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Cactodera salina n. sp. from the Estuary Plant, *Salicornia bigelovii*, in Sonora, Mexico[1]

J. G. Baldwin,[2] M. Mundo-Ocampo,[2] and M. A. McClure[3]

Abstract: Cactodera salina n. sp. (Heteroderinae) is described from roots of the estuary plant *Salicornia bigelovii* (Chenopodiaceae), in Puerto Peñasco, Sonora, Mexico, at the northern tip of the Sea of Cortez. The halophyte host is grown experimentally for oilseed in plots flooded daily with seawater. Infected plants appear to be adversely affected by *C. salina* relative to plants in noninfested plots. *Cactodera salina* extends the morphological limits of the genus. Females and cysts have a very small or absent terminal cone and deep cuticular folds in a zigzag pattern more typical of *Heterodera* and *Globodera* than of *Cactodera* spp. Many *Cactodera spp.* have a tuberculate egg surface, whereas *C. salina* shares the character of a smooth egg with *C. amaranthi, C. weissi,* and *C. acnidae.* Only *C. milleri* and *C. acnidae* have larger cysts than *C. salina.* Face patterns of males and second-stage juveniles, as viewed with scanning electron microscopy, reveal the full complement of six lip sectors as in other *Cactodera spp.* Circumfenestrae of *C. salina* are typical for the genus.

Key words: Cactodera salina, cyst nematodes, halophyte, Heteroderinae, nematode, new species, *Salicornia bigelovii,* scanning electron microscopy, Sea of Cortez, taxonomy.

Cactodera Krall and Krall, 1978 (Heteroderinae Filipjev and Schuurmans Stekhoven, sensu Luc et al., 1988) includes nine species of cyst-forming nematodes that are distinctive for their small, circumfenestrate posterior cone (Baldwin and Mundo-Ocampo, 1991). Hosts include species of *Euphorbia, Montia, Polygonum, Atriplex, Chenopodium,* and certain grasses. *Cactodera betulae* (Hirschmann & Riggs) Krall & Krall is a parasite of *Betula* and other unrelated hosts, and *C. chaubattia* Gupta & Edward is reported from soil around *Malus* (Gupta and Edward, 1973). The affinity of these two species to other *Cactodera* spp. is uncertain (Baldwin and Mundo-Ocampo, 1991). Krall and Krall (1978) suggested that the type species *C. cacti,* which is specifically associated with cactus, originated in Mexico.

Goodfriend (1997) examined the nematode community in the rhizosphere of *Salicornia bigelovii* Torr. (Chenopodiaceae), a halophyte being evaluated as an oilseed crop by the Centro de Estudios de Desiertos y Oceanos (CEDO) in Puerto Peñasco, Sonora, Mexico, at the northern tip of the Sea of Cortez. This species, native to Mexico, is grown in experimental plots with direct seawater irrigation and a salinity of 38 to 42 parts per thousand (Glenn et al., 1991). Goodfriend was assisted in her study by M. A. McClure, who discovered juveniles of cyst nematodes from soil samples extracted in a mist chamber. Further investigation revealed mature cysts, and these, along with specimens of other stages, were sent to J. G. Baldwin for identification. Cyst nematodes typically do not inhabit highly saline estuary habitats, but recently *Heterodera litoralis* Wouts & Sturhan was described from a similar habitat and host, *Sarcocornia quinqueflora* (Ung.-Sternb.) A. J. Scott in New Zealand (Wouts and Sturhan, 1996). Based on their unique morphology, the cyst nematodes from *S. bigelovii* in Sonora, Mexico, are herein described as a new, tenth species of *Cactodera.* The specific epithet of *salina* is derived from the Latin *salinus,* which means salty, and refers to the highly saline-type locality.

Materials and Methods

Females, cysts, males, and second-stage juveniles (J2) were collected from experimental plots at the type locality by extraction of sand and silt with Baermann funnels at CEDO. Young J2 were collected from funnels rather than forced from eggs. Speci-

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[2] Professor and Staff Research Associate, Department of Nematology, University of California, Riverside, CA 92521.
[3] Department of Plant Pathology, University of Arizona, Tucson, AZ 85721.
E-mail: james.baldwin@ucr.edu
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mens were killed on site with heat just to the point of becoming straight, and then were fixed in 5% formalin. Attempts were made to establish cultures on the type host in a greenhouse at the University of Arizona, Tucson, but few persisted beyond 1 year; subsequent attempts to establish greenhouse cultures were equally unsuccessful. At the University of California, Riverside (UCR), specimens were slowly infiltrated with glycerin for light microscope (LM) examination. Specimens were sensitive and sometimes collapsed in response to standard processing procedures; this sensitivity may have been the result of osmolarity changes. Specimens were infiltrated with glycerin or critical point-dried; they were sputter-coated with 20 nm gold palladium for scanning electron microscopy (SEM) with a JEOL 35C at 15kV as previously reported (Sher and Bell, 1975; Othman and Baldwin, 1985). Illustrations were prepared from digitized camera lucida drawings using Illustrator 7.0 software (Adobe Systems, Mountain View, CA). All measurements are in micrometers (μm), unless otherwise specified, and are given as the range followed by the mean and standard error in parentheses. Because the divergence of *C. betulae* and *C. chaubattia* from other *Cactodera* is so great, they were excluded from comparisons in this study.

Systematics

Cactodera salina n. sp.

Description

Holotype (female in hard glycerin jelly): L (length including neck and head) = 446; width = 182; neck length = 124; stylet length = 24.4; DGO (distance from dorsal gland orifice to base of stylet) = 2.0; excretory pore from anterior end = 114; vulva-anus distance (lateral view) = 33.2; vulva length = 12.4; thickness of cuticle = 6.1; a (length/width) = 2.5; m (length of stylet cone/total stylet length) = 4.6; O (DGO/stylet length) = 0.1; excretory pore (excretory pore to head end/L %) = 26.

Females (n = 6): L (including "neck") = 336–515 (mean 447, standard error, 28.3); width = 149–272 (194.7, 19.7); neck length = 95–178 (128.7, 12.1); stylet length = 23.5–24.4 (23.8, 0.2); DGO = 1.0–2.0 (1.6, 0.16); excretory pore from anterior end = 84–101 (92.6, 3.2); vulva-anus distance (lateral view) = 27.5–40.8 (36.9, 2.6); vulva length (n = 6 from cone mounts) = 10–12.7 (11.2, 0.5); thickness of cuticle = 6.6–9.2 (8.2, 0.5); a = 1.9–3.4 (2.4, 0.23); O = 4.3–8.7 (6.8, 0.7).

Body asymmetrical and dorsally curved with no cone in younger individuals, oval or nearly spherical with a minute posterior protuberance in large specimens (Figs. 1B,C;3B). Neck set off and usually reflexed ventrally. Young females pearly white, becoming increasingly opaque with age and following fixation. Cuticle with a pattern of deep zigzag folds; anterior to the excretory pore a gradual transition to wavy striations and finally a relatively smooth head region. Anterior to the excretory pore, a dense material covering the head and neck region. Excretory pore slightly posterior to level of median bulb and esophageal gland region elongate. Stylet slightly curved dorsally (Fig. 1A). Ovaries paired. Subcrystalline layer not observed.

Males (n = 15): L = 906–1118 (1001, 21); width = 27– 31 (28.7, 0.37); stylet = 24–28 (25.8, 0.35); DGO = 2.0– 3.1 (2.48, 0.18) esophagus length = 142–179 (161.2, 7.09); excretory pore from anterior end = 108–133 (117.8, 2.68); testis length = 415–565 (489, 29.8); spicule length (n = 1) = 40.8; gubernaculum length (n = 1) = 13.3; a = 31.7–37.8 (34.9, 0.7); b = 4.7–11.2 (8.0, 0.9); b′ = 5.5–7.1 (6.3, 0.28); O = 7.7–12.5 (9.9, 0.8); T (testis length/L) % = 39.9–55.5 (48.6, 2.84); excretory pore % = 10.5–13.2 (11.8, 0.28).

Body vermiform with little anterior or posterior tapering; posterior end of heat-killed specimens with a nearly 90° twist. Head region slightly set off with about 4–7 discontinuous annulations (Figs. 2A,5B). Labial disc elevated; in SEM, lip sectors and labial disc together forming a square; lip sectors highly irregular in pattern among individuals but generally lateral lips fused with adjacent submedial sectors (Fig. 5B). Lateral sectors much smaller than submedial sectors. Lateral field with four incisures, outer ridges

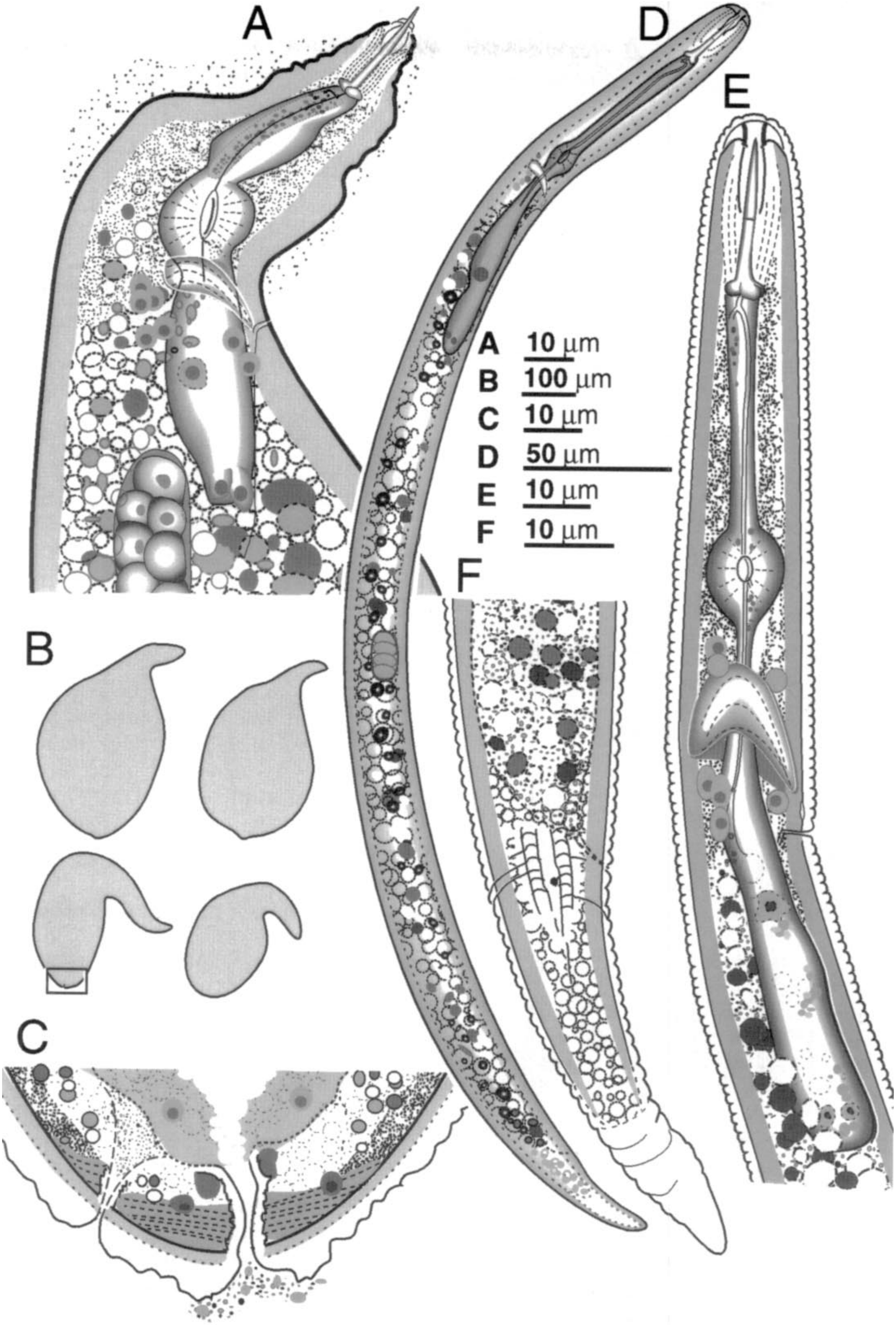

FIG. 1. Illustrations of females (A–C) and second-stage juveniles (D–F) of *Cactodera salina* in lateral views. A) Anterior portion. B) Outlines of females. C) Terminal protuberance with anus and vulva; region shown is an enlargement of the area outlined in the box in lower left of Fig. 1B. D) Entire specimen. E) Anterior portion including esophagus. F) Tail.

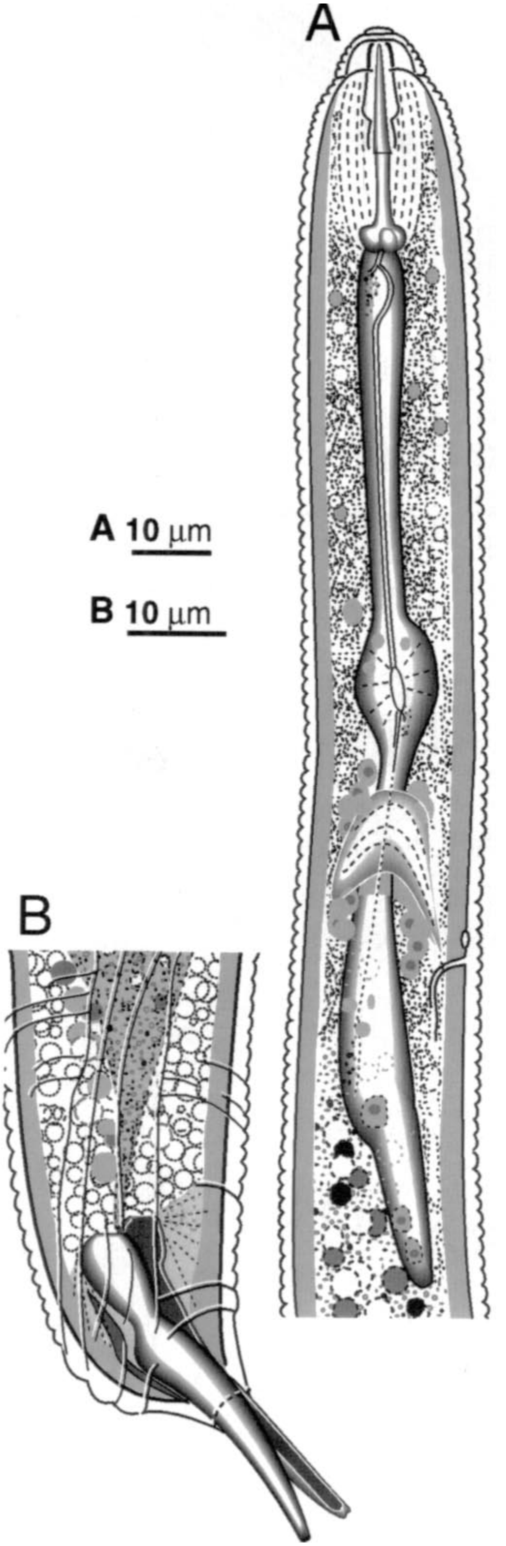

FIG. 2. Illustration of male of *Cactodera salina* in lateral view. A) Anterior portion including esophagus. B) Tail including spicules.

often areolated anteriorly, pattern of lines distorted posteriorly (Figs. 2B,5E). Hemizonid about one annule anterior to excretory pore; cephalids not observed. Stylet knobs robust and slightly flattened anteriorly (Fig. 2A). Spicules distinctly bifid; inconspicuous, elongate gubernaculum present. Phasmid openings not observed (Figs. 2B;5E,F).

Second-stage juveniles (J2) (n = 22): Measurements in Table 1. Body cylindrical and tapering posteriorly (Figs. 1D;5C). Head region not set off, with about five annules (Figs. 1B;3E;5A). Lip region in SEM consisting of oval labial disc surrounded by a full complement of six separate lip sectors; size of lateral sectors greatly reduced relative to others (Fig. 5A). Four lateral lines with outer two ridges partially areolated at the anterior and posterior ends (Figs. 1F;5C,D). Hemizonid about 1.5 annules long and positioned one annule anterior to excretory pore. Stylet knobs robust and sloping slightly posteriorly (Figs. 1E;3E). Phasmids rarely visible with LM, with SEM often seen as a minute pore within an inner lateral line just posterior to the level of the anus (Figs. 1F;5C).

Cyst (n = 20): L (including "neck") = 415–742 (603, 20); width = 193–475 (375, 14); thickness of cuticle = 4.9–9.0 (6.6, 0.3); a = 1.4–2.2 (1.6, 0.04); circumfenestra diameter = 20.4–27.6 (23.8, 0.4).

Cysts creamy yellow to light brown and oval to nearly spherical, often with a minute posterior cone (Figs. 3A;4A,B). Dense material typically persistent, covering neck region. Cyst surface with pronounced zigzag pattern (Fig. 4D). Small cone region circumfenestrate; bullae and denticles not observed. Anus visible as minute pore within a smooth region; smooth region surrounding circumfenestra (Figs. 3C,D;4C).

Egg (n = 10; one or two cell stages only): L = 107–119 (110, 1.0), width = 45.4–51.4 (49.5, 0.5). Surface of egg lacking punctations or tubercles when viewed with LM at ×1000 and with SEM at ×10,000.

Diagnosis

Cactodera salina is distinct within the genus by possession of the zigzag surface pattern

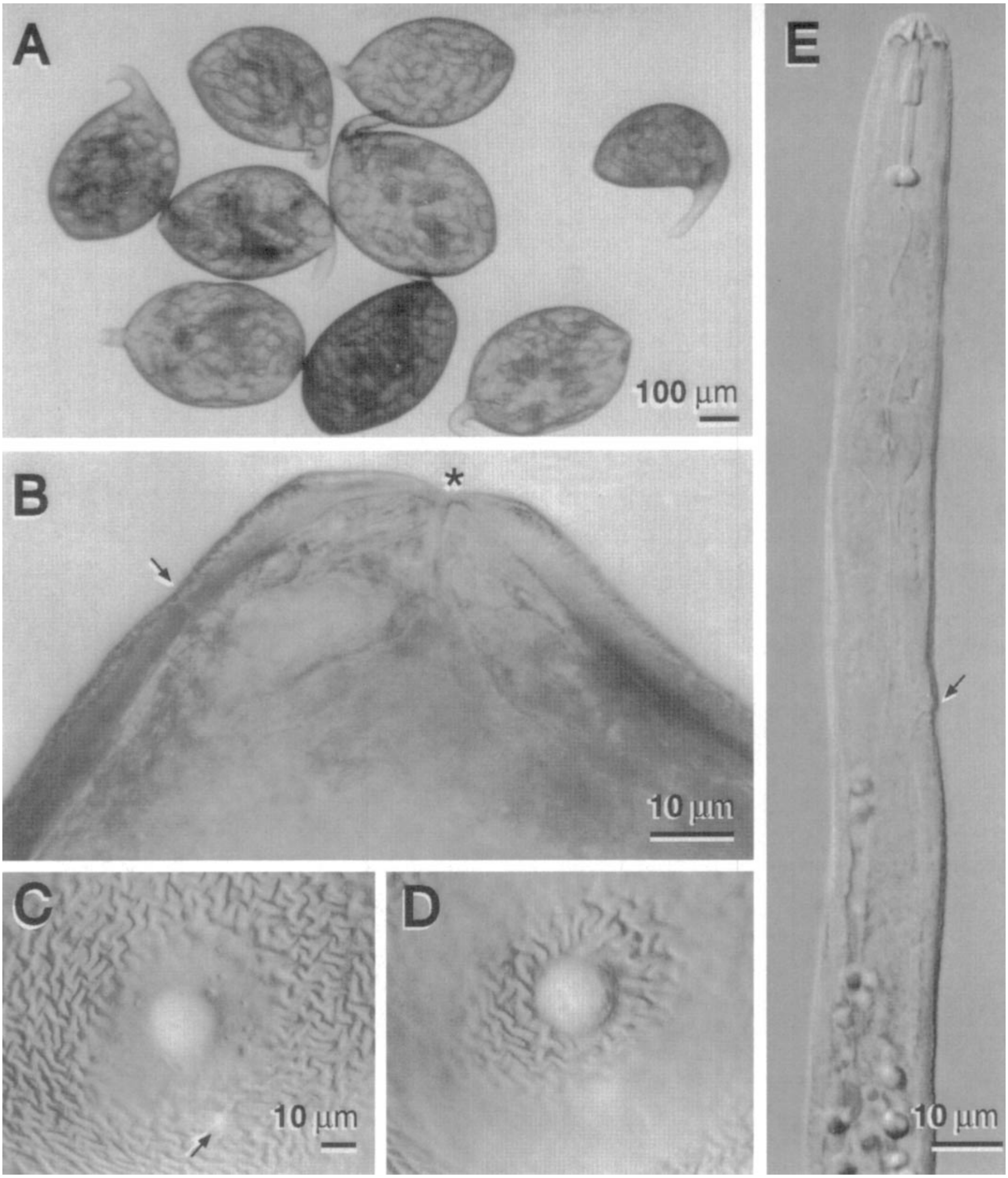

FIG. 3. Light micrographs of key features of *Cactodera salina*. A) Mature cysts filled with eggs. B) Terminal protuberance of female with anus (arrow) and vulva (asterisk). C) End view of cyst cone focused down to show cyst pattern and anus (arrow). D) End view of cyst cone focused up to show cyst pattern surrounding circumfenestra. Scale same as C. E) Lateral view of anterior end of second-stage juvenile. Arrow indicates position of excretory pore.

on females and cysts, greatly reduced or absent terminal cone, and lightly colored cysts. The cysts vary greatly in size (415–742 µm), but the mean (603 µm) and upper end of the range are greater than most *Cactodera spp.* Eggs lack punctations or surface tubercles. J2 are distinguished by a mean stylet length of 24 µm, knobs sloping slightly posteriorly, with a mean DGO-to-stylet base length of 3.2 µm.

Relationships

Cactodera amaranthi Stoyanov, *C. weissi* (Steiner) Krall & Krall, and *C. acnidae* Schu-

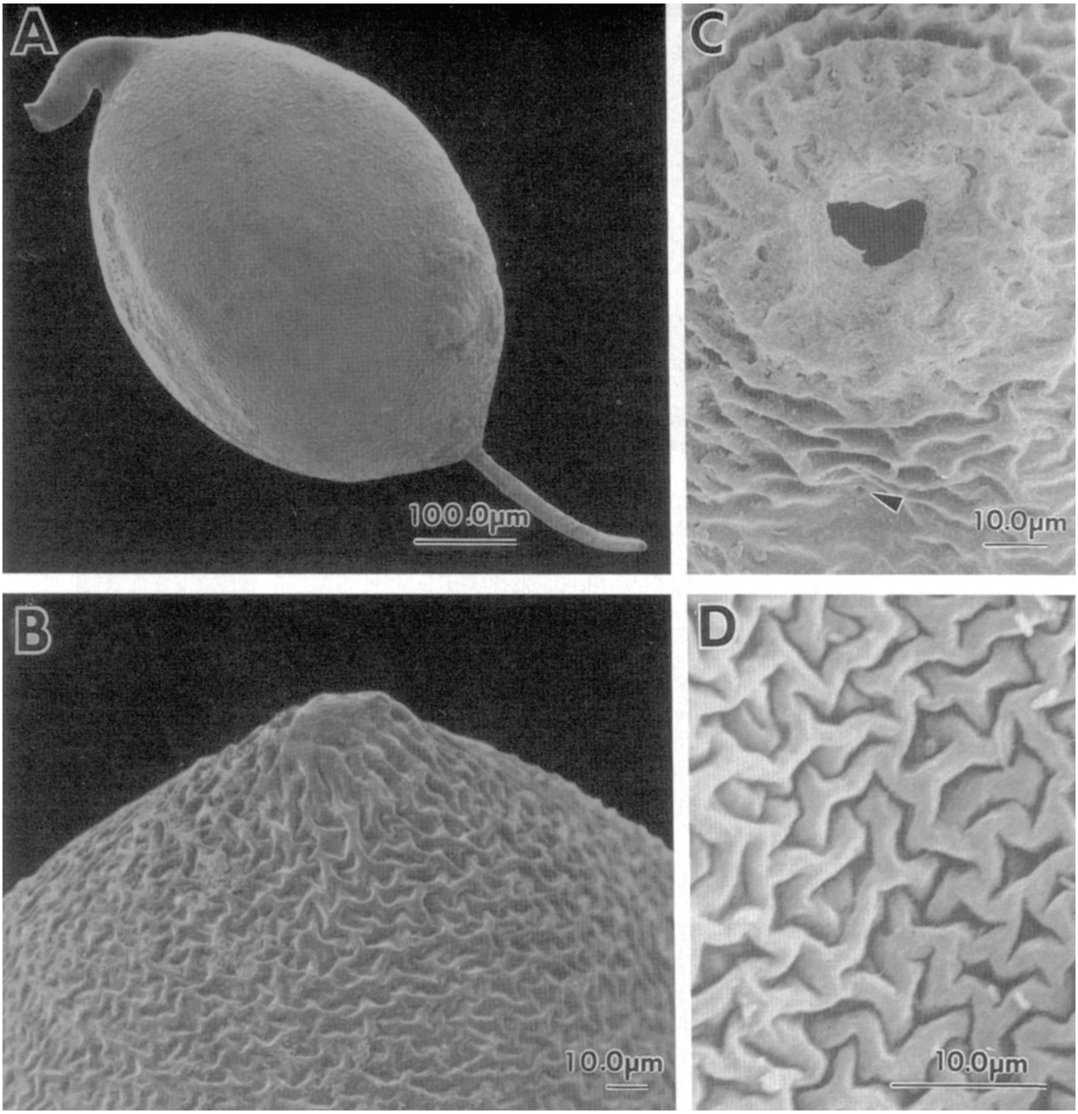

FIG. 4. SEM micrographs of *Cactodera salina* cysts. A) Young cyst. Note a second-stage juvenile emerging through the circumfenestra. B) Cone of a young cyst in which the circumfenestra is not yet ruptured. C) Ruptured circumfenestra. D) Surface cuticle in midbody region.

ster & Brezina share with *C. salina* the character of a smooth egg surface without tubercles, but *C. amaranthi* and *C. weissi* have shorter J2, shorter stylets (20.7 [Golden and Raski, 1977] and 20.4 µm, respectively) and a longer DGO (4.9 and 5.3 µm, respectively) compared to *C. salina. Cactodera acnidae* J2 also have a short DGO value (<3 µm) as in *C. salina,* but the cysts tend to be longer (698) than those of *C. salina* (603) and the J2 stylet knobs are convex versus posteriorly sloping in *C. salina. Cactodera acnidae* also has a much more pronounced posterior cone than *C. salina.* Mature cysts of *C. salina* are light-colored as are the cysts of *C. weissi,* in contrast to the dark cysts of *C. amaranthi* and *C. acnidae* (Golden and Raski, 1977; Schuster and Brezina, 1979).

Type host and locality

Roots and surrounding sand and silt of *Salicornia bigelovii* in experimental plots adjacent to the Centro de Estudios de Desiertos y Oceanos, Puerto Peñasco, Sonora,

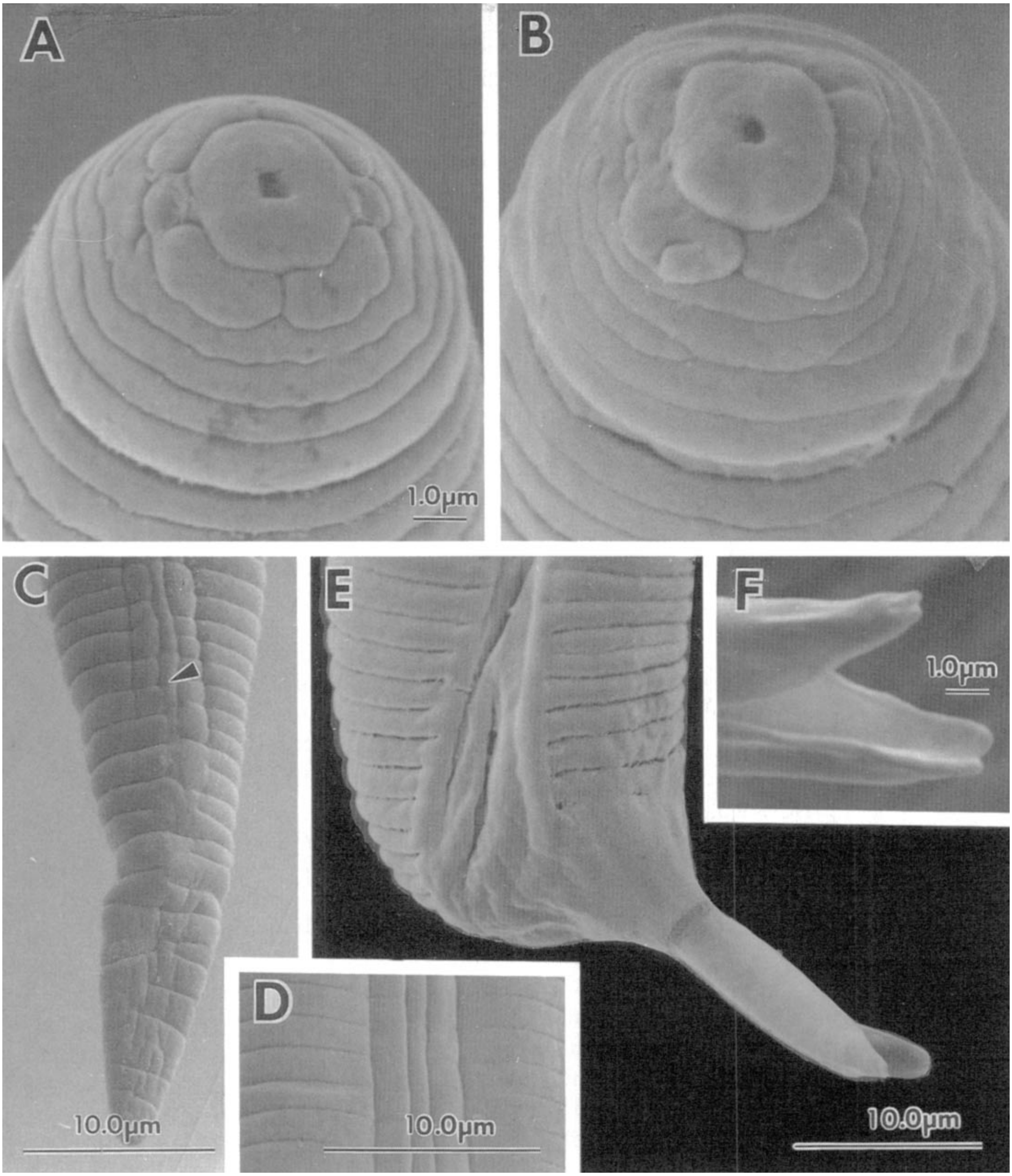

FIG. 5. SEM micrographs of second-stage juveniles (J2) and males of *Cactodera salina.* A) Face view (slightly ventral) of J2. B) Face view (slightly ventral) of male. Scale same as A. C) Tail of J2. Arrow indicates position of phasmid opening. D) Lateral field in midbody region of J2. E) Tail region of male showing spicules and lateral field. F) Distal end of the spicules showing bifid tips.

Mexico, adjacent to the northern tip of the Sea of Cortez.

Type designations

Collected by J. G. Baldwin and M. Mundo-Ocampo in August 1995. Holotype female (catalog number 30016), UCRNC, Department of Nematology, University of California Riverside. Paratypes (12 females, 72 cysts, 70 males, 88 J2) distributed in type collections as follows: 4 cysts, 2 males, 5 J2, UCDNC, Department of Nematology, University of California, Davis; 5 cysts, 3 males, 6 J2, USDANC Nematology Investigations,

TABLE 1. Measurements of second-stage juveniles of *Cactodera salina* (n = 22).

Character	Range	Mean	Standard error
Linear (μm)			
Body length	410–514	458	6.8
Body width	20.4–24.4	21.9	0.2
Stylet length	23.4–25.0	24.3	0.14
DGO	2.5–4.0	3.2	0.08
Esophagus length	130–163	146	1.6
Excretory pore to anterior end	84–107	97.8	1.3
Tail length	30.6–47.9	39.7	0.9
Tail terminus length (hyaline region)	10.2–30.6	19.6	1.07
Phasmid below anus	25.5–40.8	34.8	1.1
Ratios			
a	18.3–23.3	20.9	0.3
b′	2.9–3.5	3.2	0.03
c	9.9–16.0	11.7	0.3
Percentages			
o (DGO/stylet length)	10.2–16.0	13.2	0.3
Excretory pore	20.0–22.5	21.4	0.18

Beltsville, MD. Remaining type material, 12 females, 63 cysts, 65 males, 77 J2, in the UCRNC.

DISCUSSION

The genus *Cactodera* is widely distributed, but its primary diversification appears to be in the western hemisphere (Krall and Krall, 1978). Diversification is particularly extensive in the southwestern United States and Mexico with *C. cacti*, *C. eremica* Baldwin & Bell, *C. thornei* (Golden & Raski) Krall & Krall *C. salina*, and *C. amaranthi* (Golden and Raski, 1977; Graney and Bird, 1990; Sosa-Moss, 1986). *Cactodera salina* is striking in its unusual habitat, the interface between desert and the Sea of Cortez with extreme temperatures and high salinity in the substrate and tidal water. On the beach where the host, *S. bigelovii*, is grown for trials as an oil crop (Glenn et al., 1991), the seeds germinate in sea water and plots are irrigated daily by flooding from sea water wells. The nematode apparently survives these stresses primarily as eggs retained within cysts for much of the year. During the various times of the year that we collected field samples, we observed that cysts attached to roots were plentiful, whereas young females were much less abundant. One explanation is that reproduction and development are adapted to proceed rapidly during brief periods of moderate temperatures and rare, very limited rainfall. Another cyst nematode, *H. litoralis* occurs in a similarly high-saline habitat and on a similar host in New Zealand (Wouts and Sturhan, 1996). Although other species of *Cactodera* are not associated with high salinity, *C. cacti* and *C. eremica* typically occur in dry habitats and the type host for *C. amaranthi* is found in relatively dry or well-drained regions of the tropics. Although *Salicornia* spp. have not been previously reported as hosts of *Cactodera* spp., other members of Chenopodiaceae are common hosts of the genus (Baldwin and Bell, 1985; Golden and Raski, 1977; Graney and Bird, 1990; Stoyanov, 1972).

Cactodera salina extends the morphological limits of the genus. The midbody cuticular pattern of females and cysts of *Cactodera spp.* generally consists of straight to wavy lines, in some cases even suggesting striations (Baldwin and Bell, 1985; Graney and Bird, 1990), but *C. salina* is unusual in having a strong zigzag pattern more typical of *Heterodera* and *Globodera*. Most *Cactodera* spp., unlike other Heteroderinae, also have eggs covered with tubercles, but the absence of this character in *C. salina* apparently is shared only with *C. amaranthi*, *C. weissi*, and *C. acnidae*. Only *C. milleri* Graney & Bird and *C. acnidae* have larger cysts than *C. salina*.

The most striking distinction of *C. salina* is the terminal cone on females and cysts, which is so reduced as to approach that of the round cysts of *Globodera*. Previous transmission electron microscopy of the cones of *C. cacti* and *H. schachtii* (Schmidt) Örle (Cordero and Baldwin, 1990, 1991; Cordero et al., 1991) suggests that loss of the cone is associated with progressive deterioration of vaginal musculature and retention of eggs; this loss may be extended to its limit in certain *Cactodera* and finally in *Globodera*. Whereas previously phylogenetic analyses place *Cactodera* as part of a clade with *Heterodera* and *Afenestrata* (Baldwin and Schouest, 1990), new taxa with intermediate characters such as the cone region of *C. salina* may test alternate hypotheses that *Cactodera* and *Globodera* are sister taxa. Based on sequence data for ribosomal DNA (rDNA) for the two internal transcribed spacers (ITS1 and ITS2) and the 5.8S rRNA gene, two species of *Cactodera* (*C. milleri* and *C. weissi*) grouped more closely with *Globodera* than with *schachtii*-group species of *Heterodera* (Ferris et al., 1995). Ongoing comparisons indicate that the rDNA of *C. salina* is 95% similar to that of *C. milleri* and 93% similar to *C. weissi,* values that are comparable to those of other congeneric species (V. Ferris, personal communication).

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