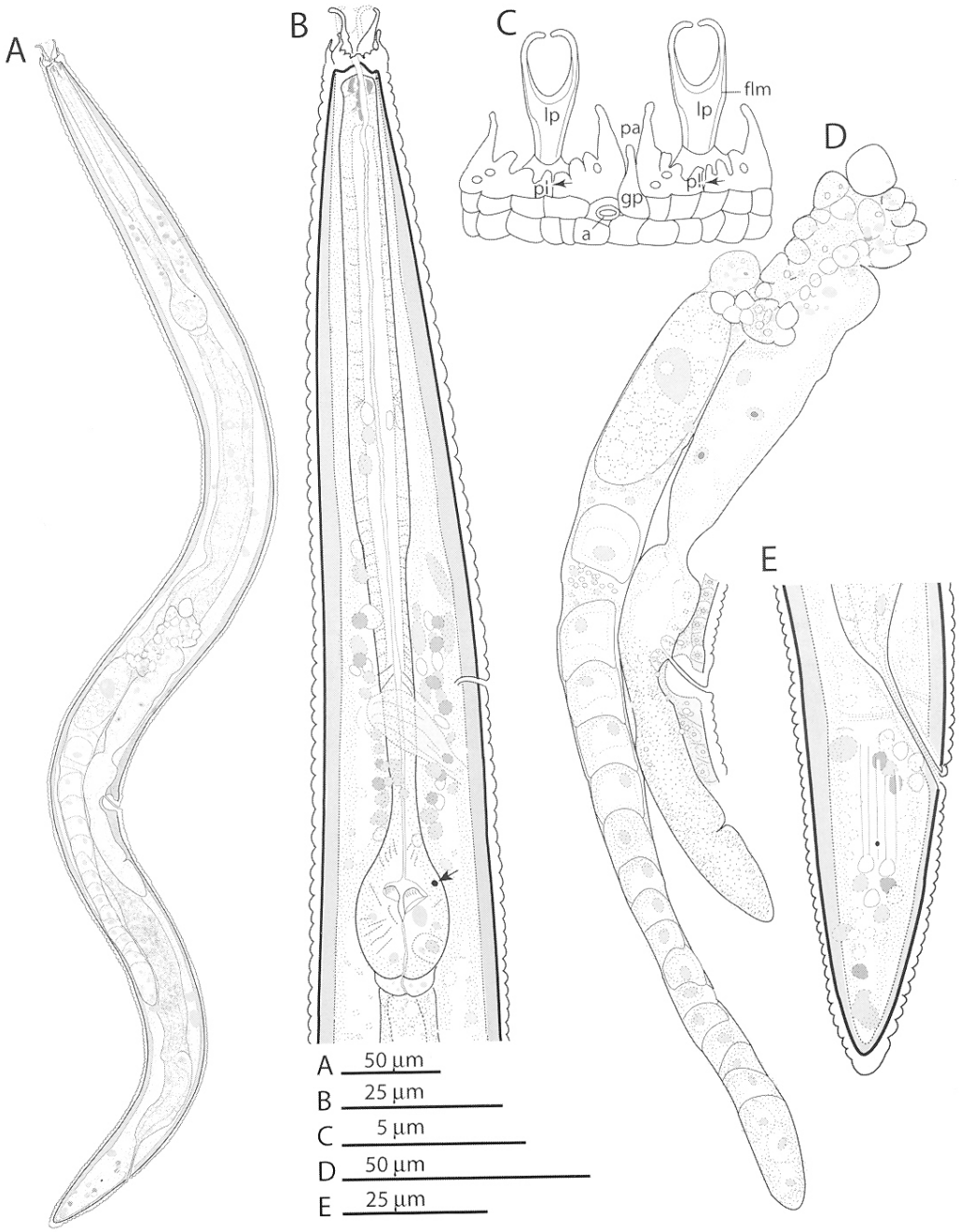

Figure 1. Camera lucida (except where otherwise indicated) drawings of *Zeldia spannata* sp. n. (female). A: Entire specimen. B: Anterior end including pharynx and deirid (arrow). C: A diagrammatic representation of a section of an "opened up" lateral view of the lip region depicting the amphid (a), single guard process (gp), primary axil (pa), secondary axil (arrows), the paired lip (pl), the spanner shaped labial probolae (lp) and flat lip margin (flm). D: Reproductive tract. E: Tail region.

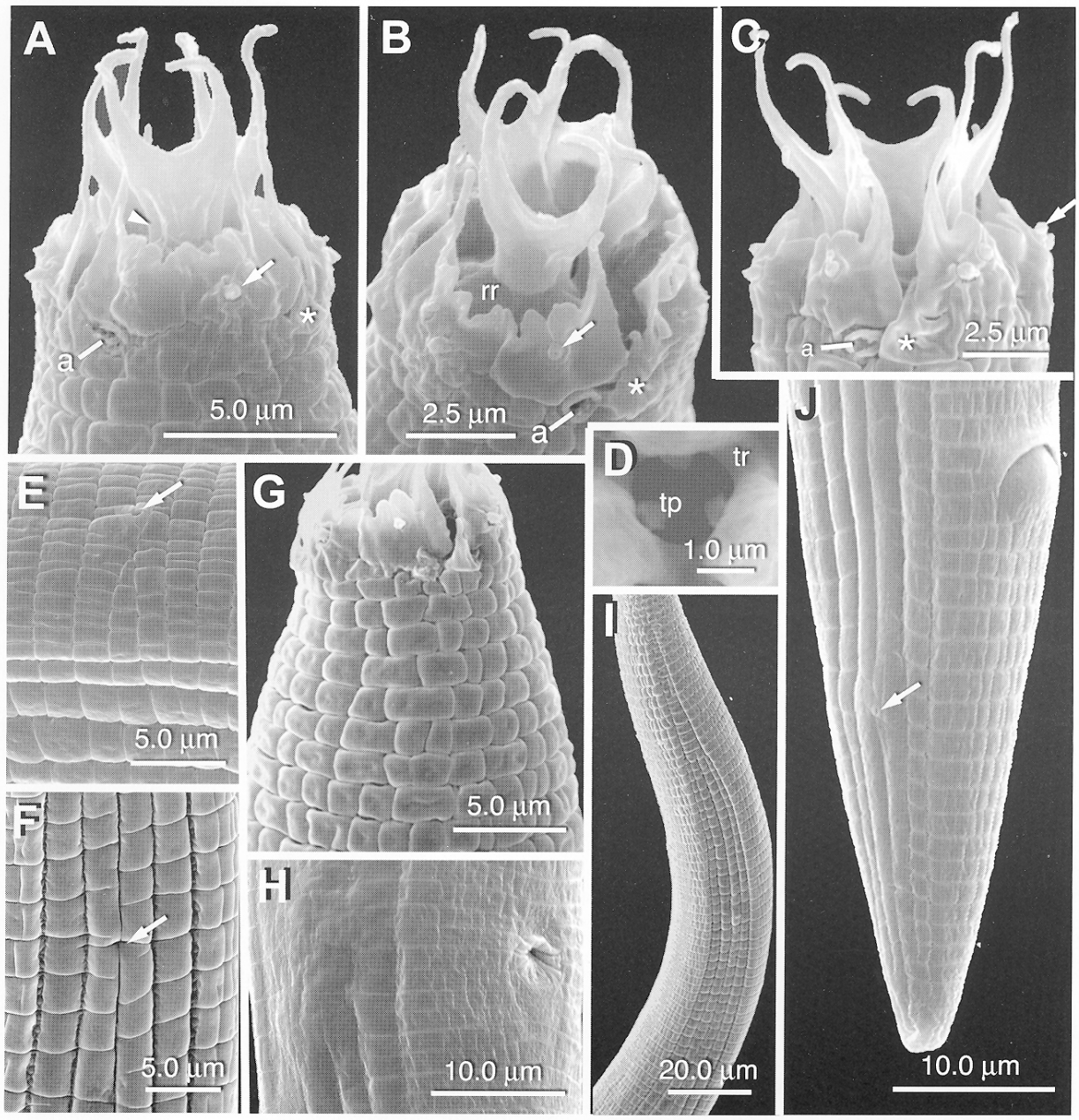

FIGURE 2. Scanning electron micrographs of female of *Zeldia spannata* sp. n. A: Lateroventral view showing the spanner-shaped labial probolae with flattened margins (arrowhead), amphid opening (a), sensory papilla (arrow) and a single guard process in primary axil (asterisk). B: Ventrosublateral view of lip region showing the radial ridge (rr), distinct stem of the labial probola and paired lips with triangular margins, amphid opening (a) and a single guard process (asterisk). C: Lateral view of lip region showing amphid (a), the single guard process (asterisk) and sensory papilla (arrow). D: *En face* view of lip region showing the tooth-like projection in the stoma (tp) and tangential ridge (tr). E: Excretory pore (arrow). F: Deirid (arrow). G: Ventrosublateral view of body wall cuticle showing cuticular tessellation. H: Vulva. I: Near lateral view showing the lateral field with the five incisures. J: Lateroventral view of tail showing anus and phasmid (arrow).

Labial probolae of Z. *spannata* sp. n. are 7-9 µm long, which is nearly twice the length of those of Z. *punctata*. Bifurcation of labial probolae occurs occasionally in *Zeldia*, having been reported in only three of fourteen species: Z. *punua* Yeates, 1967, Z. *punctata* (Allen & Noffsinger, 1972) and Z. *setosa* (Cobb, 1914) Thorne, 1937 (Thorne, 1925). As is the case for Z. *punua*, bifurcation in Z. *spannata* sp. n. occurs at about half the length of the probolae. However, while in Z. *punua* the prongs are narrow with elongate straight tips, prongs of Z. *spannata* sp. n. are broad and arch toward each other. Unlike in Z. *punua* whose labial probolae are uniform throughout their length (Allen & Noffsinger, 1972), those of Z. *spannata* sp. n. are narrow at the base with a distinct stem and widen at the level of bifurcation. In Z. *setosa*, prongs are short, arcuate, tapering, acute and have forward pointing «bristles» (tips) (Thorne, 1925). In Z. *punctata*, if bifurcation is present, it occurs in the anterior one third of the probolae and prongs are short, setose or rounded (Rashid & Heyns, 1990).

Patterns of the body wall cuticle in Z. *spannata* sp. n. differ from other members of the genus by tessellation. Z. *spannata* sp. n. has no punctations as in Z. *acuta* Allen & Noffsinger, 1972 while most other species have two and occasionally three rows of transverse punctations per annulus (Allen & Noffsinger, 1972). Z. *spannata* sp. n. is distinguished by five deep prominent lateral incisures and four raised longitudinal alae in each lateral field; the two outer incisures are crenate. Although in Z. *brevicauda* Boström, 1985 four lateral lines have been reported (Boström 1985), most of the species within this genus have three lateral lines that are shallow and less prominent. Unlike other *Zeldia* spp., the lateral field of Z. *spannata* sp. n. is areolated.

Z. *spannata* sp. n. further differs from Z. *punctata* by the shorter neck (162-179 *vs* 192-220 µm; De Ley *et al.*, 1990) and corpus (93-110 *vs* 153-171 µm; De Ley *et al.*, 1990) and longer post vulval sac (42-65 *vs* 14.5-15.0 µm; Rashid *et al.*, 1984). The excretory pore of Z. *spannata* sp. n. is more anteriorly positioned relative to Z. *punctata*, 34-37 *vs*. 52-56 annuli from anterior end (Abolafia & Peña-Santiago, 2003). The anteriad orientation of the vagina and the constriction of the anterior region of the uterus seem to be unique to Z. *spannata* sp. n. Unlike in some other *Zeldia* spp., the spermatheca is always readily observed.

The lips of this nematode are paired and have attenuated tines as in a majority of other *Zeldia* spp. Z. *spannata* sp. n. has a "tooth like" process associated with each adoral surface of the labial probolae as apparently is the case in Z. *spinula* Allen & Noffsinger, 1972, Z. *acuta*, Z. *neoacuta* Allen & Noffsinger, 1972 and Z. *solata* Allen & Noffsinger, 1972 and unlike in Z. *ondontocephala* Steiner, 1938 and Z. *tridentata* that reportedly have two and three teeth per cheilorhabdion, respectively (Allen & Noffsinger, 1972). Most of the primary and the secondary measurements of Z. *spannata* sp. n. are within the range of other *Zeldia* species.

The amplification of the ITS1-5.8S-ITS2 and flanking region of the 18S and 28S genes yielded a single PCR product whose length ranged from 768 bp (*Cervidellus* sp.) to 883 bp (*Stegelleta* sp.). The amplicon length for Z. *spannata* sp. n. was 788 bp. Divergence between sequences of *Zeldia* species ranged from 67 (8.7%) to 148 (19.6%) nucleotides. Sequences of two ITS-rDNA clones of Z. *spannata* sp. n. differed in four nucleotides (0.5%) while those of Z. *punctata* from California and Senegal differed in five substitutions (0.7%).

The length of the entire alignment for cephalobids generated by ClustalX was 915 bp. Manual removal of ambiguous positions resulted in a length of 658 bp for culled alignment. Maximum parsimony analysis of the entire alignment generated three maximum parsimonious trees, used to develop a strict consensus tree (Fig. 3). The tree obtained from analysis of the culled alignment does not strongly support relationships between several clades internal to cephalobidae. Maximum likelihood (ML) analyses (GTR+I+G model) for both alignments yielded single trees with differences in branching of weakly supported clades. Tree comparison using the Kishino-Hasegawa test did not find any differences in topology generated from MP, ML trees and a tree constraining the genus *Zeldia* to be monophyletic for both alignments (p= 0.51 and p=0.06, respectively). Therefore the maximum parsimony and maximum likelihood analyses of the ITS1, 5.8S and ITS2 of rRNA gene fragments from the 13 taxa do not allow resolution of relationships of Z. *spannata* sp. n. to other *Zeldia* species or between *Zeldia* species and other clades of Cephalobidae. Maximum likelihood tests do not reject monophyly of the genus *Zeldia*.

Type locality and habitat: Z. *spannata* sp. n. was collected by Dan Bumbarger on 19th January, 2002 in the Mojave Desert at Ashley Flats near Blythe,

TABLE 1: Measurements of *Zeldia spannata* n.sp. (all primary measurements are in µm and in the form, mean ± SD (Range).

n	Holotype ♀	Paratypes 20 ♀♀
L	722	738±45 (629-859)
a	21.2	22±2.7 (14.8-25.0)
b	4.4	4.4±0.3 (3.9-5.0)
c	15.7	16.6±1.5 (14.6–19.0)
c´	2.2	2.0±0.2 (1.6-2.4)
V	63	62.9±1.4 (58.3-65.2)
Reproductive tract length (RTL)	208	195.5±18.5 (160-235)
Tail (TL)	46	44.6±2.9 (40-50)
Vulva - anus distance (VAD)	221	229.3±25.2 (179-315)
Phasmid-anus distance	17	19.4±1.9 (16-24)
Dist. from anterior end to:		
vulva	455	463.6±23.7 (401-501)
excretory pore	115	114.7±5.5 (105-126)
nerve ring	118	115.2±6.1 (103-129)
deirid	155	153.2±3.9 (150-162)
anus	676	692.9±45.5 (589-816)
Labial probolae length (LPL)	9	8.0±0.9 (7-9)
Stoma length (StL)	12	12.3±1 (10-15)
Neck length (NL)	164	169.7±5.2 (162-179)
Corpus length (CL)	100	103±4.7 (93-110)
Isthmus length (IL)	20	25.7±3.0 (20-30)
Basal bulb length (BBL)	23	20. 8±0.9 (20-23)
Pharyngeo-intestinal valve length	7	5.7±0.6 (5-7)
LPL/LRW	1	(0.7±0.1 (0.6-1.0)
StL/LRW	1.3	1.1±0.2 (0.9-1.5)
CL/ IL	5	4.1±0.6 (3.2-5)
Nerve ring/neck %	72	67.9±3.3 (60.2-72.0)
Excretory pore/neck %	67.8	67.8±3.2 (63.7-72.8)
Deirid/neck %	94.5	90.8±2.4 (85.4-94.5)
Lip region width (LRW)	9	11.5±1.1 (9-13)
Maximum body width (MBW)	34	34.3±5.1 (28-48)
Vulval body width (VBW)	32	33±5.2 (29-48)
Spermatheca body width (SBW)	45	32.6±6.5 (20-48)
Anal body width (ABW)	21	21.3±1.3 (20-26)
Vagina length (VL)	10	10.7±0.9 (9-12)
Postvulval sac length (PVS)	58	54.3±5.6 (42-65)
Spermatheca length (SpL)	50	33.0±8.9 (23-50)
Ovary length (OL)	208	235.1±33.8 (169-306)
Rectum length (RL)	21	20.7±1.4 (18-25)
TL/VAD	0.2	0.2±0.02 (0.14-0.22)
SpL/SBW	1.1	1.04±0.3 (0.5-1.5)
VL/VBW	0.3	0.3±0.06 (0.2-0.4)
PVS/VBW	1.8	1.8±0.3 (0.9-2.0)
OL/VAD	0.3	0.3±0.1 (0.1-0.3)
RL/ABW	1	1.0±0.1 (0.8-1.2)
OL /RTL	1	1.2±0.2 (1.0-1.5)
RTL as % L	28.8	26.6±2.6 (21.7-33.0)
Ovary length as % L	28.8	31.9±4.2 (23-43)
Phasmid as % of tail	37	43.8±5.1 (37–53)
Annuli from ant. end to nerve ring	39	39±3 (36-45)
Annuli from ant. end to Ex. pore	35	35±1 (33-37)
Annuli from ant. end to deirid	46	46±1 (44-49)

California, USA (N 33° 25'7", W 114° 56'05"). The sample was from sandy soil in the vicinity of a honey mesquite tree (*Prosopis glandulosa*).

Type material: Holotype slide no. 30397 and seventeen female paratypes slides (30398-30402) are deposited at the University of California Riverside Nematode Collection (UCRNC), Riverside, CA, USA. Seven paratype females are deposited at the University of California Davis Nematode Collection (UCDNC) and 5 paratypes are at Kenyatta University Herbarium Unit, Nairobi, Kenya.

DISCUSSION

Z. spannata sp. n. expresses key morphological characters of the genus including lips paired as asymmetrical triangles and a single guard process in each primary axil, but it also includes a range of features more typically associated with other genera of Cephalobidae. Indeed, Abolafia and Peña-Santiago (2003) noted that *Zeldia* has features intermediate between *Chiloplacus* and *Nothacrobeles* and this continuum is extended by *Z. spannata* sp. n. Specifically, the high bifurcate probolae of *Z. spannata* sp. n. resemble those of certain *Chiloplacus* or *Stegelleta* Thorne, 1938. The overall asymmetry and triangular shape and pattern of the lips and presence of tines are similar to those of certain *Nothacrobeles*. Unlike the probolae of *Nothacrobeles*, those of *Z. spannata* sp. n. lack a prominent basal ridge. The five lateral incisures and tessellation of *Z. spannata* sp. n. while found in other Cephalobidae, have not been previously reported in *Zeldia*. As is the case throughout the family, discovery of new species, growing insight into morphology and phylogenetic patterns emerging from molecular studies raise new questions about the reliability of some classical morphological characters for defining genera including *Zeldia*. Mapping morphological characters on preliminary molecular phylogenetic analyses suggest that the feature of "lacking guard process or one guard process" may define a major clade placing *Zeldia* in a group distinct from taxa with two guard processes including *Nothacrobeles* (Nadler *et al.*, submitted). However, many classical characters are highly convergent among clades. The ITS tree, while excluding *Nothacrobeles* as a sister to *Zeldia* spp., does resolve relationships between several cephalobid clades. This includes supporting a sister relationship between a species of *Nothacrobeles* Allen and Noffsinger, 1971 and *Stegelletina* Andrassy, 1984 as well as between *Heterocephalobellus* Rashid et al., 1985 and *Metacrobeles* Loof, 1962. It is noteworthy that outgroups for this ITS tree were determined based on the more comprehesive phylogeny based on the large subunit rDNA for Cephalobina Nadler *et al.* (submitted).

Sequences of ITS are frequently used in delimiting nematode species and have been shown to be highly conserved among geographically diverse populations of the same species (Anderson *et al.*, 1998). In the present study populations of *Z. punctata* from Senegal and California varied by only five nucleotides (0.7%), consistent with intraspecific variation demonstrated for other species (Subbotin *et al.*, 2003). *Z. spannata* sp. n. and an undescribed *Zeldia* species (JB 118) are identified as *Zeldia* on the basis of morphology including a single guard process in the primary axil. A high level of sequence divergence and substitution autapomorphies further strengthen the status of these two as unique species. Whereas phylogenetic analysis is consistent with JB 118 as a sister to the type species of *Zeldia*, the position of *Z. spannata* sp. n. is unresolved (Fig. 3). Sequence data (ITS), while useful for diagnosis of *Zeldia*, is not adequately informative for resolution of phylogenetic relationships among these species. It is noteworthy, however, that maximum likelihood tests do not reject monophyly of the genus *Zeldia*. Additional genes and taxa may further resolve these relationships.

In addition to diagnostic characters, there are a number of additional features in *Z. spannata* n. sp that are worth noting. The "tooth-like" process associated with the lining of each cheilostom in *Z. spannata* sp. n. may occur throughout the genus, apparently having been first described for *Z. ondontocephala* and later included in the generic diagnosis (Allen & Noffsinger, 1972). We note that similar structures at the anterior end of the stoma lining may occur throughout Cephalobidae, having been observed in a range of representatives with improved SEM resolution of the buccal region (De Ley *et al.*, 1999; Poiras *et al.*, 2002; Chiu *et al*, 2002; Mundo-Ocampo *et al.*, 2002; Taylor *et al.*, 2004). Among taxa, these stomatal processes are diverse in number and overall structure and with further sampling and analysis they may prove to have phylogenetic significance.

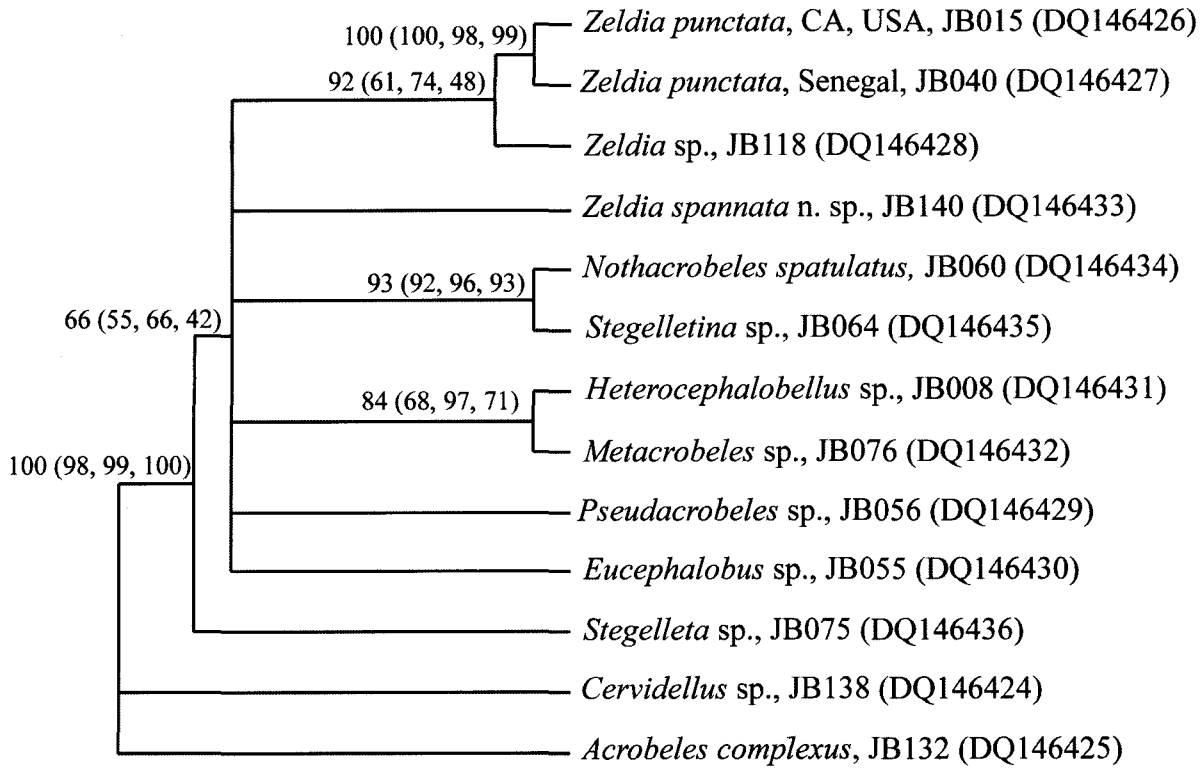

FIGURE 3. Consensus of the three equally maximum parsimonious trees generated from analyses of thirteen sequences of cephalobid nematodes (number of informative characters = 325; tree length = 1342; CI excluding uninformative characters = 0.5502; HI excluding uninformative characters = 0.4498). Bootstrap values are given for appropriate clades (in brackets for MP tree from the culled alignment, ML tree from entire alignment and ML tree from culled alignment).

Z. spannata sp. n. lacks punctations, a feature that is variable and undoubtedly phylogenetically convergent among *Zeldia* species as well as among individuals of a given species population (Allen & Noffsinger, 1972). The tessellation of *Z. spannata* sp. n., while not previously reported in *Zeldia*, is widespread and convergent throughout the family including some species of *Acrobeles* von Linstow, 1877, *Acromoldavicus* Nesterov, 1970, *Cervidellus* Thorne, 1937, *Metacrobeles* Loof, 1962, *Nothacrobeles*, *Paracrobeles* Heyns, 1968, *Placodira* Thorne, 1937, *Stegelleta* Thorne, 1938 and *Stegelletina*.

The female reproductive tract of *Z. spannata* sp. n. is typical for Cephalobidae and as in some other taxa, varies among individuals with respect to overall length and development of the spermatheca. In some specimens the ovary is particularly long and this added length is associated with a secondary flexure at its distal end. There are two types of spermatheca in *Z. spannata* sp. n., one type being a sac-like cluster of cells and the other being more clearly defined and attenuated. In some taxa, such differences might suggest that the more developed spermatheca is a response to insemination, but in the present case the diverse morphology was observed in females from cultures in which no males were found. Whereas we anticipate that *Z. spannata* sp. n. is parthenogenic, differences in morphology could suggest an alternate facultative mode of reproduction.

Expanded analyses, including broader sampling of additional species, is needed to further test monophyly of *Zeldia* and additional extant genera, as

well as to test morphological synapomophies relative to characters that are highly convergent or otherwise not useful for defining phylogenetic taxa. However, based on present morphological and molecular understanding, a revised diagnosis is proposed to more broadly define *Zeldia* to accommodate *Z. spannata* sp. n.

Zeldia Thorne, 1937

Diagnosis (emended): Cephalobidae: Body varying from 0.5 – 0.9 mm. Cuticle with large annuli and with or without punctations or tessellation. Lateral field with 3-5 lines, outer lines sometimes crenate. Lateral field with or without areolation. Labial probolae low and rounded to elongate and shallowly to deeply bifurcate at one level; without tines. Lips flap-like asymmetrical triangular pairs, with or without tines. Deep primary axils each with a single triangular or elongate sclerotized guard process. Shallow secondary axils. Lining of cheilostom without or with 1-3 tooth-like processes. Stegostom lining with distinct set of sclerotizations. Pharyngeal corpus long and cylindrical, isthmus short and posterior bulb with distinct transverse valves. Deirid variable in position between excretory pore and anterior region of basal bulb. Female reproductive system cephalobid; distal end of the ovary sometimes with double flexure. Spermatheca and postvulvar sac present and variable in length. Phasmid at about 50% of tail length. Female tail short, conoid to elongate conoid, ventrally arcuate/sigmoid conoid; terminus acute, subacute or rounded. Males rare or unknown in most species.

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