

A new cyst nematode, *Cactodera torreyanae* sp. n. (Tylenchida: Heteroderidae), parasitising romerito, *Suaeda torreyana*, in Texcoco, Mexico

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Summary – A new species of cyst nematode, *Cactodera torreyanae* sp. n., parasitising romerito plants, *Suaeda torreyana* (Chenopodiaceae), found in highly saline soils in Texcoco, Mexico, is described. The new species is morphologically and molecularly related to *C. weissi*, from which it differs in smaller fenestral diam., longer body and shorter tail lengths of second-stage juveniles (J2) and to *C. rosae*, from which it differs in smaller cyst size, longer body length of J2 and the presence of a smooth eggshell surface. Phylogenetic relationships within populations and species of the Punctoderinae and *Cactodera* are given based on the analysis of the D2-D3 expansion segments of 28S rRNA and the ITS rRNA gene sequences.

Keywords – *Betulodera*, *Cactodera galinsogae*, *Cactodera rosae*, *Cactodera weissi*, key, molecular, morphology, morphometrics, phylogeny, plant-parasitic nematode, Punctoderinae, taxonomy.

During the spring of 2012, second-stage juveniles (J2) of a cyst-forming nematode were detected from soil around plants of the coastal succulent *Suaeda torreyana* S. Watson (Chenopodiaceae), known in Mexico under the common name ‘romeritos’ and used for the preparation of a traditional Christmas dish. Further soil samplings revealed the presence of cysts, and large numbers of white females attached to the roots were also found during the rainy season. A detailed study of the vulval cones showed the typical circumfenestrate pattern, identifying these nematodes as belonging to *Cactodera* Krall & Krall, 1978. Morphological and molecular comparison of our material with the other 13 valid *Cactodera* species (Subbotin *et al.*, 2010) revealed the nematode to be a new species. Herein, we describe this nematode under the name *Cactodera torreyanae* sp. n. The new species shares the same saline environment with *C. salina* Baldwin, Mundo-Ocampo & McClure, 1997, which was described from the rhizosphere of *Saliconia bigelovii* Torr, a plant

growing in a typically saline soil in Mexico (Baldwin *et al.*, 1997).

Material and methods

NEMATODE ISOLATES

Females, males, cysts and J2 of *C. torreyanae* sp. n. were collected from the natural habitat of the host plant, *S. torreyana*, in the Colegio de Postgraduados, Montecillo Campus, Mexico State. For the molecular study, several other circumfenestrate cyst nematodes were collected and included in the analysis: *C. galinsogae* Tovar Soto, Cid Del Prado, Nicol, Evans, Sandoval Islas & Martinez Garza, 2003 from *Galinsoga parviflora* Cav., La Raya Municipio de Singuilucan, Estado de Hidalgo, Mexico; *C. rosae* Cid Del Prado & Miranda, 2008 from *Hordeum vulgare* L., San Juan Ixtimaco, Municipio de Apan, Hidalgo State, Mexico; *Betulodera betulae* (Hirschmann & Riggs, 1969) Sturhan, 2002 from an unknown tree, Oak

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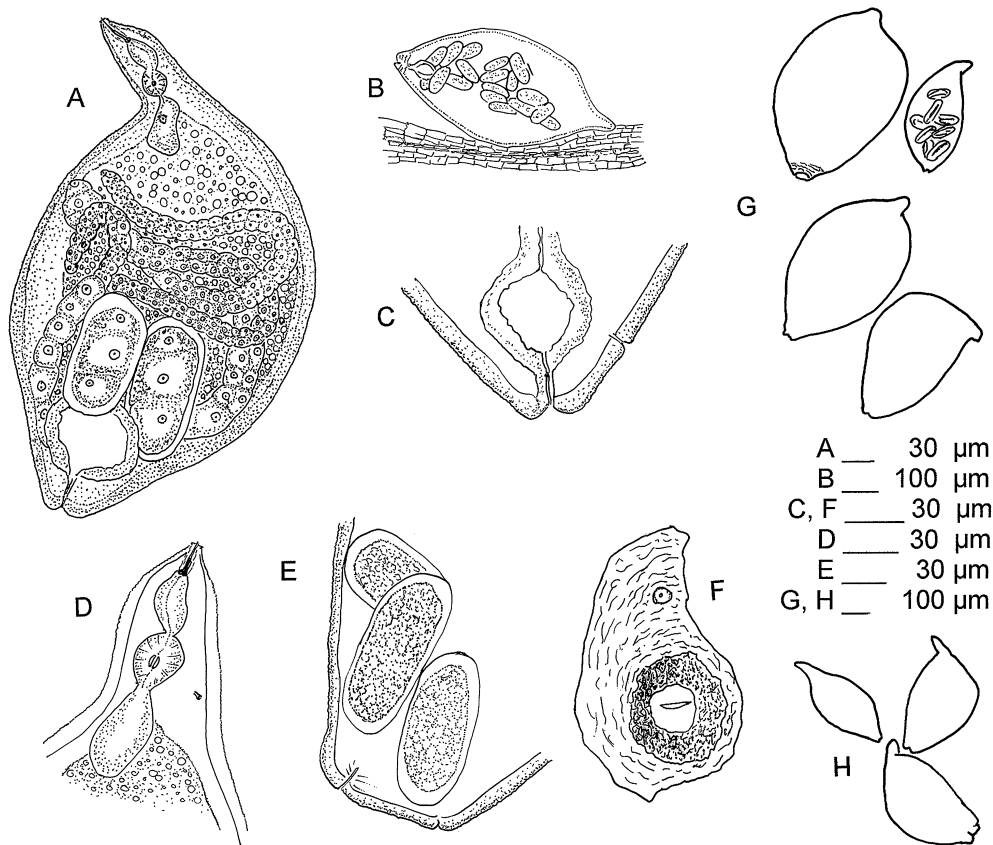


Fig. 1. *Cactodera torreyanae* sp. n. Female. A: Entire body (lateral view); B: Female (lateral) on a root; C, E, F: Vulval cones with anus and vulva; D: Neck (lateral view); G: Outline of cysts; H: Outline of females.

Creek, vicinity of Sedona, Arizona, USA; and *Betulodera* sp., Merced River, Mariposa County, Sierra National Forest, California, USA. Cysts, juveniles and males were extracted from soil samples using standard centrifugal-flotation and the Fenwick methods. White females were picked directly from the roots under a binocular microscope.

MORPHOLOGICAL STUDY

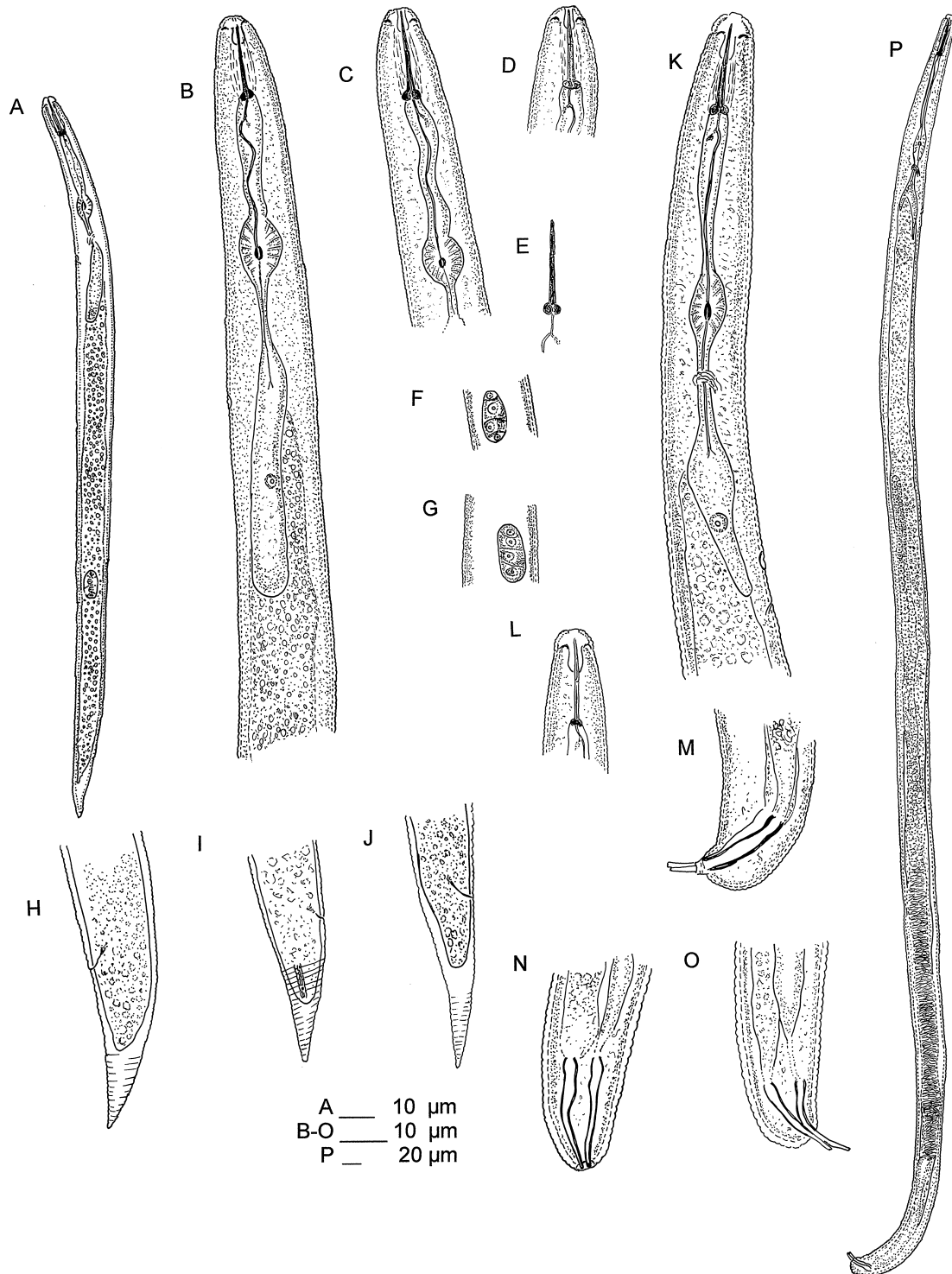
Nematodes were killed by heating in a Petri dish with water. Formalin (8%) was added to a Petri dish to achieve a final fixative concentration of 4% formalin. The covered dish was stored at room temperature for 10 days and then placed in a small desiccator containing 95% ethanol and incubated at 40°C for 3 days. The nematodes were then processed to glycerin using a modification of the Seinhorst (1959) method as described by Cid Del Prado Vera & Subbotin (2012) and mounted on slides. Measurements and drawings were made using a

drawing tube mounted on an American Optical compound microscope.

For scanning electron microscopy, some specimens were treated in phosphate buffer for 15 min and dehydrated in an alcohol series (10-100%) for 15 min at each concentration (Cid Del Prado Vera *et al.*, 2012). The specimens were critical point-dried and coated with gold-palladium before observation under a scanning electron microscope (Jeol JSM-6390) at 10 kV.

MOLECULAR STUDY

DNA was extracted from a single cyst containing J2 and eggs using extraction buffer containing Proteinase K. Detailed protocols for DNA extraction, PCR, cloning and sequencing for all studied samples are described by Tanha Maafi *et al.* (2003). The forward primer TW81 (5'-GTTTCCGTAGGTGAACCTGC-3') and the reverse primer AB28 (5'-ATATGCTTAAGTTCAGCGGGT-3') amplifying the ITS1-5.8S-ITS2 of rRNA and the forward



A — 10 μm
 B-O — 10 μm
 P — 20 μm

Fig. 2. *Cactodera torreyanae* sp. n. Second-stage juvenile. A: Entire body; B-D: Anterior region; E: Stylet; F: Genital primordium, after eclosion; G: Genital primordium, advanced development; H-J: Tail. Male. K, L: Anterior region; M-O: Tails with spicules in different views; P: Entire body.

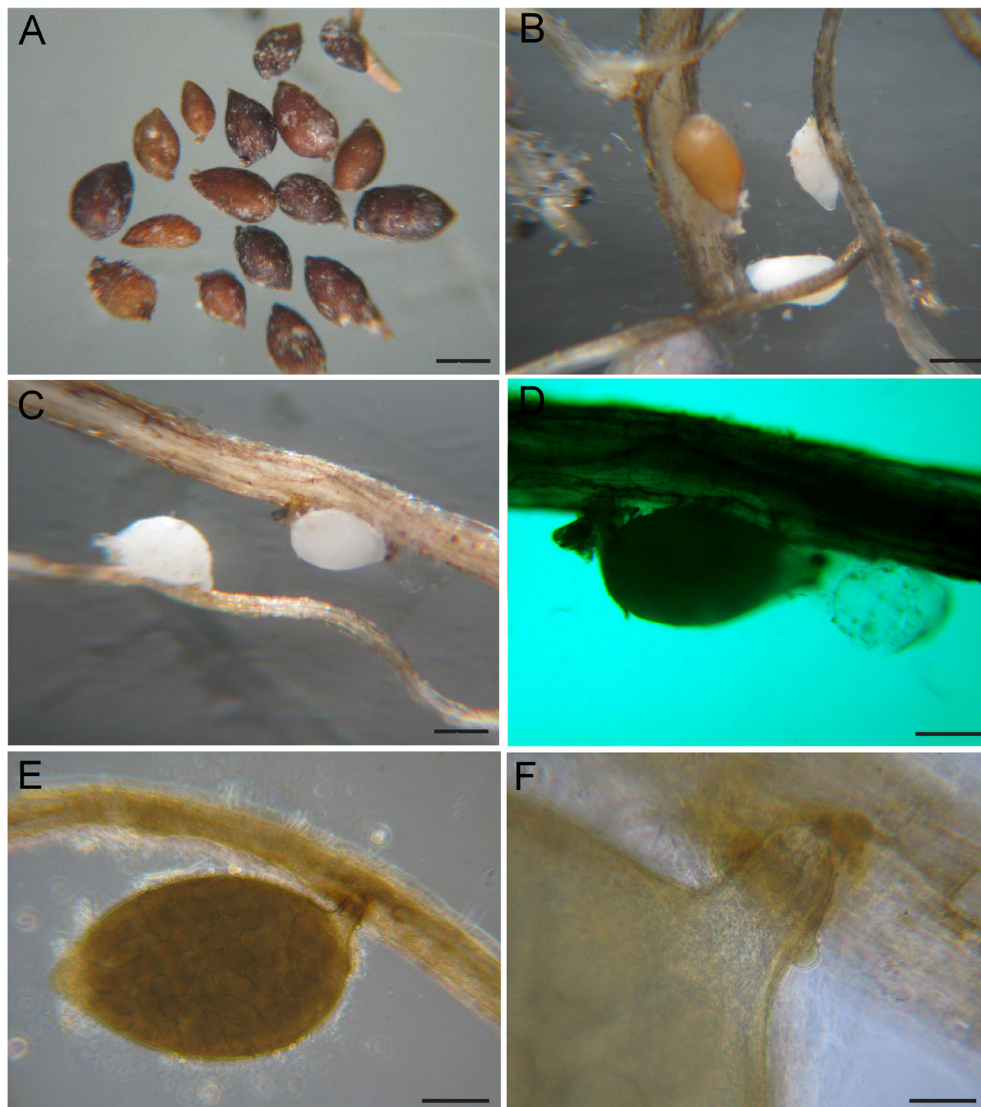


Fig. 3. Light microscopic photos of *Cactodera torreyanae* sp. n. A: Cysts; B: White females and cysts on roots; C: White females on roots; D: White female with a gelatinous sac on a root; E: Cyst on a root; F: Head embedded into a root. (Scale bars: A = 250 μm , B, C = 300 μm , D = 120 μm , E = 100 μm , F = 35 μm .) This figure is published in colour in the online edition of this journal, which can be accessed via <http://booksandjournals.brillonline.com/content/15685411>.

primer D2A (5'-ACAAGTACCGTGAGGGAAAGTTG-3') and reverse primer D3B (5'-TCGGAAGGAACCAGC TACTA-3') amplifying the D2-D3 expansion segments of 28S rRNA gene were used in PCR. New sequences obtained in the present study were submitted to the GenBank database under the accession numbers KF214747-KF214755.

The newly obtained sequences for the partial 28S rRNA and ITS rRNA gene were aligned using ClustalX

1.83 with default parameters with corresponding gene sequences for circumfenestrated cyst nematodes or *Cactodera*, respectively, deposited in GenBank (Ferris *et al.*, 1999, 2004; Subbotin *et al.*, 2001, 2011; Tanha Maafi *et al.*, 2003; Bernard *et al.*, 2010; Duan *et al.*, 2012; Handoo & Subbotin, unpubl.; Peng & Xu, unpubl.; Chan *et al.*, unpubl.; Chen *et al.*, unpubl.; Wei *et al.*, unpubl.). Outgroup taxa for each dataset were chosen according to the results of previously published data (Subbotin *et*

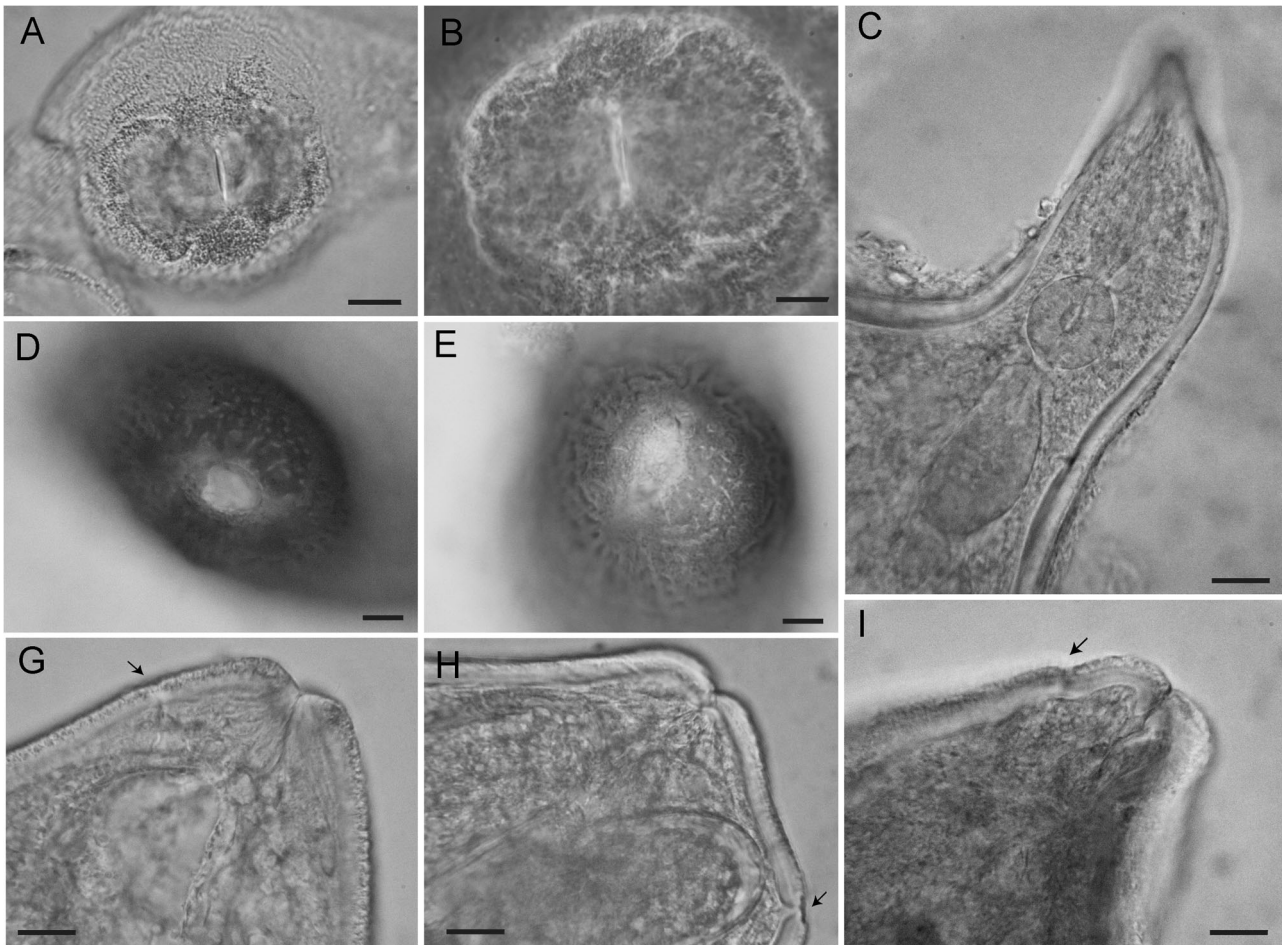


Fig. 4. Light microscopy photos of *Cactodera torreyanae* sp. n. A, B: Posterior ends of female showing vulval slit; C: Neck of female; D, E: Fenestrae of cysts; G-I: Vulval cones. Anus is indicated by arrow. (Scale bars: A, B = 25 μm , C = 10 μm , D-I = 20 μm .)

al., 2001, 2006, 2011). Sequence datasets for each gene fragment were analysed separately with Bayesian inference (BI) using MrBayes 3.1.2 (Huelsenbeck & Ronquist, 2001). BI analysis under the GTR + I + G model for each gene was initiated with a random starting tree and was run with four chains for 1.0×10^6 generations. The Markov chains were sampled at intervals of 100 generations. Two runs were performed for each analysis. The topologies were used to generate a 50% majority rule consensus tree. Posterior probabilities (PP) are given on appropriate clades. Trees were visualised with the TreeView program. Sequence analyses of alignments were performed with PAUP* 4b10 (Swofford, 2003). Pairwise divergences between taxa were computed as absolute distance values and as percentage mean distance values based on whole alignment, with adjustment for missing data.

Results

*Cactodera torreyanae** sp. n. (Figs 1-7)

MEASUREMENTS

See Tables 1 and 2.

DESCRIPTION

White female

Body oval-shaped with conspicuous vulval cone. Most white females pearly-white. Gelatinous sac protruding

* The specific epithet is derived from the Latin name of the host plant species.

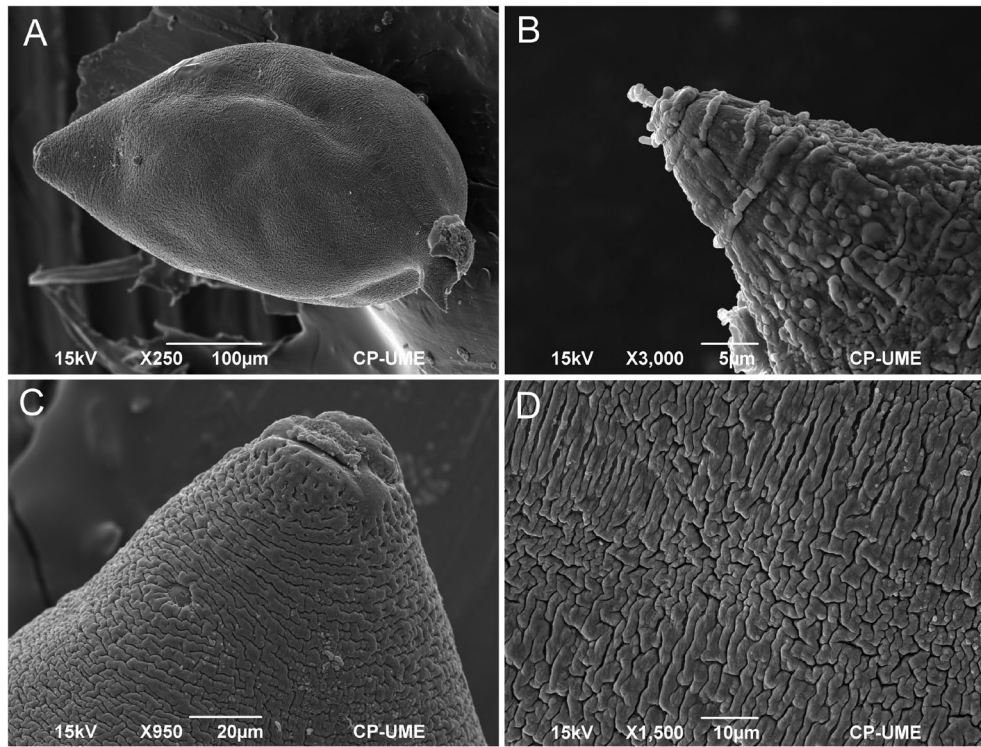


Fig. 5. SEM photos of *Cactodera torreyanae* sp. n. A: Cyst; B: Female anterior end; C: Vulval cone of a cyst with anus; D: Surface of cyst cuticle at mid-body.

from vulval cone and without eggs. Irregular annulation of posterior end of some mature females observed. Stylet slightly curved dorsally, with knobs slightly directed posteriorly. Excretory pore located at same level as end of isthmus. Most females with vulval cones having slightly protruding lips. Uterus oval to almost spherical in shape with thick wall.

Cyst

Lemon-shaped, from light to dark brown with distinct vulval cone. Vulva not in a depression. Cyst surface with zigzag pattern at mid-body and not prominent on surface of vulval cone. Cone without bullae and denticles. Anus a minute pore in a small depression with smooth surface.

Male

Body vermiform with slightly tapering anterior end, posterior end not twisted. Labial region slightly set off with *ca* five irregular annuli. *En face* pattern as viewed in SEM showing an oval-shaped labial disc, six labial sectors of irregular shape with lateral sectors smaller than submedian sectors. Stylet knobs rounded. Lateral field with four incisures, outer ridges with incomplete areolation,

posterior end with middle ridge wider than outer ridges. Hemizonid *ca* one annulus anterior to excretory pore. Spicules with slightly bifid end. Gubernaculum small. Cloacal tubus present. Phasmid not observed.

J2

Body cylindrical, tapering posteriorly. Lip region not or slightly set off with four annuli. In *en face* view, lip disc oval-rectangular and surrounded by six rectangular lip sectors, two dorsal and two ventral and two small lateral lips. Stylet knobs rounded, subventral ones sloping slightly posteriorly. Lateral field with four lines with outer two ridges partially areolated along body. Excretory pore immediately, or in some specimens three annuli, posterior to hemizonid. Phasmid a minute pore at level of beginning of hyaline part of tail.

Egg

Shell surface smooth, lacking punctations, only found inside cysts, 99 ± 0.9 (84-104) \times 41 ± 0.8 (34-53) μm in size (paratypes; n = 26).

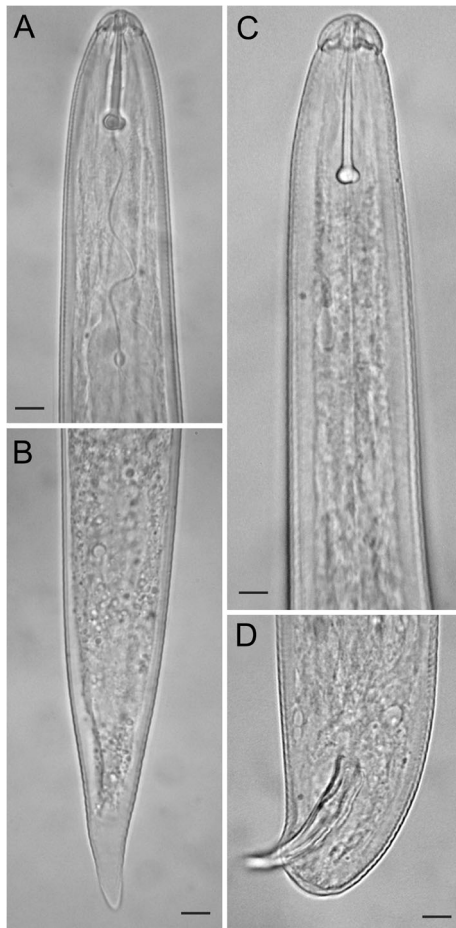


Fig. 6. Light microscopy photos of *Cactodera torreyanae* sp. n. Second-stage juvenile. A: Anterior region; B: Tail. Male. C: Anterior region; D: Tail. (Scale bars = 5 μm .)

TYPE HOST AND LOCALITY

Romerito plant, *Suaeda torreyana* (Chenopodiaceae), in Campus Montecillo, Colegio de Postgraduados, Texcoco, México State, Mexico. Coordinates: 19°27.850'N, 98°54.812'W, 2230 m a.s.l.

TYPE MATERIAL

Holotype female and ten paratype cysts, ten paratype white females, ten paratype males and ten paratype J2 deposited in the Colección Nacional de Helminthos (CNHE) Instituto de Biología, Laboratorio de Helminthología, UNAM (holotype CNHE 5684, paratype males CNHE 5685, paratype J2 CNHE 5686). Paratype slides with ten

cysts, ten white females, ten males and ten J2 are also deposited in the Colección Nematologica del Colegio de Postgraduados; paratypes of cysts (3), white females (3), males (3) and J2 (6) are in the University of California, Riverside, CA, USA, and paratypes of cysts (3), white females (3), males (3) and J2 (5) are in the USDANC Nematology Investigations, Beltsville, MD, USA.

DIAGNOSIS AND RELATIONSHIPS

The new species is characterised by the light- to dark-brown-coloured, lemon-shaped cysts with distinct vulval cone lacking bullae and denticles; J2 having a lip region with four annuli, not, or slightly, set off from the rest of the body and rounded stylet knobs, and eggs with smooth surface.

Cactodera torreyanae sp. n. is morphologically similar to *C. weissi* (Steiner, 1949) Krall & Krall, 1978, but differs from this species in a smaller mean fenestral diam. (16-20 vs 34 μm) for cysts, and in the J2 by the longer body length of 440 (390-550) vs 407 (372-489) μm , shorter tail length of 38 (32-45) vs 46 (43-50) μm and stylet knobs rounded or sloping slightly posteriorly vs anteriorly concave. *Cactodera torreyanae* sp. n. is distinguished from *C. salina* by cyst shape (lemon-shaped with distinct vulval cone vs oval or nearly spherical-shaped without a prominent vulval cone), shorter stylet length of 22.0 (21.0-23.0) vs 24.3 (23.4-25.0) μm for the J2 and eggshell smooth vs punctate, and from *C. rosae* in the smaller cyst size of 575 (364-712) \times 303 (92-432) vs 654 (460-840) \times 433 (280-560) μm , longer J2 body length of 440 (390-550) vs 397 (348-472) μm and eggshell surface smooth vs punctate.

BIOLOGY

Cactodera torreyanae sp. n. is found in Mexico in highly saline soils with a pH ranging from 8.6 to 10.1 and electrical conductivity from 1.25 to 11.22 dS/m at 0-30 cm depth. The species has a semi-endoparasitic, sessile habit. The juveniles only penetrate with the anterior portion of their body into the roots, the posterior part of the body protruding from the root surface. Juveniles develop into swollen females or males, which also remain attached to the roots by the anterior portion of their bodies. A gelatinous matrix protrudes from the posterior end of the female body. The semi-endoparasitic sessile habits for juveniles and females was reported for *Heterodera mediterranea* Vovlas, Insearra & Stone, 1981 (Castillo *et*

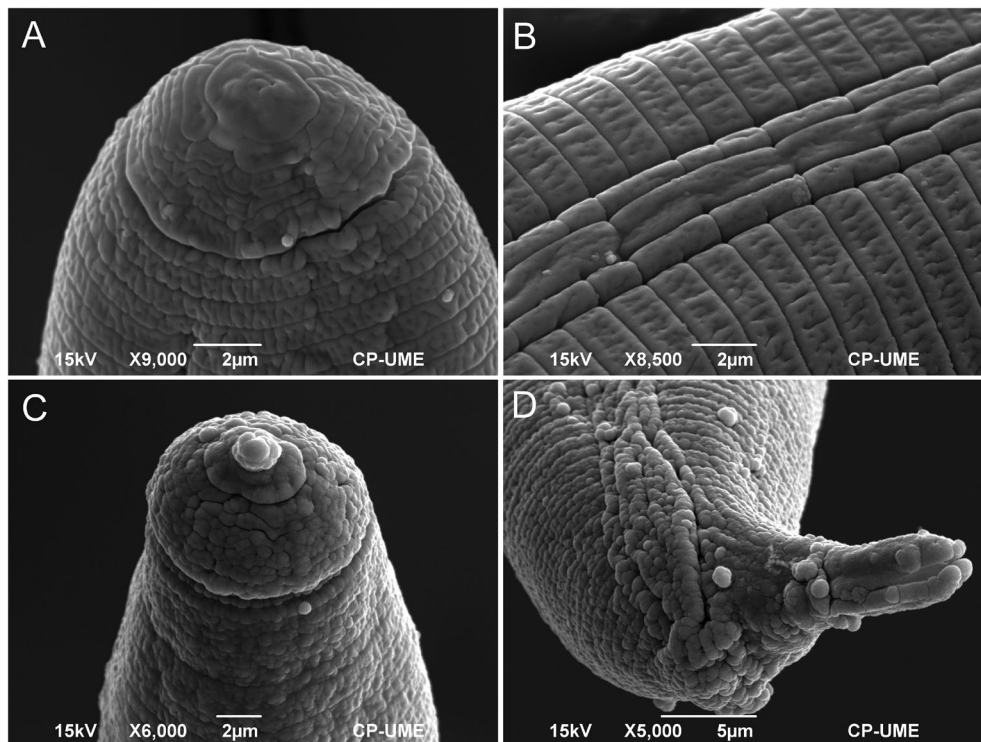


Fig. 7. SEM photos of *Cactodera torreyanae* sp. n. Second-stage juvenile. A: Face view; B: Lateral field at mid-body. Male. C: Face view; D: Tail region with spicules and lateral field.

Table 1. Morphometrics of white females and cysts of *Cactodera torreyanae* sp. n. All measurements are in μm and in the form: mean \pm s.d. (range).

Character	Female		Cyst
	Holotype	Paratypes	Paratypes
n	–	26	22
L (inc. neck)	398	490 \pm 20 (350-830)	575 \pm 24.2 (364-712)
W	154	235 \pm 14.7 (154-376)	303 \pm 22.5 (92-432)
L/W	2.6	1.2-2.5	1.9 \pm 0.2 (1.4-2.9)
Neck length	80	30-100	–
Stylet length	25	24.8 \pm 0.9 (22-27)	–
DGO	3.0	2.0-3.0 (n = 5)	–
Excretory pore from anterior end	96	98 \pm 5.6 (70-116) (n = 7)	–
Vulva-anus distance	35	43 \pm 2.2 (32-72) (n = 18)	46 \pm 4.0 (35-58)
Cuticle thickness	5.0	9.2 \pm 0.8 (5.0-22.0)	7.6 \pm 0.4 (4.0-10.0)
Vulval slit	–	43 \pm 2.2 (32-72) (n = 2)	–
Fenestral length	–	–	20 \pm 2.6 (15-30) (n = 5)
Fenestral width	–	–	16 \pm 2.6 (10-23) (n = 5)

al., 1999) which parasitises wild pistachio growing along the coastal dunes of Italy, and for *H. graminophila* Golden & Birchfield, 1972 parasitising barnyard grass (Inserra *et al.*, 1989).

MOLECULAR CHARACTERISATION AND POSITION OF *CACTODERA TORREYANAE* SP. N. WITHIN *CACTODERA*

In the phylogenetic tree inferred from the D2-D3 expansion segments of the 28S rRNA gene, *C. torreyanae*

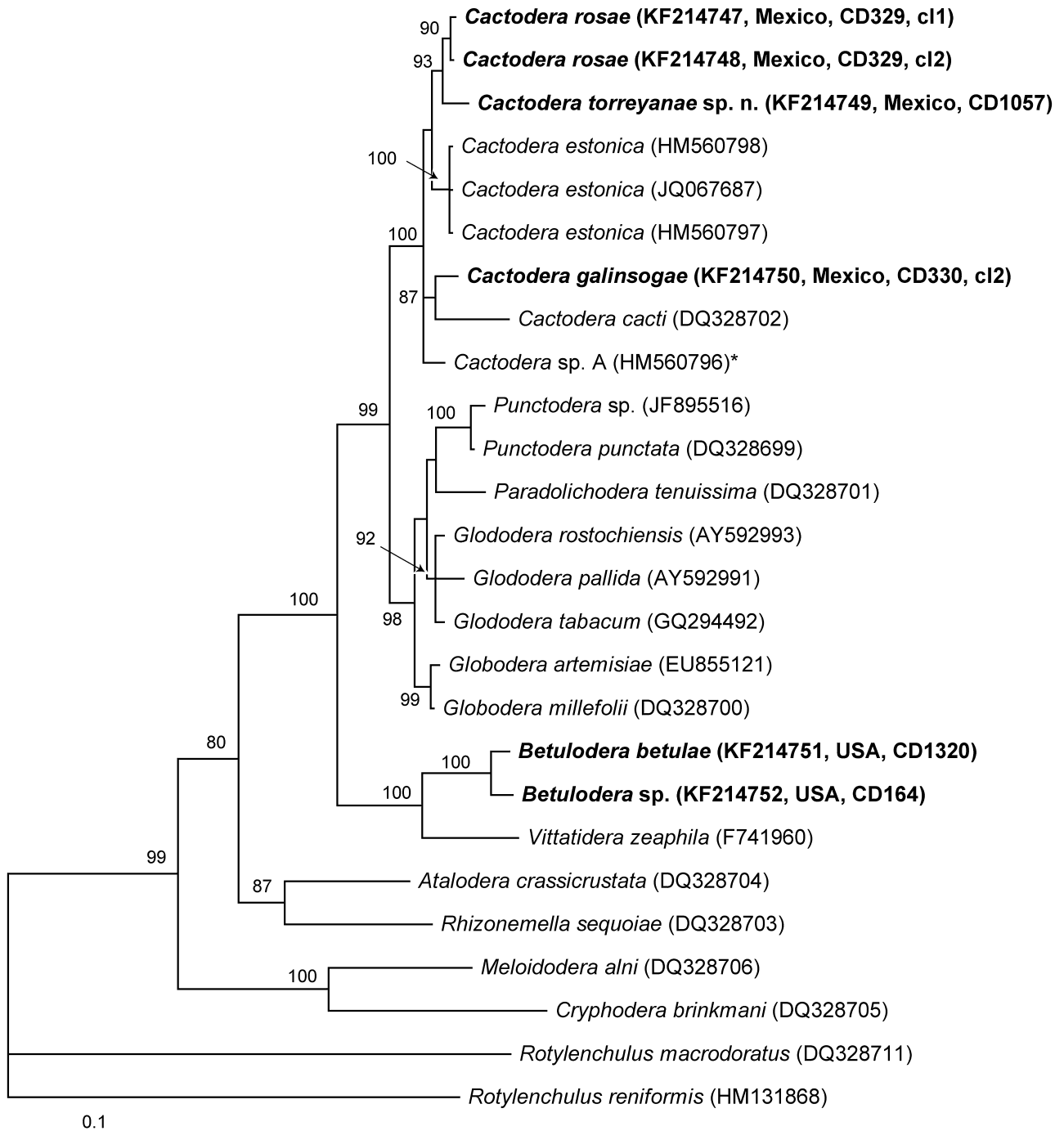


Fig. 8. Phylogenetic relationships within populations and species of Punctoderinae sensu Krall & Krall, 1978. The 50% majority rule consensus trees from Bayesian analysis generated from two runs as inferred from the analysis of the D2-D3 of 28S rRNA gene sequences under the GTR + G + I model. PP values are given in appropriate clades. Newly sequenced samples are indicated by bold font. * – originally identified as *Cactodera estonica* in GenBank.

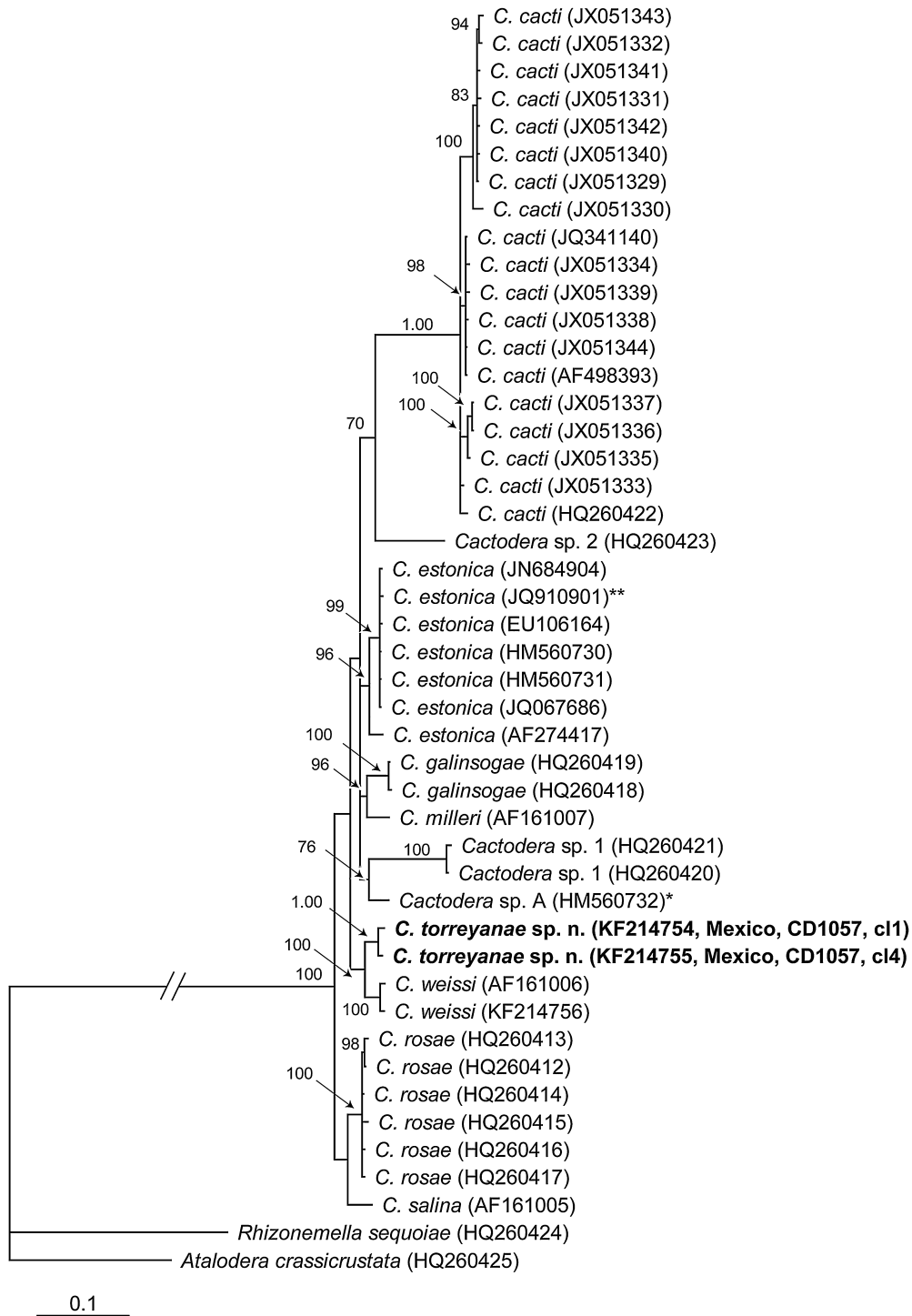


Fig. 9. Phylogenetic relationships within populations and species of *Cactodera*. The 50% majority rule consensus trees from Bayesian analysis generated from two runs as inferred from the analysis of the ITS rRNA gene sequences under the GTR + G + I model. PP values are given in appropriate clades. Newly sequenced samples are indicated by bold font. * – originally identified as *Cactodera estonica* in GenBank; ** – originally identified as *Cactodera eremica* in GenBank.

Table 2. Morphometrics of males and J2 *Cactodera torreyanae* sp. n. All measurements are in μm and in the form: mean \pm s.d. (range).

Character	Male	J2
	Paratypes	Paratypes
n	26	31
L	1170 \pm 23.4 (943-1437)	440 \pm 10.0 (390-550)
a	39.8 \pm 0.7 (34-45)	21.3 \pm 0.3 (19.4-23.3)
b	9.6 \pm 0.2 (7.2-10.9)	5.0 \pm 0.1 (3.0-6.1)
b'	6.8 \pm 0.2 (5.7-8.5)	–
c	252 \pm 27.5 (120-719)	11.6 \pm 0.2 (9.9-13.8)
c'	0.33 \pm 0.12 (0.12-0.50)	3.0 \pm 0.1 (1.9-4.4)
Stylet length	26 \pm 0.3 (24-28)	22 \pm 0.1 (21-23)
DGO	4.3 \pm 0.2 (3.0-6.0)	3.0 \pm 0.2 (2.0-4.0)
Pharynx length	121 \pm 4.5 (104-163)	89 \pm 2.5 (66-122)
Pharyngeal gland end from anterior end	175 \pm 6.2 (140-229)	145 \pm 3.8 (101-189)
Excretory pore from anterior end	132 \pm 4.6 (99-159)	95 \pm 2.2 (72-125)
Median bulb length	–	13 \pm 0.3 (11-16)
Median bulb diam.	–	10 \pm 0.4 (6.0-13)
Genital primordium from anterior end	–	260 \pm 4.5 (215-307)
Max. body diam.	29 \pm 0.6 (25-36)	20.9 \pm 0.4 (18-25)
Phasmid-anus distance	–	23 \pm 1.2 (20-25) (n = 4)
Hyaline tail length	–	19 \pm 0.4 (16-25)
Tail length	5.4 \pm 0.4 (2-10)	38 \pm 0.7 (32-45)
Spicules	30.8 \pm 0.5 (25-40)	–
Gubernaculum	6, 9 (n = 2)	–
o	0.17 \pm 0.01 (0.11-0.24)	–
T	54.6 \pm 2.8 (31-71)	–

sp. n. formed a clade with *C. rosae* (Fig. 8) and differed from this species by 9-10 bp (1.3-1.4%). In the tree obtained from the ITS rRNA gene sequence alignment, *C. torreyanae* sp. n. formed a clade with *C. weissii* (Fig. 9) and differed from this species by 26-29 bp (2.9-3.2%). The ITS intraspecific sequence variation for *C. cacti* was 0-24 bp (0-2.7%) and for *C. estonica* was 0-18 bp (0-2.1%).

Key to species of *Cactodera*

(modified from Subbotin *et al.*, 2010)

1. Cyst generally two times or more longer than wide, mean L/W ratio = 2.3 *C. estonica*
– Cyst usually less than twice as long as wide, mean L/W ratio = 1.1-1.9 2
2. Eggshell punctate 3
– Eggshell smooth 9
3. Mean stylet length of J2 \geq 26 μm 4
– Mean stylet length of J2 < 26 μm 5

4. J2 tail length = 48-64 μm , J2 hyaline region = 23-28 μm , fenestral diam. = 23-41 μm *C. thornei*
– J2 tail length = 37-48 μm , J2 hyaline region = 17-24 μm , fenestral diam. = 14-25 μm *C. eremica*
5. Mean J2 body length \geq 411 μm , mean tail length \geq 42 μm 6
– Mean J2 body length < 411 μm , mean tail length < 42 μm 7
6. Fenestral diam. = 23-35 μm , vulval slit = 10-15 μm , J2 stylet = 21-27 μm *C. cacti*
– Fenestral diam. = 7-22 μm , vulval slit = 14.3-17.8 μm , J2 stylet = 21-23 μm *C. milleri*
7. Fenestral diam. < 25 μm 8
– Fenestral diam. \geq 25 μm *C. galinsogae*
8. Mean cyst size = 654 \times 433 μm *C. rosae*
– Mean cyst size = 460 \times 335 μm *C. evansi*
9. Mean J2 tail length < 40 μm 10
– Mean J2 tail length \geq 40 μm 12
10. Mean J2 body length < 406 μm , mean hyaline region length < 16 μm *C. amaranthi*

- Mean J2 body length $\geq 406 \mu\text{m}$, mean hyaline region length $\geq 16 \mu\text{m}$ 11
- 11. Cyst with distinct vulval cone, J2 stylet length = 21.0-23.0 μm *C. torreyanae* sp. n.
 - Cyst without distinct vulval cone, J2 stylet length = 23.4-25.0 μm *C. salina*
- 12. J2 stylet knobs anterior surface concave, DGO = 4.5-5.6 μm 13
 - J2 stylet knobs anterior surface convex, DGO = 2.5-3.0 μm *C. acnidae*
- 13. Vulval denticles present, J2 tail length = 43-50 μm *C. weissi*
 - Vulval denticles absent, J2 tail length = 46-60 μm *C. radiale*

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