

## A SECOND CYST-FORMING NEMATODE PARASITE OF BARLEY (*HORDEUM VULGARE* L. VAR. *ESMERALDA*) FROM MEXICO

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### ABSTRACT

Cid del Prado, V. I. and B. L. Miranda. 2008. A second cyst-forming nematode parasite of barley (*Hordeum vulgare* L. var. *Emeralda*) from Mexico. *Nematropica* 38:105-114.

A new species of cyst nematode, *Cactodera rosae* n.sp., is described and illustrated from specimens obtained from roots of barley, *Hordeum vulgare* L. variety *Emeralda*, and associated soil, from San Juan Ixtimaco, Apan Hidalgo State. *Cactodera rosae* n.sp. cysts are characterized by their lemon shape, dark brown to black color, 460-840 µm length, with a length / width ratio of 1.2-2.1, strongly striated pattern on the cuticle, a prominent vulval cone with circumfenestrate vulva, and an anus located 25-60 µm from the fenestra in a depression in the cuticle. Egg surfaces bear conspicuous punctations. The second stage juvenile is characterized by the presence of 4-5 lip annulations on the head, a strong stylet 16-26 µm long with rounded stylet knobs, a tail 31-68 µm long and 6-14 µm wide with a hyaline portion of 11.2-28 µm in length, and lateral fields with four incisures and incomplete areolation. A key to the species of *Cactodera* is presented.

*Key words:* Barley, *Cactodera rosae*, cyst-forming nematode, Heteroderinae, new species, taxonomy.

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### RESUMEN

Cid del Prado, V. I. and B. L. Miranda. 2008. Nemátodo quiste parásito secundario de la cebada (*Hordeum vulgare* L. var. *Emeralda*) de México. *Nematropica* 38:105-114.

Se describe e ilustra una nueva especie, *Cactodera rosae* n.sp. con base en el estudio de especímenes obtenidos de suelo y raíces del cultivo de cebada (*Hordeum vulgare* L.) variedad *Emeralda* de San Juan Ixtimaco, Apan Estado de Hidalgo. *Cactodera rosae* n.sp. se caracteriza por la forma de lima del quiste, su color café oscuro a negro, de 460-840 µm de largo, y prominente cono vulvar con circumfenestra terminal; el ano se encuentra a 25-60 µm de la fenestra en una conspicua depresión cuticular; la cutícula es fuertemente estriada; la relación L/A es de 1.2-2.1; el corion de los huevos tiene puntuaciones conspicuas; el segundo estadio juvenil tiene cuatro a cinco anillos en la cabeza, su estilete mide 16-26 µm de largo y los nódulos del estilete son redondeados; el campo lateral tiene cuatro incisuras con aerolación incompleta; la cola mide 31-68 µm de largo y 6-14 µm de ancho y una porción hialina de 11.2-28 µm. Se incluye una clave de identificación de especies.

*Palabras clave:* *Cactodera rosae*, cebada, Heteroderinae, nematodos formadores de quistes, nueva especie, taxonomía.

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### INTRODUCTION

During our 2001-2002 research projects, a nematode population with lemon shaped cysts was isolated from soil and the roots of barley *Hordeum vulgare* L. cultivar *Emeralda*, in the High Valleys

near San Juan Ixtimaco, Apan County, Hidalgo State, México. Preliminary morphological examination of cysts, second stage juveniles (J2) and eggs indicated that the population represented a species of *Cactodera* Kral and Kral, 1978; and was very closely related to *C. milleri* and *C. amaranthi*

morphologically. This population has dark brown to black colored cysts with a prominent circumfenestrate vulva cone. It is different from *Cactodera galinsogae* Tovar S. and Col. 2003, which has smaller light brown cysts with an almost inconspicuous vulval cone. *Cactodera galinsogae* was also found parasitizing barley and weed species including *Galinsoga parviflora* Cav., *Bidens odorata* Cav., and *Avena fatua* L.

The objective of this study was to conduct a detailed examination of the morphology and morphometric observations of cysts, J2s and eggs to determine species present. A revised key to the genus *Cactodera* is provided, which includes a description of a new species, *Cactodera rosae* n.sp.

#### MATERIALS AND METHODS

Soil and root samples were collected from a barley field. Centrifugal-flotation and the Fenwick can techniques were used to extract nematodes from soil and roots (Fortuner, 1991; Shepherd, 1986). Nematodes were relaxed and killed in hot water and then fixed in 4% formaldehyde. The specimens were later dehydrated with glycerin dilutions (Seinhorst, 1959) and mounted in 100% glycerin on Cobb slides, using the paraffin wax ring method (De Maeseneer and d'Herde, 1963). Measurements and drawings were made with a camera lucida mounted on an American Optical compound microscope.

For scanning electron microscopy, the specimens were treated according to Eisenback (1991). The samples were fixed in 2% glutaraldehyde and post-fixed with 2% osmium tetroxide overnight. Afterwards, the specimens were washed with pH 7.0 phosphate buffer and dehydrated in an alcohol series (10 to 100%). The specimens were critical point dried and coated with gold-palladium before observation under the scanning electron microscope at 15 kv.

#### RESULTS

##### *Cactodera rosae* n.sp.

Figs. 1 A-F; 2 A-D; 3 A-E; 4 A-D

(Table 1)

Cyst (n = 40): L including neck = 460-840 ( $654 \pm 26.6$ )  $\mu\text{m}$ ; body width = 280-560 ( $433 \pm 22$ )  $\mu\text{m}$ ; a: 1.2-2.1 ( $1.5 \pm 0.1$ ); neck length: 48-120 ( $78.2 \pm 4.8$ )  $\mu\text{m}$ ; circumfenestra diameter = 10-21 ( $17.6 \pm 1.0$ )  $\mu\text{m}$ .

Most of the cysts are big and lemon shaped; the smaller ones are oval to spherical in shape. The color varies from dark brown to black with some translucent brown. The neck is curved and oriented posteriorly; the vulval cone is very conspicuous with a rounded end. The cuticular pattern varies with the age and size of the cyst; transversal lines of the mature cyst form an irregular net around of the cyst and in the posterior part are continuous with wavy lines. The vulval cone has parallel vertical lines; in some cysts small denticles are present around the fenestra. The anus is conspicuous, and is found in a clear depression, circular or oval in shape, and separated from the edge of the circumfenestra by 25-60 ( $27.5 \pm 1.02$ )  $\mu\text{m}$ .

Young female: not observed.

Male: not observed.

Egg (n = 55): L = 92-156 ( $109 \pm 3.0$ )  $\mu\text{m}$ ; width = 37-62 ( $48 \pm 1.7$ )  $\mu\text{m}$ ; L/ W ratio = 1.7-2.7 ( $2.3 \pm 0.1$ ).

Eggs are oval in shape with rounded ends. The eggshell is ornamented with fine punctations over its entire surface.

Second stage juvenile (J2) (n = 30) L = 348-472 ( $397 \pm 10.7$ )  $\mu\text{m}$ ; a = 15-26.5 ( $21.3 \pm 1.1$ ); tail 31-68 ( $39.2 \pm 3.2$ )  $\mu\text{m}$ ; c = 5.8-14.4 ( $10.5 \pm 0.7$ ); c' = 2.5-6.8 ( $3.8 \pm 0.5$ ); anterior end to excretory pore = 76-92 ( $87 \pm 12.0$ )  $\mu\text{m}$ ; stylet length = 16-26 ( $20 \pm 1.0$ )  $\mu\text{m}$

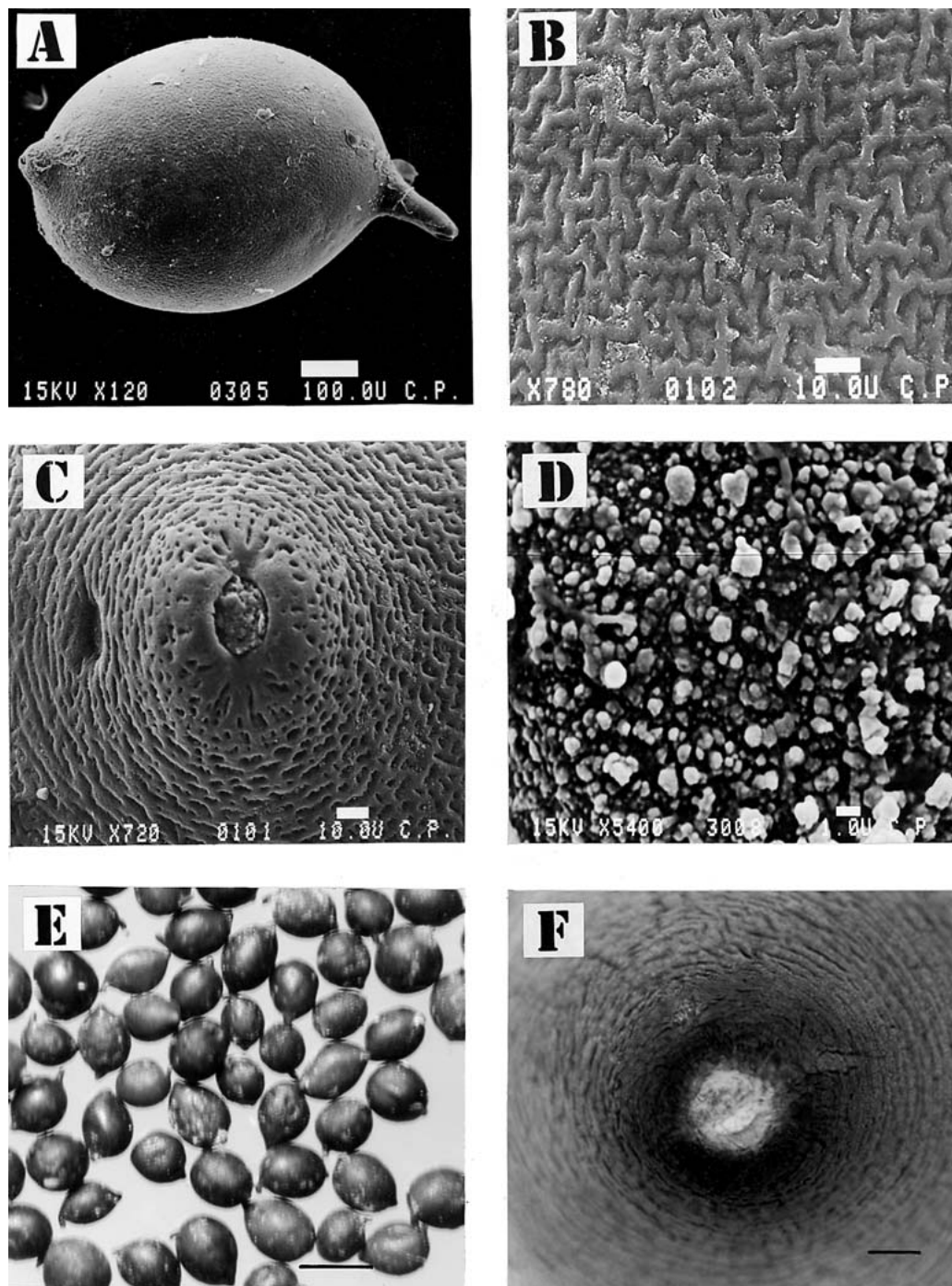


Fig. 1. *Cactodera rosae* n.sp. Female A-F. A. Scanning electron micrographs A-D. A) Entire body. B) Cuticular pattern C) Fenestra and anus view. D) Corion ornamentation. E) Shape cysts. F) Fenestra view and anus.

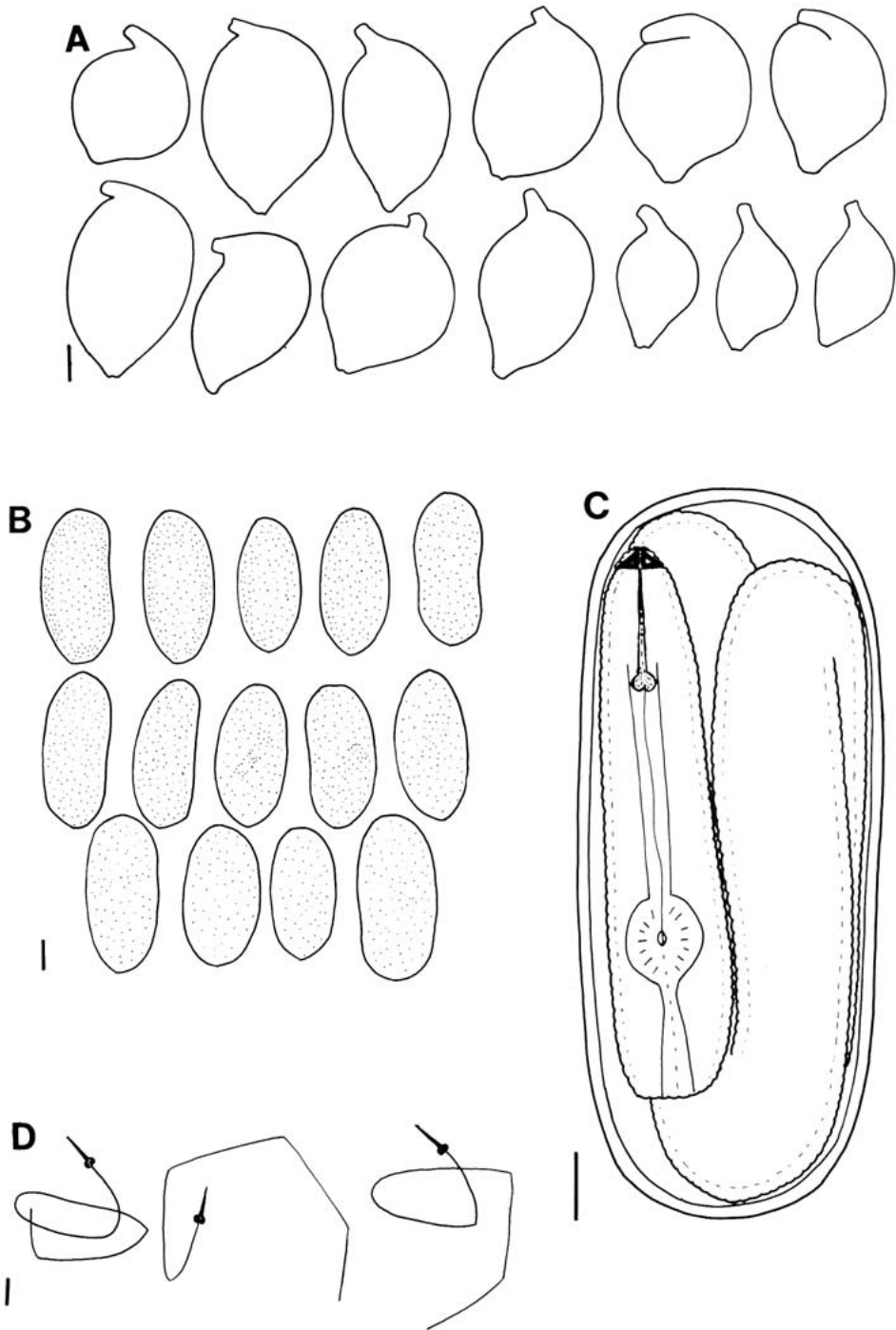


Fig. 2. *Cactodera rosae* n.sp. Female A) Variation cyst shape. B) Eggs. Second stage juvenile. C) Inside egg. D) Twisty body variation into egg.

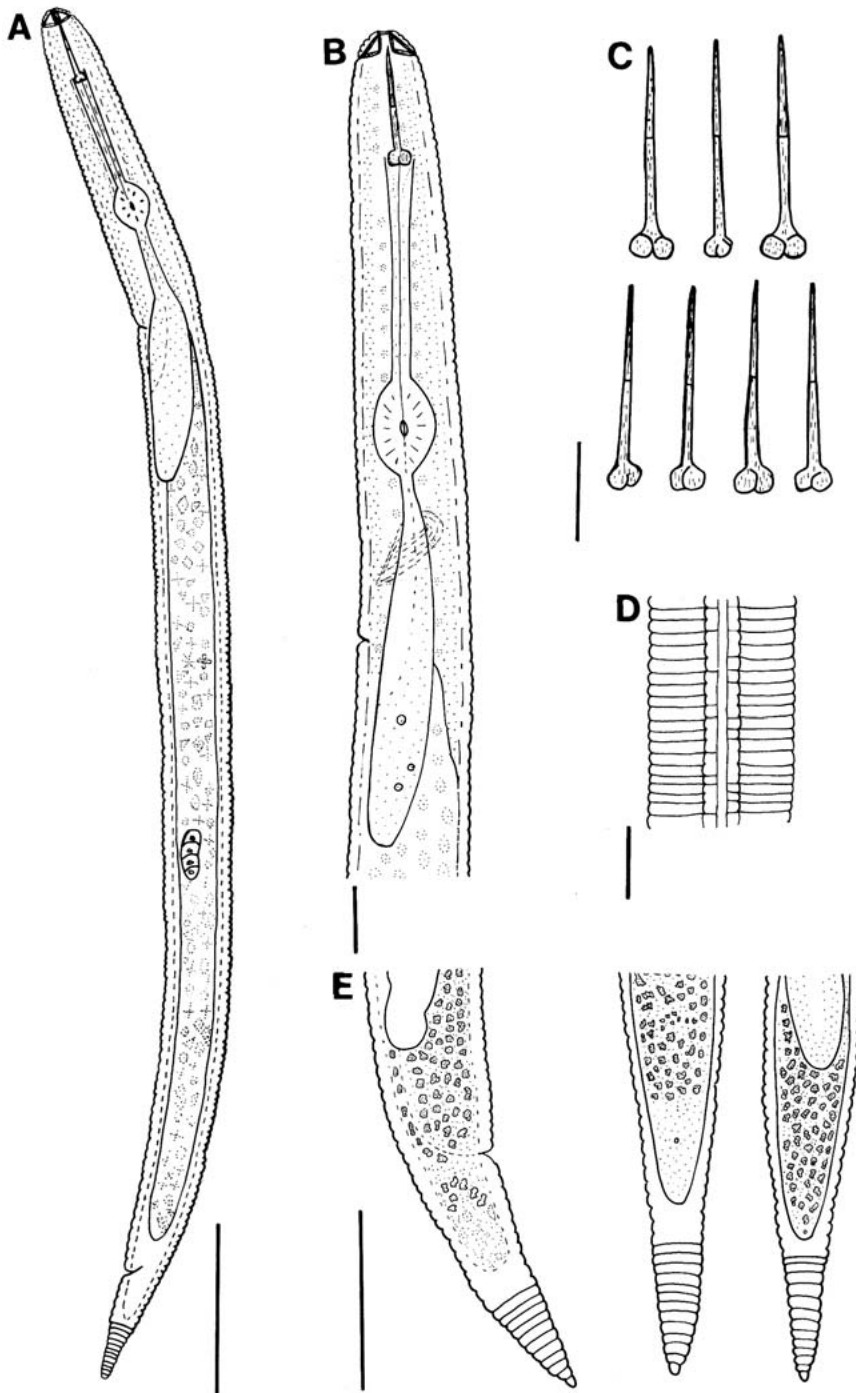


Fig. 3. *Cactodera rosae* n.sp. Second stage juvenile A-E. A) Entire body. B) Anterior end body. C) Stylet shape. D) Lateral field view. E) Tails.

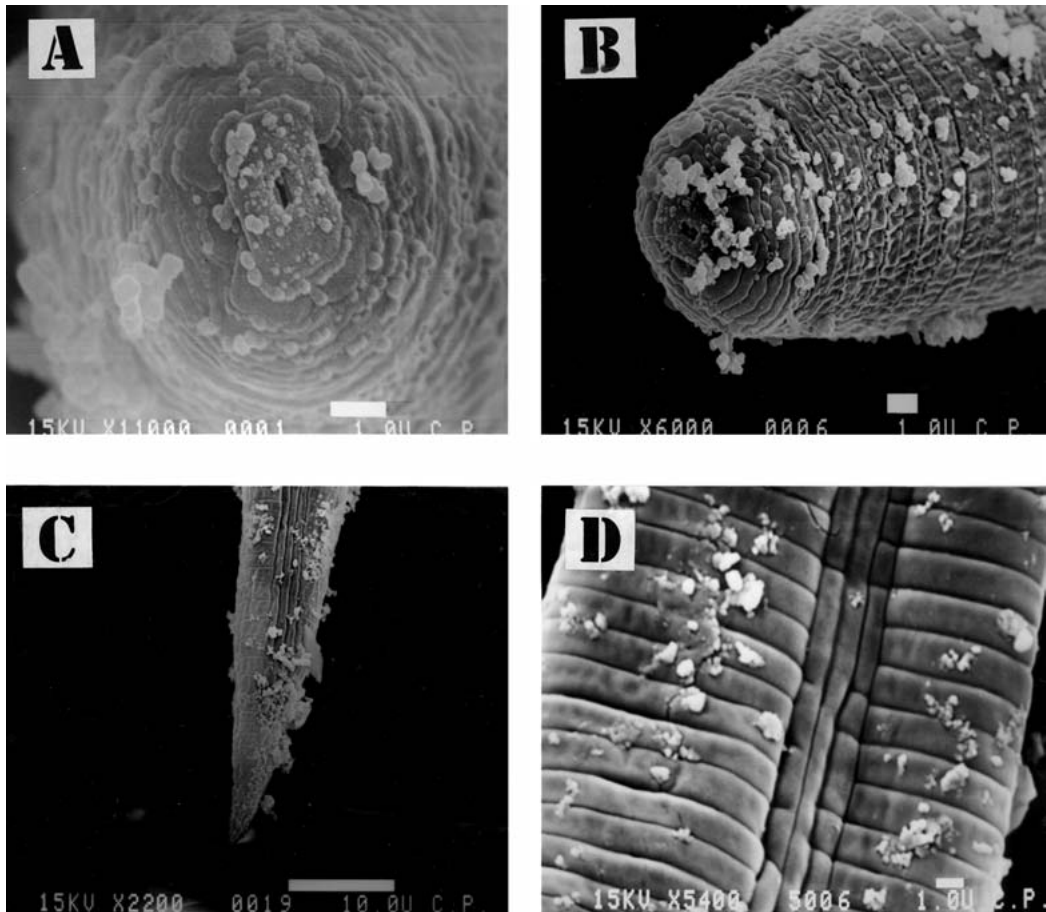


Fig. 4. *Cactodera rosae* n.sp. Second stage juvenile. Scanning electron micrographs. A) Face view. B) Anterior end. C) Tip tail. D) Lateral field view.

The body of the second stage juvenile is slightly curved, with the anterior end rounded and the posterior end with an acute terminus; head with four to five annules, separated from the body by a constriction; labial disc elongated rectangular shape, with six pseudolips; the ventral and dorsal rounded and the lateral reduced. The knobs of the stylet are almost round with the anterior end concave and are  $1.6-4.0$  ( $2.6 \pm 4.4$ )  $\mu\text{m}$  long and  $3.6-5.6$  ( $4.4 \pm 0.3$ )  $\mu\text{m}$  wide; metacarpus oblong,  $8-12$  ( $11 \pm 1.2$ )  $\mu\text{m}$  long and  $5.6-10.8$  ( $7.9 \pm 1.2$ )  $\mu\text{m}$

wide. Genital primordium is oval in shape,  $11.2-16$  ( $13.8 \pm 4.1$ )  $\mu\text{m}$  long and  $6-8$  ( $7.3 \pm 1.5$ )  $\mu\text{m}$  wide. Lateral field has four incisures having incomplete areolation. The tail has a conical end  $6-14$  ( $10.7 \pm 0.8$ )  $\mu\text{m}$  long, hyaline portion  $4.0-8.0$  ( $6.3 \pm 0.6$ )  $\mu\text{m}$  long.

*Type locality and habitat:*

*Cactodera rosae* n.sp. was collected from soil around roots of barley in San Juan Ixtimaco, Municipio de Apan, Hidalgo State,

Table 1. Morphometric comparison of eggs, second state juveniles and cysts among *Cactodera amaranthi*, *C. milleri* and *C. rosae*. (Measurements in  $\mu\text{m}$  except indexes and ratio L/W).

Character	<i>C. amaranthi</i> (Stoyanov,1972)	<i>C. milleri</i> (Graney & Bird, 1990)	<i>C. rosae</i> n. sp
<b>Cysts</b>			
L with neck	621 (297-841)	700 (550-849)	654 (460-840)
width	416 (242-485)	506 (419-598)	432 (280-560)
L without neck	546 (263-713)	632 (514-730)	576 (400-760)
Neck	75 (34-128)	68 (56-119)	78 (48-120)
Relation L/W	1.5 (1.2-1.7)	1.4 (1.2-1.6)	1.5 (1.2-2.1)
Fenestra diameter	13.2	19 (14-22)	17.6 (10-21)
<b>Eggs w/larvae</b>			
L	97 (86-108)	102 (93-114)	109 (92-156)
width	38 (36-44)	44 (38-51)	48 (37-62)
Relation L/W	2.4 (2.2-2.4)	2.4 (1.9-2.8)	2.3 (1.7-2.7)
Surface	Smooth	Punctated	Punctated
<b>Second stage juvenile (J2)</b>			
L	409 (372-447)	246 (370-479)	397 (348-472)
Body width	21 (17-24)	22 (19.5-25)	19 (16-25)
Stylet length	23 (22-24)	22 (21-23)	20 (16-26)
Stylet knobs length	2.5	—	2.6 (1.6-4)
Stylet knobs width	4	4.6 (3.9-4.9)	4.4 (3.6-5.6)
Excretory pore to anterior end	96 (88-107) 100 (90-111)	87 (76-92)	
Tail length	39 (34-47) 43 (37-49)	39 (31-68)	
Tail width	13.5 (13-15.6) 13.3 (12.2-15.4)	10.7 (6-14)	
Hyaline portion length	-18.2 (14.6-22)	18.8 (11.2-28)	
<b>Indices</b>			
a	22 (20-24) 19 (17-21)	21 (15-27)	
c	-10 (9-10.8)	10.5 (5.8-14)	
ć	2.9 (2.3-3.2) 3.2 (2.8-3.6)	3.8 (2.5-6.8)	

México, latitude North 19°39'.467; longitude West W 98°26'.829, and 2,562 m above sea level.

*Type specimens:*

Accession numbers of type specimens deposited in the University of California Davis Nematode Collection (UCDNC) are:

holotype female (cyst), paratype females (cysts), and paratype juveniles (J2). Other type material is deposited in the University of California Riverside Nematode Collection (UCRNC), Wageningen University Nematode Collection (WUNC), and the Colegio de Postgraduados, México (CPNC).

*Etymology*

This species is named in honor of my mother, the late Mrs. Rosa Vera Díaz.

DIAGNOSIS

*Cactodera rosae* n.sp. is characterized their lemon shaped cysts that are dark brown to black in color, a prominent circumfenestrate vulval cone, strongly striated cuticle, and with the anus found in a conspicuous cuticle depression; the eggs bear punctations; the J2 have a short stylet 16-26 µm in length and the size of the J2 body is 400 (348-472) µm long.

DISCUSSION

Of the species of *Cactodera* with lemon shaped cysts and with the eggshell bearing punctations, *Cactodera rosae* n.sp. differs distinctly from *C. thornei* (Golden and Raski, 1977) Mulvey and Golden, 1983, *C. eremica* Baldwin and Bell, 1985 and *C. cacti* (Filipjev and Schuurmans-Stekhoven, 1941) Krall and Krall, 1978, in the size of the stylet, and J2 body length.

*Cactodera rosae* n.sp. is similar to *C. milleri* Graney and Bird 1990, in the color and cuticular pattern of the cysts and in the ornamentation of the eggshell; however, it differs by the size of the cysts (654 µm *C. rosae* and 700 µm *C. milleri*), and the J2 body length, (397 µm *C. rosae* and 246 µm *C. milleri*). *Cactodera rosae* n.sp. is like *C. amaranthi* (Stoyanov, 1972) (Golden and Raski, 1990) in shape and color of the cyst and J2 stylet length and tail shape. *Cactodera rosae* n.sp. cyst mean size is larger (654 µm) compared to *C. amaranthi* (621 µm). Second-stage juvenile body and stylet length are smaller for *C. rosae* as compared to *C. amaranthi* with an average body and stylet length of 397 and 20µm and 409 and 23 µm, respectively. There is an incomplete areolation in the *C. rosae* J2 lateral field while *C. amaranthi* is smooth. The surface of the *C. rosae* eggshell is ornamented. *C. amaranthi* eggshell are smooth.

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**Key to the genus *Cactodera* Krall and Krall, 1978**

- 1a. Lemon shaped (citriform) cysts, length × 2 width, brown dark in color, relation L/A less of 2.5; egg corion without punctuations . . . . . *C. estonica*
- 1b. Lemon shape or rounded cysts, length less than 2× width, relation L/A 1.1-1; eggshell smooth or with punctuations . . . . . 2
- 2a. Eggshell corion with punctuations . . . . . 3
- 2b. Eggshell corion without punctuations . . . . . 7
- 3a. Lemon shaped cysts . . . . . 4
- 3b. Rounded cysts. . . . . 5
- 4a. J2 stylet length 27 µm; J2 body length 554 µm; tail > 55 µm long . . . . . *C. thornei*
- 4b. J2 stylet length 26.5 µm; J2 body length 400 µm; L/W ratio in cysts 1.5 . . . . . *C. eremica*
- 4c. J2 stylet length 20 µm; J2 body length 397 (348-472) µm; J2 tail length < 40 µm . . . *C. rosae*
- 4d. J2 stylet length > 20 µm; J2 body length > 400 µm; J2 tail length > 40 µm. . . . . 4e



- 4e. J2 stylet length 21  $\mu\text{m}$ ; J2 body length 426  $\mu\text{m}$ ; citriform cyst; L/W ratio 1.4 . . . *C. milleri*
- 4f. J2 stylet length 23  $\mu\text{m}$ ; J2 body length 460  $\mu\text{m}$ ; lemon shaped cysts prominent vulva cone; L/W ratio 1.1 . . . . . *C. cacti*
- 5a. Rounded cysts with small vulval cone; mean cyst length 523  $\mu\text{m}$ ; J2 body length 401  $\mu\text{m}$ ; tail length 36.5  $\mu\text{m}$  . . . . . *C. galinsogae*
- 5b. Rounded cysts with prominent vulva-cone . . . . . 6
- 6a. Average cyst length 459  $\mu\text{m}$ , brown in color; J2 tail length 40.4  $\mu\text{m}$ ; hyaline caudal portion length 21.1  $\mu\text{m}$  . . . . . *C. evansi*
- 6b. Rounded cyst, mean length 576  $\mu\text{m}$ , dark brown color; J2 tail length 53.2  $\mu\text{m}$ ; hyaline caudal portion length averages 26.7  $\mu\text{m}$  . . . . . *C. johanseni*
- 7a. High salinity habitat of cyst and J2. . . . . *C. salina*
- 7b. Habitat of cyst and J2 not saline . . . . . 8
- 8a. J2 length < 400  $\mu\text{m}$ ; J2 tail length 39  $\mu\text{m}$ ; hyaline caudal length 15  $\mu\text{m}$  . . . *C. amaranthi*
- 8b. J2 length > 400  $\mu\text{m}$ ; hyaline caudal portion length > 20  $\mu\text{m}$ ; cyst lemon shaped—round . . . . . 9
- 9a. Rounded cysts, mean length > 750  $\mu\text{m}$  . . . . . *C. betulae*
- 9b. Lemon shaped cyst . . . . . 10
- 10a. Cyst length and width < 600 and 350  $\mu\text{m}$ ; J2 DGO = 5.3  $\mu\text{m}$  behind stylet knobs . . . . . *C. weissii*
- 10b. Cyst length and width > 600  $\mu\text{m}$  an 417  $\mu\text{m}$ ; DGO in J2 = 3.0  $\mu\text{m}$  . . . . . *C. acnidae*

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