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Cactodera solani n. sp. (Nematoda: Heteroderidae), a new species of cyst-forming nematode parasitising tomato in Mexico

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Summary – A new species of cyst-forming nematode, *Cactodera solani* n. sp., from Mexico is described. The new species was found in a glasshouse in Mexico City parasitising tomato and common lambsquarter. This cyst nematode has light brown to almost black cysts, presents a small vulval cone with circumfenestra and morphologically it most resembles *C. milleri. Cactodera solani* n. sp. can be differentiated from *C. milleri* by having smaller cysts that are 417 (291-581) × 324 (204-505) *vs* 632 (515-730) × 506 (419-598) μm in size and a longer stylet in the second-stage juveniles of 25 (24-27) *vs* 22 (21-23) μm. Phylogenetic relationships within populations and species of *Cactodera* are given based on the analysis of the ITS rRNA and the partial *COI* gene sequences. The ITS rRNA and *COI* gene sequences clearly differentiated *C. solani* n. sp. from other *Cactodera* species. This new cyst-forming nematode has several generations per year and its life cycle can be completed in 49 days on tomato and in 40 days on common lambsquarter at 20-25°C.

Keywords – *Chenopodium album*, common lambsquarter, life cycle, molecular, morphology, morphometrics, phylogeny, *Solanum lycopersicum*, taxonomy.

Tomato (*Solanum lycopersicum* L.) is one of the most important crops in the world due to increasing commercial and dietary value, widespread production, as well as its use as a model plant for research (Kimura & Sinha, 2008; Melomey *et al*., 2019). In 2017, 182 million tonnes were produced worldwide, with China, India, Turkey, and the USA being the leading producers (FAOSTAT, 2020). Tomato belongs to the Solanaceae family and originated in the Andean region, although it is presumed that Mexico was the actual site of domestication (Robertson & Labate, 2006; Melomey *et al*., 2019; Sharma *et al*., 2019). In 2017, Mexico was the ninth largest producer of tomato worldwide with a production of 4 million tonnes (FAOSTAT, 2020).

In October 2018, cysts and white females of a cyst-forming nematode were found attached to tomato roots and common lambsquarter (*Chenopodium album* L.) in the glasshouse of the Escuela Nacional de Ciencias Biológicas-Instituto Politécnico Nacional (ENCB-IPN) in Mexico City, Mexico. Tomato plants were stunted and chlorotic, whereas in common lambsquarter symptoms were not observed. The nematode had a small vulval cone and a circumfenestrate pattern, which is typical of the genus *Cactodera* Krall & Krall, 1978. Morphological and molecular analyses of this nematode revealed that it belongs to a new species, differing from the 15 known valid species of the genus (Subbotin *et al*., 2010; Feng *et al*., 2018; Handoo & Subbotin, 2018). In this paper we describe *Cactodera solani* n. sp. and study the life cycle of the nematode on two hosts, tomato and common lambsquarter, under glasshouse conditions.

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Materials and methods

Nematode population

Females, males, cysts and second-stage juveniles (J2) of *C. solani* n. sp. were extracted from a plastic pot sown with tomato 'Rio Grande' in the glasshouse of ENCB-IPN (19°27′14″N, 99°10′19″W; 2255 m a.s.l.) and were used for the morphological and molecular characterisation (cysts) of the nematode (code MT3HE). Males, J2 and cysts were extracted from 200 cm^3 of soil by the centrifugal flotation technique (Jenkins, 1964) and by the Fenwick can method (Fenwick, 1940), respectively. Females were picked directly from tomato roots under a stereomicroscope. In addition, cysts of a population of *C. solani* n. sp., obtained from the nematology collection of ENCB-IPN, were also used for the molecular study (code M124E). This sample was collected from a field sown with broccoli in Palmar de Bravo, Puebla, Mexico (18°53′36″N, 97°39′52″W; 2158 m a.s.l.)

Morphological characterisation

Males and J2 were killed in a water bath at 65°C for 5 min, fixed with a mixture of ethanol, acetic acid and formalin (20:6:1) and processed to glycerin. The specimens were mounted on permanent slides using anhydrous glycerin with a paraffin wax seal (s'Jacob & Van Bezooijen, 1984; Hooper, 1986). For observations of the vulval cone, cysts were soaked in 45% lactic acid for 15 min, transferred to water and the posterior region dissected and mounted in glycerin with a paraffin seal (De la Jara-Alcocer *et al*., 1994). All measurements were taken using an Olympus CX31 microscope with Infinity Analyze software v6.5.2. and drawings were made from pictures using the software Inkscape v0.92.

For scanning electron microscopy, J2, males and cysts were processed. The nematodes were fixed in 4% glutaraldehyde, post-fixed in 1% osmium tetroxide and dehydrated in increasing concentrations of ethanol (30-100%). Samples were critical point-dried and coated with gold palladium for viewing in a scanning electron microscope (Shepherd & Clarke, 1986).

Life cycle

Two hosts, tomato and common lambsquarter, were used for studying the life cycle of *C. solani* n. sp. Fifty-four pots, each containing 700 g of naturally infested soil (average 2380 J2), were put in the glasshouse at 20-25°C; 30 of these were sown with eight seeds of common lambsquarter each, and 24 pots with five seeds of tomato 'Rio Grande' each. Different dates for plant examination were selected. For common lambsquarter, three pots were destructively sampled at 2, 4, 6, 10, 15, 20, 25, 30, 35 and 40 post-germination days (PGD). For tomato, three pots were destructively sampled every 7 days for 56 PGD. The plants were removed from the pots, carefully washed with tap water and the roots examined under a stereoscopic microscope for white females and cysts. Then, roots were stained by the acid fuchsin lactoglycerol technique to observe nematode stages inside the roots (Byrd *et al*., 1983). The soil was used for the extraction of males and J2 by the centrifugal flotation technique (Jenkins, 1964).

Molecular study

DNA was extracted from several single cysts containing J2 and eggs from two populations of *C. solani* n. sp., one from the glasshouse of ENCB-IPN, Mexico City, Mexico (MT3HE), and one from Palmar de Bravo, Puebla, Mexico (M124E). Extraction was done using proteinase K. A sample of *Cactodera* sp. 2 from Cumuatillo, Mexico (Subbotin *et al*., 2011), was also included in this study. Detailed protocols for DNA extraction, PCR, cloning and sequencing for all studied samples are described by Tanha Maafi *et al*. (2003). The forward TW81 (5′-GTT TCC GTA GGT GAA CCT GC-3′) and reverse AB28 (5′-ATA TGC TTA AGT TCA GCG GGT-3′) primers (Tanha Maafi *et al*., 2003) amplifying the ITS1-5.8S-ITS2 of rRNA gene and the forward JB3 (5′-TTT TTT GGG CAT CCT GAG GTT TAT-3′) and the reverse JB4 (5′-TAA AGA AAG AAC ATA ATG AAA ATG-3′) primers (Derycke *et al*., 2010) amplifying the partial *COI* gene were used in PCR. New sequences obtained in the present study were submitted to the GenBank database under the accession numbers MN994488-MN994493 and MN993285-MN993289.

The newly obtained sequences for the ITS rRNA and partial *COI* gene were aligned using ClustalX 1.83 with default parameters with the corresponding gene sequences for *Cactodera* deposited in GenBank (Subbotin *et al*., 2001, 2011; Tanha Maafi *et al*., 2003; Cid del Prado Vera & Subbotin, 2014; Saranya *et al*., 2017; Feng *et al*., 2018; Skantar *et al*., 2019, and others). Outgroup taxa for each dataset were chosen according to the results of previously published data (Cid del Prado Vera & Subbotin, 2014). Sequence datasets for each gene fragment were analysed separately with Bayesian inference (BI) using MrBayes 3.1.2 (Huelsenbeck & Ronquist, 2001). BI analysis under

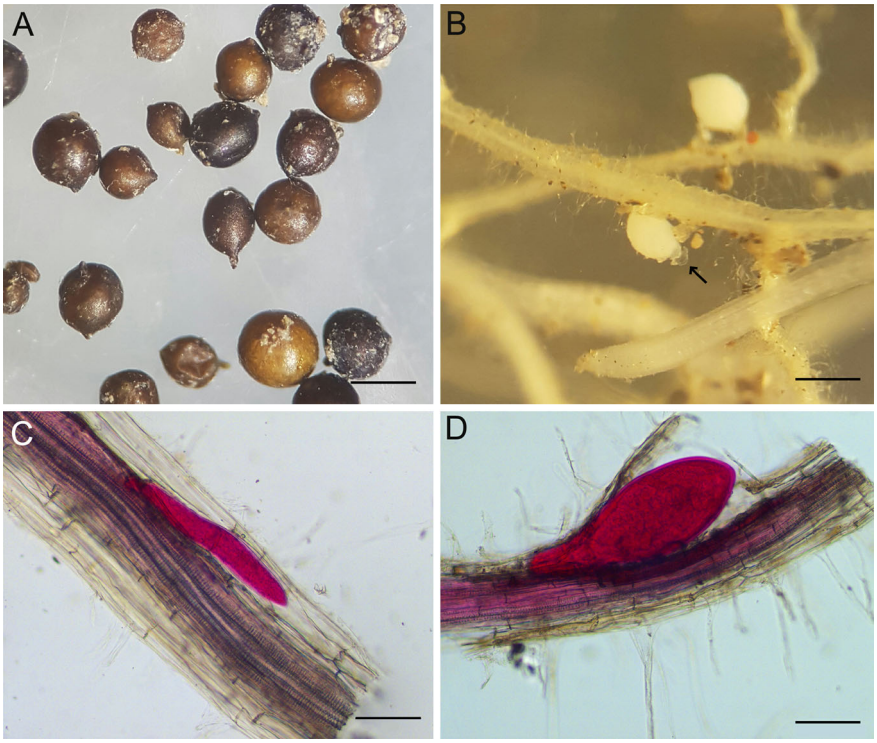

Fig. 1. Light microscopy photos of cysts, females and third-stage juvenile (J3) of *Cactodera solani* n. sp. A: Cysts; B: White females on roots (arrow indicates gelatinous sac); C: Semi-endoparasitic J3; D: Adult female on roots. C and D stained by the acid fuchsin lactoglycerol technique. (Scale bars: A, B = 500 μm; C, D = 100 μm.)

the GTR + I + G model for each gene was initiated with a random starting tree and was run with four chains for 1×10^6 generations. Posterior probabilities (PP) are given on appropriate clades. 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 solani* **n. sp.**
(Figs 1-6)

MEASUREMENTS

See Table 1.

* Specific epithet formed after the generic name of the host, *Solanum lycopersicum*.

DESCRIPTION

Female

Body pearly white, rounded to lemon-shaped with small vulval cone. Subcrystalline layer not observed. Small gelatinous sac present without eggs (Fig. 1B). Neck distinctively offset. Stylet and knobs well developed. Excretory pore located posterior to median bulb at same level as end of isthmus. Some females with vulval cones showing slightly protruding lips.

Cyst

Rounded to lemon-shaped, light brown to almost black with small vulval cone (Figs 1A; 2B; 3E). Cyst surface with zigzag pattern, not prominent, on surface of vulval cone (Fig. 2A). Vulval cone circumfenestrate with denticles sometimes present (Figs 3F; 4). Bullae absent.

Male

Body vermiform with slightly tapering anterior end (Fig. 5A, B). Labial region slightly offset with oval-shaped labial disc presenting six lip sectors. Stylet well

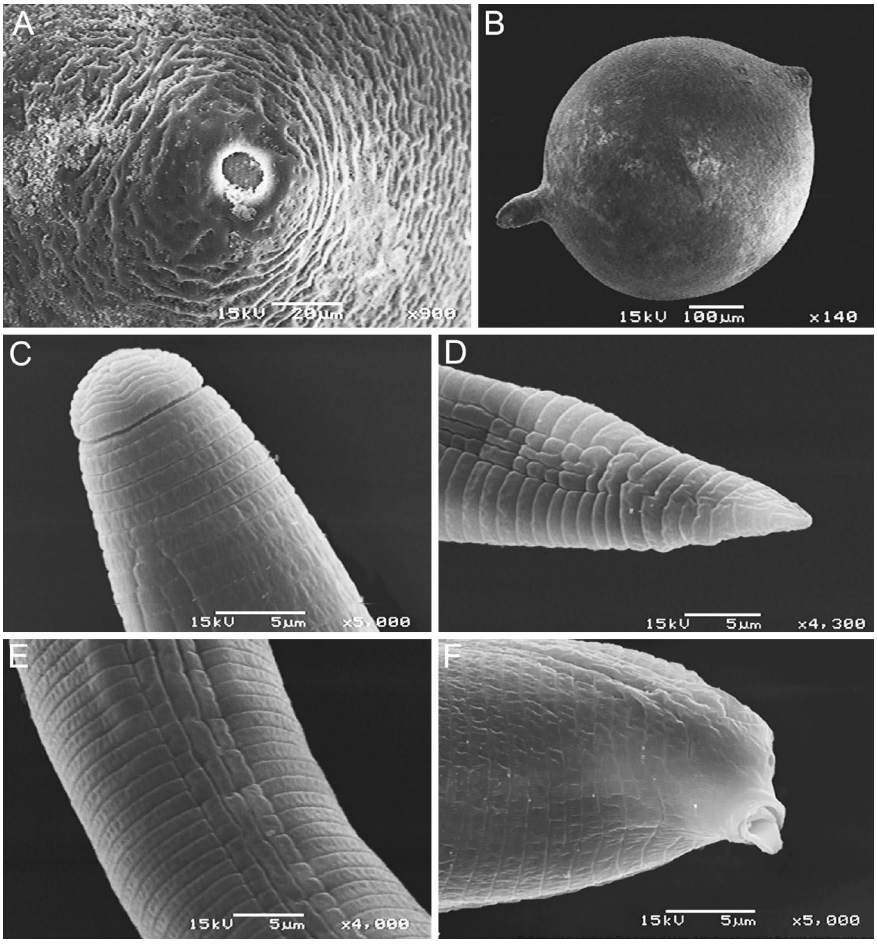

Fig. 2. SEM micrographs of cyst, second-stage juvenile (J2) and male of *Cactodera solani* n. sp. A: Circumfenestrate vulval cone; B: Cyst; C: Anterior region of J2; D: Tail of J2 with lateral field; E: Lateral field of male; F: Male tail showing spicules and anus.

developed with rounded knobs (Figs 3C; 5B). Lateral field with four lines with outer two ridges partially areolated along body (Fig. 2E). Spicules curved with tips slightly notched. Tail short, phasmids not observed (Figs 2F; 3D; 5C).

Second-stage juvenile

Body vermiform, tapering at extremities, more so posteriorly (Figs 2D; 5D). Head region offset (Figs 2C; 3A). Stylet well developed, knobs rounded to slightly projecting anteriorly (Fig. 5E). Pharyngeal glands overlapping ventrally. Excretory pore located near level of gland lobe. Lateral field with four lines with outer two ridges partially areolated along body (Fig. 5F). Tail tapering, hyaline region clearly demarcated by a U-shaped outline (Figs 3B; 5G).

Eggs

Exterior shell with small, distinct punctations visible with the light microscope (Fig. 6).

Type host and locality

Tomato, *Solanum lycopersicum*, in the glasshouse of Escuela Nacional de Ciencias Biológicas-Instituto Politécnico Nacional, Mexico City, Mexico. Coordinates: 19°27′14″N, 99°10′19″W; altitude: 2255 m a.s.l. The glasshouse was selected as the type locality as the morphological, morphometric and molecular characterisation of *C. solani* n. sp. was based from this population.

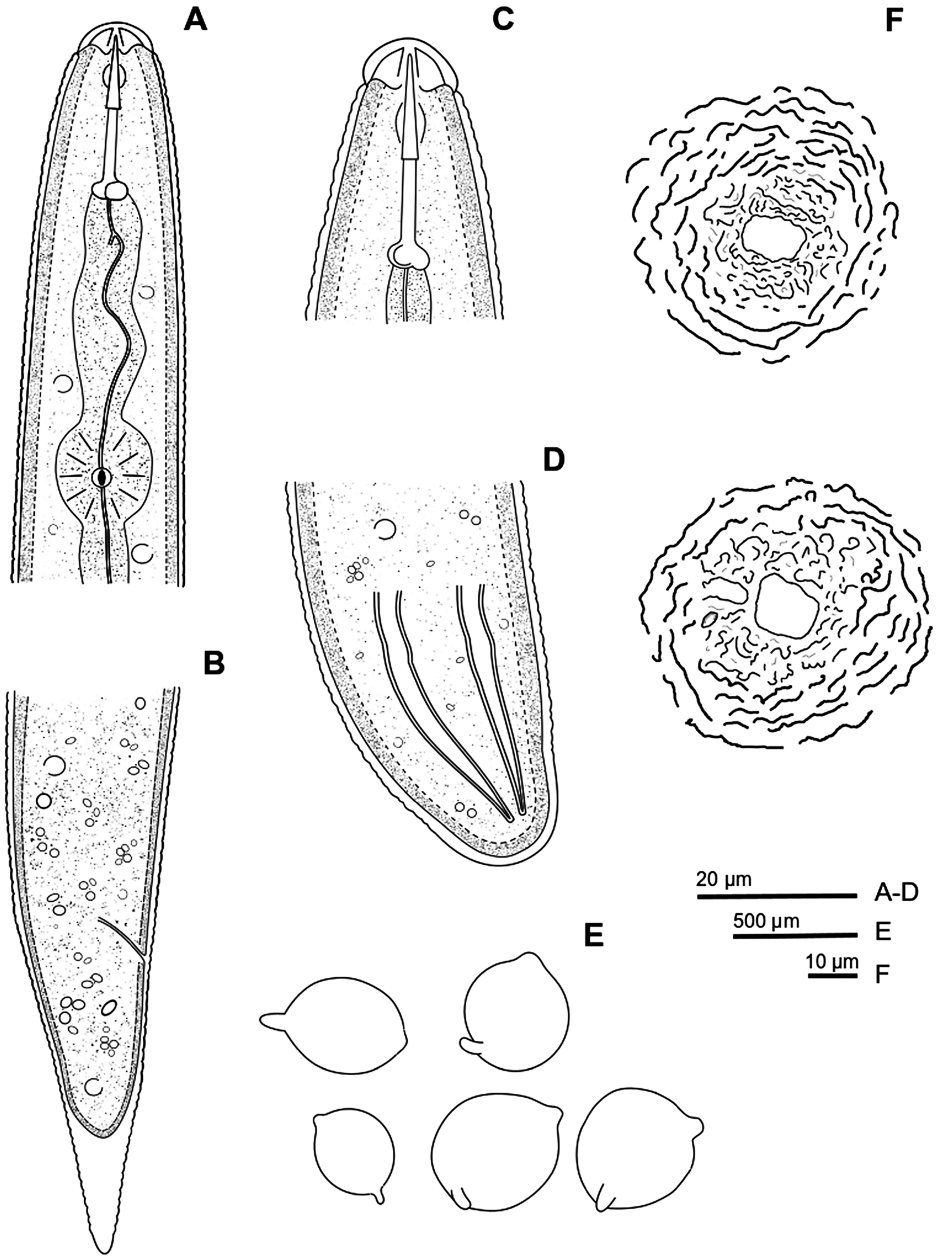

Fig. 3. Drawings of *Cactodera solani* n. sp. A: Second-stage juvenile (J2) anterior region; B: J2 tail. C: Male anterior region; D: Male tail; E: Entire cysts; F: Circumfenestrate vulval cone.

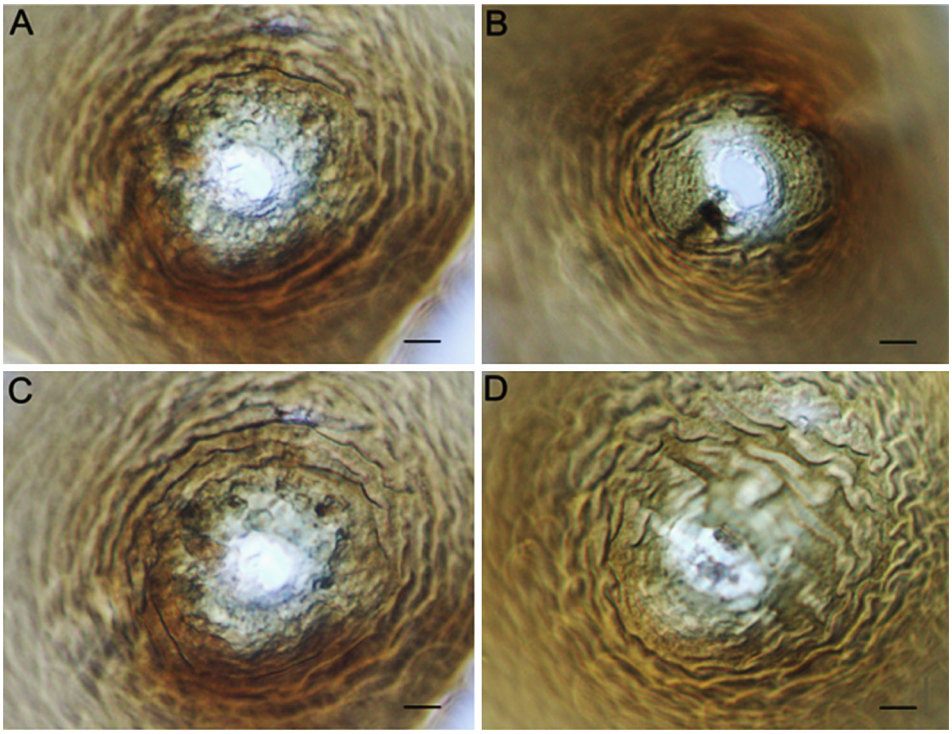

Fig. 4. Circumfenestrate vulval cones of *Cactodera solani* n. sp. (Scale bar = 5 μm.)

Other hosts and other localities

Common lambsquarter, *Chenopodium album*, in the glasshouse of Escuela Nacional de Ciencias Biológicas-Instituto Politécnico Nacional, Mexico City, Mexico. Coordinates: 19°27′14″N, 99°10′19″W; altitude: 2255 m a.s.l.

Soil from a broccoli field in Palmar de Bravo, Puebla, Mexico. Coordinates: 18°53′36″N, 97°39′52″W; altitude: 2158 m a.s.l.

Type material

Thirty paratype cysts, males and J2 deposited in the Colección de Nematología de la Escuela Nacional de Ciencias Biológicas-Instituto Politécnico Nacional, Mexico.

Diagnosis and relationships

Cactodera solani n. sp. has small rounded to lemon-shaped, light brown to almost black cysts 417 (291-581) × 324 (204-505) μm in size and with a circumfenestrate vulval cone (Table 2). Females are pearly white with a small gelatinous sac present without eggs. The exterior shell of the eggs has small, distinct punctations. The J2 are vermiform, tapering at the extremities and 434 (379-511) μm long, the stylet is well developed with the knobs rounded to slightly projecting anteriorly, the lateral field has four lines, and the hyaline region is clearly demarcated by a U-shaped outline.

The morphometric and molecular data show that the new species mostly resembles *C. milleri* Graney & Bird, 1990. Morphometrically, *C. solani* n. sp. can be differentiated from *C. milleri* by having smaller-sized cysts (417 (291-581) × 324 (204-505) *vs* 632 (515-730) × 506 (419-598) μm), larger mean L/W cyst ratio of 1.3 (1.2-1.4) *vs* 1.2 (1.1-1.4), and the longer J2 stylet of 25 (24-27) *vs* 22 (21-23) μm (Table 2). This new cyst-forming nematode species also can be differentiated from the other 15 valid species of *Cactodera* by the size of the cysts, *C. solani* n. sp. having the smallest cysts described for the genus at 417 (291-581) × 324 (204-505) μm in size. Another valuable characteristic for the diagnosis is the host, *C. solani* n. sp. being the only species of the genus known to parasitise tomato.

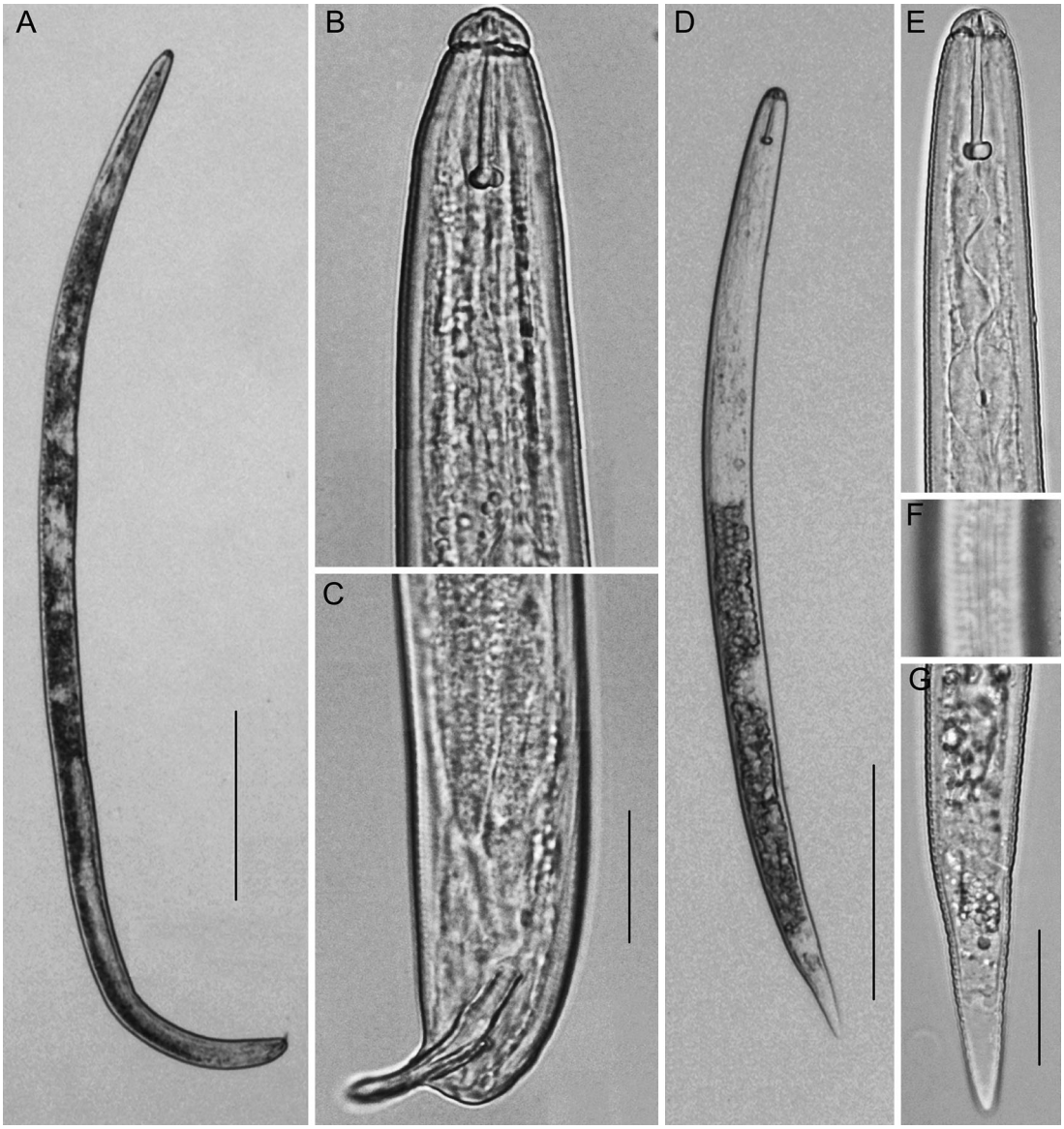

Fig. 5. Light microscopy photos of *Cactodera solani* n. sp. A: Entire male; B: Male anterior region; C: Male tail; D: Entire second-stage juvenile (J2); E: J2 anterior region; F: J2 lateral field; G: J2 tail. (Scale bars: A = 200 μm; B, C = 20 μm; D = 100 μm; E-G = 20 μm.)

BIOLOGY

Cactodera solani n. sp. was found attached to roots of tomato and common lambsquarter in the glasshouse of ENCB-IPN, Mexico City, Mexico (Fig. 1B, D). This new species was shown to be endoparasitic to semi-endoparasitic in habit. Some endoparasitic stages (J2 and third-stage juveniles (J3)) were observed, using the acid fuchsin lactoglycerol technique, with the posterior body portion located outside of the roots on both hosts (Fig. 1C). Occasionally, females produce a small gelatinous sac, although no eggs were observed within (Fig. 1B). *Cactodera solani* n. sp. has several generations per year. White females, cysts and J2 can be found in the glasshouse of ENCB-IPN during spring to autumn, but not in winter.

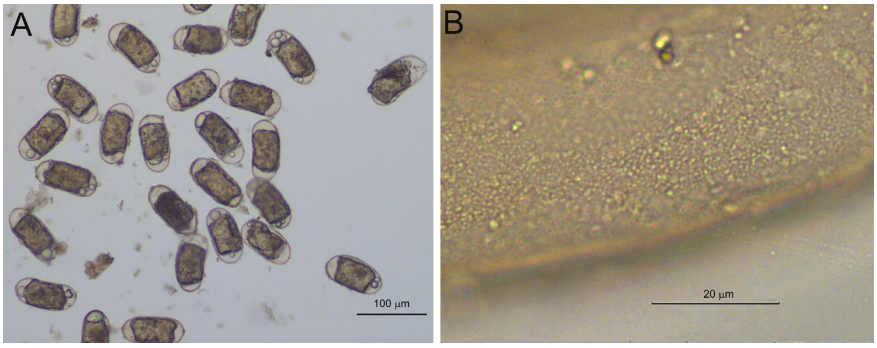

Fig. 6. Light microscopy photos of (A) eggs and (B) punctations in the eggshell of *Cactodera solani* n. sp.

Life cycle

The life cycle of *C. solani* n. sp. was completed in 49 days in tomato and in 40 days in common lambsquarter at 20-25°C. In tomato, the J2, J3, fourth-stage juveniles (J4), females and cysts were found inside and on the roots at 21, 28, 35, 42 and 49 PGD, respectively. Males were found in soil extractions from 42 to 49 PGD. On common lambsquarter, the J2, J3, J4, females and cysts were found inside and on the roots at 2, 10, 15, 30 and 40 PGD, respectively. Males were found in soil extractions from 30 to 35 PGD.

Molecular study and phylogenetic analysis

In total, six ITS rRNA and four partial *COI* gene sequences of *C. solani* n. sp. were obtained: two ITS rRNA (MT3HEacl1-TW and MT3HEacl2-TW) and two partial *COI* gene sequences (MT3HEa and MT3HEb) from Mexico City, and four ITS rRNA (M124Ebcl1, M124Ebcl2, M124Ebcl1-Vrain, M124Ebcl2-AB) and two partial *COI* gene sequences (M124Ea and M124Eb) from Palmar de Bravo, Puebla. A partial *COI* gene sequence of a putative new *Cactodera* species was also obtained. The phylogenetic relationships of *Cactodera* are given in Figures 7 and 8. In the tree obtained from the ITS rRNA gene sequence alignment, *C. solani* n. sp. formed a clade with *C. milleri* (Fig. 7), differing from this species by 7-13 bp (0.8-1.4%). In the tree obtained from the partial *COI* gene sequence alignment, *C. solani* n. sp. formed a clade with *Cactodera* sp. 2 (Fig. 8), differing by 59-64 bp (16-17%).

Discussion

Cactodera previously had 15 valid species and with the present description of *C. solani* n. sp. the total number is now 16 (Subbotin *et al*., 2010; Cid del Prado Vera & Subbotin, 2014; Feng *et al*., 2018). This genus is considered to be endemic to Mexico and has a recorded host range that includes plants from the families Cactaceae, Amaranthaceae, Poaceae and Chenopodiaceae (Cid del Prado Vera *et al*., 2018). Seven species of the genus are reported in Mexico: *C. amaranthi* (Stoyanov, 1972) Krall & Krall, 1978, *C. cacti* (Filipjev & Schuurmans Stekhoven, 1941) Krall & Krall, 1978, *C. evansi* Cid del Prado Vera & Rowe, 2000, *C. galinsogae* Tovar Soto, Cid del Prado Vera, Nicol, Evans, Sandoval Islas & Martinez Garza, 2003, *C. rosae* Cid del Prado Vera & Miranda, 2008, *C. salina* Baldwin, Mundo-Ocampo & McClure, 1997 and *C. torreyanae* Cid del Prado Vera & Subbotin, 2014 (see Sosa-Moss, 1986; Baldwin & Mundo-Ocampo, 1991; Tovar-Soto *et al*., 2006; Escobar-Avila *et al*., 2018), of which five were described in Mexico.

Cactodera solani n. sp. was found in Mexico City parasitising tomato (Solanaceae) and common lambsquarter (Chenopodiaceae), and in Palmar de Bravo, the latter being a municipality that belongs to an important vegetable-producing area in Mexico known as the Tepeaca Valley in Puebla State, Mexico. It is likely that the new species got into the glasshouse of ENCB-IPN as a result of one of the several nematological surveys carried out by our research group in this area, although more sampling is needed to determine the distribution of this nematode. This is the first species described of the genus *Cactodera* that has as a host a member in the Solanaceae family. The new species was shown to be endoparasitic to semi-endoparasitic in habit, a characteristic that has also been reported for other species of the genus, such as *C. torreyanae* and *C. chenopodiae* (Cid del Prado Vera & Subbotin, 2014; Feng *et al*., 2018). The life cycle of *C. solani* n. sp. was completed in 49 days on tomato and 40 days on common lambsquarter (20-25°C), in both cases being

Table 1. Morphometrics of females (n = 30), cysts (n = 30), second-stage juveniles (J2; n = 30), males (n = 5) and eggs (n = 30) of *Cactodera solani* n. sp. All measurements are in μm and in the form: mean ± s.d. (range).

Character	Holotype	Paratypes
Female	–	
Length	524	544 ± 42.1 (467-632)
Width	325	344 ± 40.9 (270-437)
L/W	1.6	1.57 ± 0.1 (1.34-1.75)
Cyst		
L (excluding neck)	552	417 ± 72.4 (291-581)
Width	382	324 ± 75.9 (204-505)
L/W	1.4	1.3 ± 0.16 (1.2-1.4)
Fenestral diam.	32.5	26.3 ± 4.6 (20.0-36.0)
J2		
L		434 ± 32.6 (379-511)
a		17.9 ± 1.7 (14.5-21.3)
b		2.6 ± 0.2 (2.3-3.2)
b′		2.3 ± 0.2 (2.1-2.7)
c		10.8 ± 1.5 (9.2-13.5)
c′		2.8 ± 0.3 (2.2-3.3)
Stylet length		25.3 ± 0.9 (23.5-27.0)
Stylet knob height		2.8 ± 0.4 (2.0-3.5)
Labial region height		4.2 ± 0.4 (3.4-4.9)
Labial region diam.		10.0 ± 0.8 (8.7-12.2)
DGO		5.0 ± 0.9 (3.7-6.9)
Anterior end to:		
median bulb valve		70 ± 5.0 (61-79)
end of pharyngeal glands		188 ± 14.6 (150-220)
excretory pore		103 ± 9.0 (89-120)
Pharynx length		162 ± 17.4 (107-196)
Body diam. at mid-body		24.2 ± 2.9 (18.4-29.7)
Body diam. at anus		14.6 ± 2.09 (10-18.2)
Tail length		40.4 ± 5.2 (28-49.3)
Hyaline region length		16.4 ± 2.2 (12.3-22.8)
Male		
L		1238 ± 55.5 (1184-1295)
Body diam.		37.5 ± 2.5 (34.9-39.9)
Labial region height		4.9 ± 0.4 (4.6-5.6)
Labial region diam.		10.8 ± 0.1 (10.6-10.9)
Anterior end to median bulb valve		152 ± 10.5 (140-165)
Anterior end to excretory pore		85.7 ± 5.2 (80-92)
Pharynx length		144 ± 7.8 (136-151)
Stylet length		23.2 ± 1.7 (21.3-24.5)
Genital tract length		605 ± 68.1 (529-661)
Spicule length		34.7 ± 2.7 (31.8-37.2)
Gubernaculum length		10.0 ± 2.0 (7.8-11.9)
Egg		
Length		111 ± 5.5 (102-120)
Diam.		44 ± 1.5 (41-47)
L/W		2.5 ± 0.2 (2.2-2.8)

shorter than the 56 days required for *C. galinsogae* on barley (*Hordeum vulgare* L.) at 16-20°C, and more than the 25 days for *C. torreyanae* in *Suaeda edulis* Flores Olv. & Noguez, at 20°C (Tovar-Soto *et al*., 2008; Evans *et al*., 2015). The difference in time needed to complete the life cycle of a cyst-forming nematode depends upon the co-evolution of the species with its host and the environmental conditions. In temperate regions, life cycles are completed in about 30 days (Moens *et al*., 2018). Under glasshouse conditions, *C. solani* n. sp. (20-25°C) has several generations per year with white females and cysts being found attached to tomato and common lambsquarter roots during the spring to autumn, but not in winter. The number of generations per year varies between cyst-forming nematode species, most of the temperate species completing one or two generations, corresponding to the natural life cycle of its host combined with the optimal temperature range. However, where favourable environmental conditions are more constant throughout the year, as in Mexico, multiple generations occur (Moens *et al*., 2018).

Sequences of *C. solani* n. sp. formed a single clade and showed it to be phylogenetically closely related to *C. milleri* based on the ITS rRNA, differing from this species by 7-13 bp (0.8-1.4%). These results agree with those obtained in our morphological study, where this new species is closest morphometrically to *C. milleri*. These two taxa can be considered as sibling species because of the similarities in their morphological and molecular characteristics. The partial *COI* gene tree also showed a single clade for sequences of *C. solani* n. sp.

The mitochondrial cytochrome c oxidase subunit 1 (*COI*) gene is known as the standard molecular barcode for many animals and is used to facilitate the correct identification of specimens and the discovery of new species. This region is translated into an evolutionarily conserved protein and presents a high degree of polymorphism, therefore generating a higher possibility of discovering intraspecific polymorphism than rRNA genes. The *COI* gene and other mtDNA genes are also used for phylogeographical analysis of cyst nematode populations (Subbotin *et al*., 2018, 2020).

Sequences of the *COI* gene of *Cactodera* are scarce in GenBank. Previous to this study, there were only two sequences published, one for *Cactodera* sp. and one for *C. chenopodiae* (Feng *et al*., 2018; Powers *et al*., 2019). Generating more sequence information for *Cactodera* is necessary to understand the phylogenetic

Table 2. Morphological and morphometric characters of cysts, eggs and second-stage juveniles (J2), useful for identification of *Cactodera* species. All measurements are in μm (modified from Subbotin *et al*., 2010).

Species	Stage							
	Cyst			Egg	J2			
	Size	L/W ratio	Fenestral diam.	Shell surface	L	Stylet	Hyaline region	Tail
C. acnidae	635 (543-756) × 417 (319-493)	1.5 (1.1-2.1)	30 × 33	Smooth	411 (361-448)	21 (19-25)	22 (17-26)	44 (43-48)
C. amaranthi	642 (525-774) × 472 (370-550)	1.4 (1.1-1.7)	31 (25-38)	Smooth	374 (340-406)	21 (20-21)	15 (11-18)	35 (31-40)
C. cacti	442-587 × 328-475	1.1-1.4	26-38	Punctate	368-504	22-26	16-25	41-55
C. chenopodiae	486 (423-585) × 334 (283-398)	1.5 (1.2-1.7)	23 (20-26)	Punctate	490 (438-539)	24 (22-26)	23 (17-28)	46 (39-51)
C. eremica	620 (530-810) × 434 (290-590)	1.5 (1.2-1.9)	21 (14-25)	Punctate	480 (440-510)	27 (25-28)	18 (17-23)	40 (36-47)
C. estonica	852 (686-1014) × 383 (312-468)	2.3 (2.0-2.4)	18-30	Smooth	428-440	23	17-19	34-40
C. evansi	459 (416-528) × 334 (284-384)	1.4 (1.2-1.7)	18-23	Punctate	387 (358-420)	20 (20-24)	21 (16-23)	40 (34-44)
C. galinsogae	523 (453-675) × 384 (284-508)	1.4 (1.1-1.7)	41 (33-56)	Punctate	401 (358-443)	22 (19-31)	18 (10-24)	37 (26-45)
C. milleri	632 (515-730) × 506 (419-598)	1.2 (1.1-1.4)	13-18	Punctate	426 (370-479)	22 (21-23)	18 (15-21)	43 (37-49)
C. radicale	682 (553-986) × 438 (220-626)	1.6 (1.3-2.6)	24 (17-28)	Smooth	488 (467-520)	25 (20-27)	20 (15-28)	52 (46-60)
C. rosae	654 (460-840) × 433 (280-560)	1.5 (1.2-2.1)	18 (10-21)	Punctate	397 (348-472)	20 (16-26)	6 (4-8)	39 (31-68)
C. salina	603 (415-742) × 375 (193-475)	1.6 (1.4-2.2)	24 (20-28)	Smooth	458 (410-514)	24 (23-25)	20 (10-31)	35 (31-48)
C. solani n. sp.	417 (291-581) × 324 (204-505)	1.3 (1.2-1.4)	26 (20-36)	Punctate	434 (379-511)	25 (24-27)	16 (12-23)	40 (28-49)
C. thornei	548-656 × 432-448	1.3-1.5	29-34	Punctate	457-554	25-27	25-27	52-56
C. torreyanae	575 (364-712) × 303 (92-432)	1.9 (1.4-2.9)	20 × 16	Smooth	440 (390-550)	22 (21-23)	19 (16-25)	38 (32-45)
C. weissi	524-598 × 350-394	1.7 (1.2-2.3)	34 (29-38)	Smooth	407-489	20-21	20-24	46

and phylogeographical relationships of the species in this genus.

Cactodera solani n. sp is closely related to *C. milleri*, these sibling species being morphometrically differentiated by the size of the cysts and the length of the J2 stylet (see Diagnosis and relationships section). The ITS rRNA and *COI* gene sequences formed a single clade that clearly differentiated *C. solani* n. sp. from other *Cactodera* species, supporting this new species as an independent lineage.

Cactodera spp. are not considered to cause significant economic crop loss in Mexico; however, even though pathogenicity tests have not been conducted for *C. solani* n. sp., this species should be considered of importance as it is a parasite of tomato, an important crop worldwide. Further studies on the biology, host range, distribution and pathogenicity of *C. solani* n. sp. are planned.

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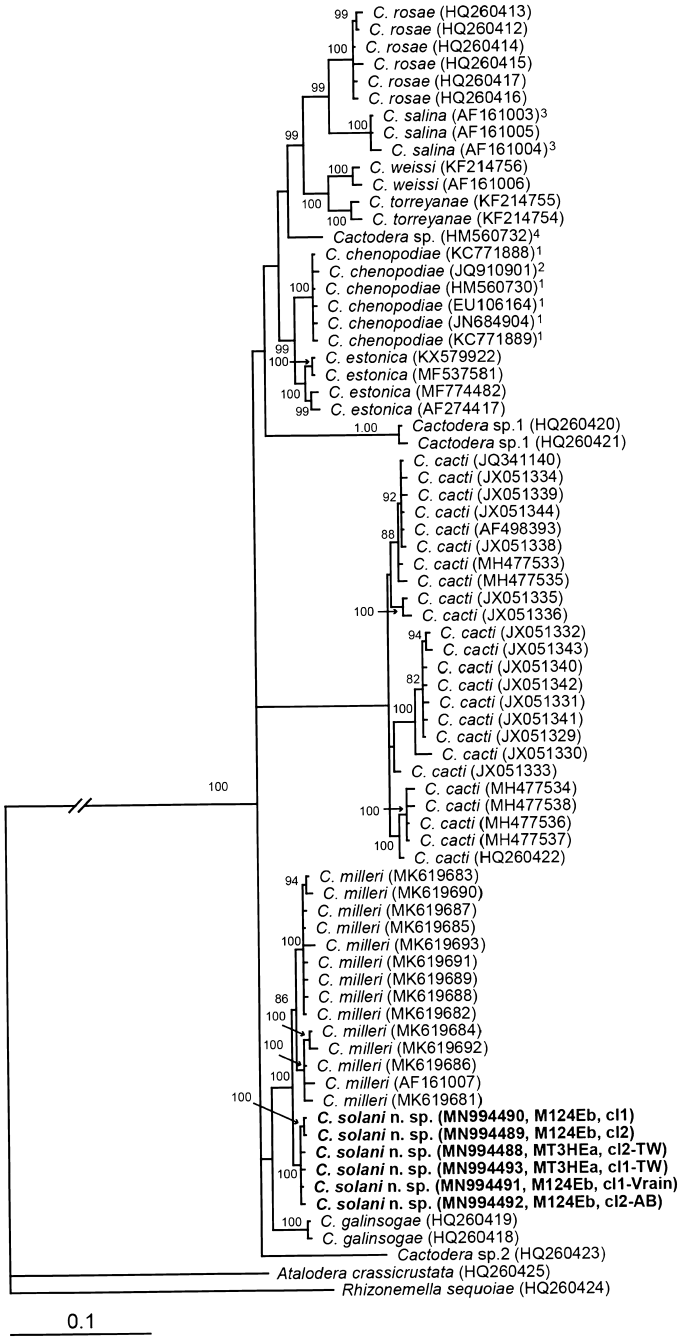

Fig. 7. Phylogenetic relationships within populations and species of *Cactodera*. The 50% majority rule consensus trees from Bayesian analysis generated from two runs as inferred from the analysis of the ITS rRNA gene sequences under the GTR + G + I model. PP values are given in appropriate clades. Newly sequenced samples are indicated by bold font. *Cactodera solani* n. sp. from Mexico City: MT3HEacl1-TW and MT3HEacl2-TW; and from Palmar de Bravo, Puebla: M124Ebcl1, M124Ebcl2, M124Ebcl1-Vrain, M124Ebcl2-AB. Identification of some sequences were corrected based on the results of the present sequence and phylogenetic analysis: [1] – originally identified in GenBank as *C. estonica* by Wei *et al*. (unpubl.), Zhang & Hu (unpubl.), and Peng *et al*. (unpubl.); [2] – originally identified in GenBank as *C. eremica* by Duan *et al*. (unpubl.); [3] – originally identified in GenBank as *Globodera millefolii* or *G. artemisiae* by Ferris *et al*. (1999); [4] – originally identified in GenBank as *C. estonica* by Peng *et al*. (unpubl.).

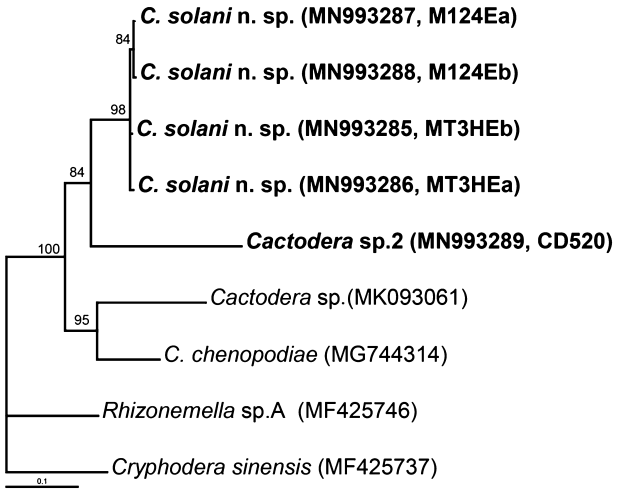

Fig. 8. Phylogenetic relationships within populations and species of *Cactodera*. The 50% majority rule consensus trees from Bayesian analysis generated from two runs as inferred from the analysis of the partial *COI* gene sequences under the GTR + G + I model. PP values are given in appropriate clades. Newly sequenced samples are indicated by bold font. *Cactodera solani* n. sp. from Mexico City: MT3HEa and MT3HEb; and from Palmar de Bravo, Puebla: M124Ea and M124Eb.

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