A new species, *Cactodera herba* sp. n. (Nematoda: Heteroderidae), with molecular characterisation of some cyst nematodes from Mexico

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Summary. In 2018-2021, during nematological surveys in several regions of Mexico, *Cactodera solani*, *C. rosae*, *Cactodera* sp., a new species, *C. herba* sp. n. and three unidentified cyst nematode species belonging to an unknown genus were recovered and distinguished from soil and root samples. *Cactodera herba* sp. n. collected from liverseed grass, *Urochloa panicoides*, growing around peach trees at the Campus Montecillo, Colegio de Postgraduados, Mexico State, Mexico, is described and illustrated. The new species is characterised by cysts 444-752 µm long, with light to dark brown colour, a small vulval cone, and average fenestral diameter of 36.4 µm. The second-stage juvenile of the new species is 400-490 µm in body length with stylet 22-28 µm long. Eggshell with distinct punctation. Five new D2-D3 of 28S rRNA, 15 ITS rRNA and 3 *COI* gene sequences were obtained from 12 samples of seven cyst nematode species. These gene sequences differentiated *C. herba* sp. n. from other molecularly characterised *Cactodera* species. Phylogenetic relationships within species of *Cactodera* and other Heteroderidae are given based on the analysis of the D2-D3 of 28S rRNA, ITS rRNA and the partial *COI* gene sequences. In the ITS rRNA gene tree, sequences of three species (cyst nematode sp.A, sp.B, sp.C) belonging to an unknown genus formed a separate lineage within Punctoderinae and had sister relationships with the clade of the genus *Globodera* parasitising solanaceous plants.

Key words: 28S rRNA gene, *Cactodera*, *COI* gene, grass host, taxonomy, phylogeny.

In 2018-2021, during nematological surveys, cysts of several cyst nematodes were collected in several states of Mexico. Two known and two unknown species of the genus *Cactodera* Krall & Krall, 1978 were recovered from soil and root samples. One of the *Cactodera* species was determined to be new. The new species collected from roots of a grass in a peach orchard at the Colegio de Postgraduados, Montecillo Campus, Mexico State is described herein. Several unidentified cyst nematode species belonging to an unknown genus were also collected. Molecular characterisation of cyst nematodes found during these surveys is provided in this study.

MATERIALS AND METHODS

Nematode populations. Cysts, second-stage juveniles (J2) and males were extracted from soil samples (Table 1) using the decanting, sieving and sugar flotation method (Jenkins, 1964). Specimens were hand-picked under a stereomicroscope and used for microscopic study or transferred to 70% ethanol for molecular analysis.

Morphological study. Hand-picked nematodes were killed by heating in a drop of water and fixed in Golden solution (Hooper, 1970). They were transferred to 20 ml vials and stored at room temperature for 10 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 for light microscopy (LM). Measurements and drawings were made using a drawing tube and an American Optical compound microscope.

The specimens for scanning electron

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Table 1. Species and populations of cyst nematodes from Mexico characterised in this study.

Species	Location	GPS coordinates: latitude, longitude, altitude	Associated plants	Sample code	GenBank accession number		
					D2-D3 of 28S rRNA	ITS rRNA	*COI*
Cactodera herba sp.n.	Mexico State, Texcoco County, Campus Montecillo CP	19. 45936, -98.90423, 2230 m	*Urochloa panicoides*	CD2995, CD3554	PP464030	PP464037, PP464038	PP441948, PP441949
C. rosae	Tlaxcala State, Huamantla, Francisco Villa Tecoac	19.38455, -97.92855, 2440 m	Unknown plants	CD3562	-	PP464043	PP441950
C. solani	Tlaxcala State, Huamantla, Francisco Villa Tecoac	19.38455, -97.92855, 2440 m	*Chenopodium* sp.	CD3552b, CD3586a, b,c	PP464028	PP464033-PP464036	-
Cactodera sp.3	Mexico, Queretaro State, Tequisquiapan County	20.3070, -99.55061, 1941 m	*Opuntia* sp. and grasses	CD3612, CD3613, CD3616, CD3553	PP464029	PP464039-PP464042	-
Cyst nematode sp.A	Morelos State, Juchitepec Road 113 to Oaxtepec town, Oak forest	19.10200, -98.93365, 2830 m	Solanaceous plants	CD2810	PP464031	PP464046	-
Cyst nematode sp.B	Hidalgo State, Singuilucan, Rincon del Puerto	20.04541, -98.51611, 2550 m	*Solanum rostratum* and grasses	CD3011k	PP464032	PP464044, PP464045	-
Cyst nematode sp.C	Hidalgo State, Singuilucan, Rincon del Puerto	20.04541, -98.51611, 2550 m	*Solanum rostratum* and grasses	CD3571	-	PP464046	-

microscope (SEM) studies were washed in magnesium buffer solution, pH 7.2, for 20 min and dehydrated in an ethanol solution series, from 10 to 100% in ten stages for 20 min each. Samples were then critical-point dried before coating with gold/palladium (80/20%) for 4 min followed by observation in a Jeol JSM 6390 microscope at 10 kV (Cid Del Prado Vera *et al.,* 2012).

Molecular study. DNA was extracted from single cysts using proteinase K. DNA extraction and PCR protocols were conducted according to Subbotin (2021a). Three primer sets were used for amplification of nematode genes: *i*) D2A (5' - ACA AGT ACC GTG AGG GAA AGT TG - 3') and D3B (5' - TCG GAA GGA ACC AGC TAC TA - 3') amplifying the D2-D3 expansion segments of 28S rRNA gene and *ii*) TW81 (5' - GTT TCC GTA GGT GAA CCT GC - 3') and AB28 (5' - ATA TGC TTA AGT TCA GCG GGT - 3') amplifying the ITS rRNA gene; and *iii*) Het-coxiF (5' - TAG TTG ATC GTA ATT TTA ATG G - 3') and Het-coxiR (5' - CCT AAA ACA TAA TGA AAA TGW GC - 3') amplifying the partial *COI* gene of mtDNA (Subbotin, 2021a). The successfully amplified fragments were purified and then sequenced by Azenta (MA, USA) with the primer pairs used in PCR.

New sequences were aligned using ClustalX 1.83 with corresponding selected and published gene sequences for nematodes (Subbotin *et al.,* 2011; Cid del Prado Vera & Subbotin, 2014; Feng *et al.,* 2018; Escobar-Avila *et al.,* 2021; Li *et al.,* 2021; Ni *et al.,* 2024). Outgroup taxa for each dataset were chosen according to the results of previously published data (Cid del Prado Vera & Subbotin, 2014). Sequence alignments were analysed with Bayesian inference (BI) using MrBayes 3.1.2 (Huelsenbeck & Ronquist, 2001). BI analysis for each gene was initiated with a random starting tree and was run with four chains for 1.0×10^6 generations as described by Subbotin (2021b). Posterior probabilities (PP) are given on appropriate clades. The new sequences were submitted to the GenBank database under accession numbers: PP464028 - PP464032 (28S rRNA gene), PP464033 - PP464047 (ITS rRNA gene), PP441948 - PP441950 (*COI* gene).

RESULTS

Using traditional morphological taxonomic characters integrated with molecular criteria, a new species, *Cactodera herba* sp. n. (Figs 1-5A, B, G, H), two known species, *C. solani* Escobar-Avila *et al.,* 2021 (Fig. 5C, D, I, J), *C. rosae* Cid del Prado Vera & Miranda, 2008, one unidentified species, *Cactodera* sp.3 (Fig. 5E, F, K, L), and three unidentified cyst nematode species (cyst nematode sp.A, sp.B, sp.C) belonging to an unknown genus were distinguished in soil samples collected in five states of Mexico. Cysts, J2 and two males were extracted for *C. herba* sp. n., whereas only a few cysts were recovered from samples for other cyst nematode species. Morphological description is given for the new species only.

Cactodera herba sp. n. (Figs 1-5A, B, G, H)

Measurements:

Holotype cyst: L (including neck) = 470 µm; W = 330 µm; L/W =1.42; vulva-anus distance = 50 µm.

Paratype cysts (n=25): L (including neck) = 555±79 (444-752) µm; W = 418±55 (323-550) µm; L/W=1.3±0.2 (1.0-2.0); fenestral diam. = 36.4±8.0 (25-50) µm; vulva slit = 28.4±4.0 (23-35) µm; vulva-anus distance = 53.8±9.1 (41-66) µm.

Paratypes second-stage juveniles (n=29): L = 440±20 (400-490) µm; W = 19.8±2.0 (17.9-28.0) µm; a = 22.4±1.7 (16.5-24.9); b = 4.4±0.6 (3.1-5.4); c = 9.6±0.8 (8.3-11.1); lip region height = 4.1±0.4 (3.8-5.0) µm; lip region width = 8.9±0.5 (8.0-10.0) µm; stylet length = 23.8±1.3 (21.6-28) µm; stylet knobs width = 3.8±0.4 (2.8-5.0) µm; tail length = 45.9±3.9 (40-55.5) µm; tail width at anus level = 12.1±0.8 (10.0-13.2) µm; hyaline part of tail length = 24.2±2.9 (19-30) µm; phasmid-anus distance = 15.3±2.1 (13-18) µm.

Paratype males (n=2): L = 620, 930 µm; a = 6.5, 8.2; b = 6.5, 8.2; c = 123.6, 233; stylet = 23.0 µm; T% = 42.0; spicules = 18, 24 µm.

Eggs (n=30): L = 109.6±6.4 (96-122) µm; W = 46.2±3.7 (40-56) µm; L/W = 2.4±0.1 (2.1-2.8).

Cysts. Lemon-shaped, with short neck and small vulval cone, light to dark brown colour (Fig. 2). Vulval cone circumfenestrate (Fig. 4). Bullae absent. Exterior of cyst wall at mid-body marked by parallel transverse lines and abundant punctuations; the lines are wavy and broken in last third of the body, especially on the vulval cone. Eggs retained in cyst body. No egg-masses.

Second-stage juvenile. Body vermiform, tapering anteriorly and posteriorly, slightly curved ventrally after heat treatment. Labial region slightly offset, hemispherical (Fig. 5A, B). Stylet well developed, dorsal stylet knob rounded or slight anteriorly projected. Pharyngeal glands overlapping ventrally. Hemizonid just anterior to, or at level of, excretory pore. Lateral field with four incisures, areolated. Transition to hyaline region demarcated with outline usually U-shaped. Tail gradually tapering with rounded terminus (Fig. 5 G, H).

Male. Labial region nearly continuous with body, usually with four annuli. Anterior surface of dorsal stylet knob concave. Hemizonid four annuli anterior to excretory pore. Lateral field with four incisures. Testis single, sperm present. Tail 4 to 5 µm long. Spicules with bifid tips.

Egg. Eggshell with distinct punctation visible under LM (Fig. 3C).

Etymology. The specific epithet is derived from the Spanish word for grass.

Type host and locality. Liverseed grass, *Urochloa panicoides* P. Beauv. growing in a peach orchard at the Colegio de Postgraduados (CP), Campus Montecillo, Mexico State, Mexico.

Type material. Slides with holotype cyst (CNHE 11602), and paratype cysts, second-stage juveniles and males (CNHE 11603) were deposited in the Laboratorio de Helmintología del Instituto de Biología, UNAM, Mexico and the Colegio de Postgraduados Nematode Collection (CPNC) (A-115). ZooBank: urn:lsid:zoobank.org:act: C2CB8536-5FC6-42AE-AF65-6F4E891D9EA

Differential diagnosis. *Cactodera herba* sp. n. is morphologically similar to *C. milleri* Graney & Bird, 1990, *C. solani* and *C. weissi* (Steiner, 1949) Krall & Krall, 1978.

The new species can be differentiated from *C. milleri* by smaller cyst length (444-752 *vs* 550-849 µm) and longer hyaline part of tail length for J2 (19-30 *vs* 14.6-21.2 µm). It differs from *C. solani* by the larger cyst length (444-752 *vs* 291-581 µm) and smaller male body length (620, 930 *vs* 1184-1295 µm). *Cactodera herba* sp. n. can be distinguished from *C. weissi* by the longer J2 (400-490 *vs* 372-420 µm) and shorter male spicules (18, 24 *vs* 30.8-33.6 µm).

Cactodera herba sp. n. can be also differentiated from several species that have not been characterized molecularly. It differs from *C. acnidae* (Schuster & Brezina, 1979) Wouts, 1985, *C. amaranthi* (Stoyanov, 1972) Krall & Krall, 1978 and *C. radicale* Chizhov, Udalova & Nasonova, 2008 by presence of eggshell punctations. It differs from *C. thornei* (Golden & Raski, 1977) Mulvey &

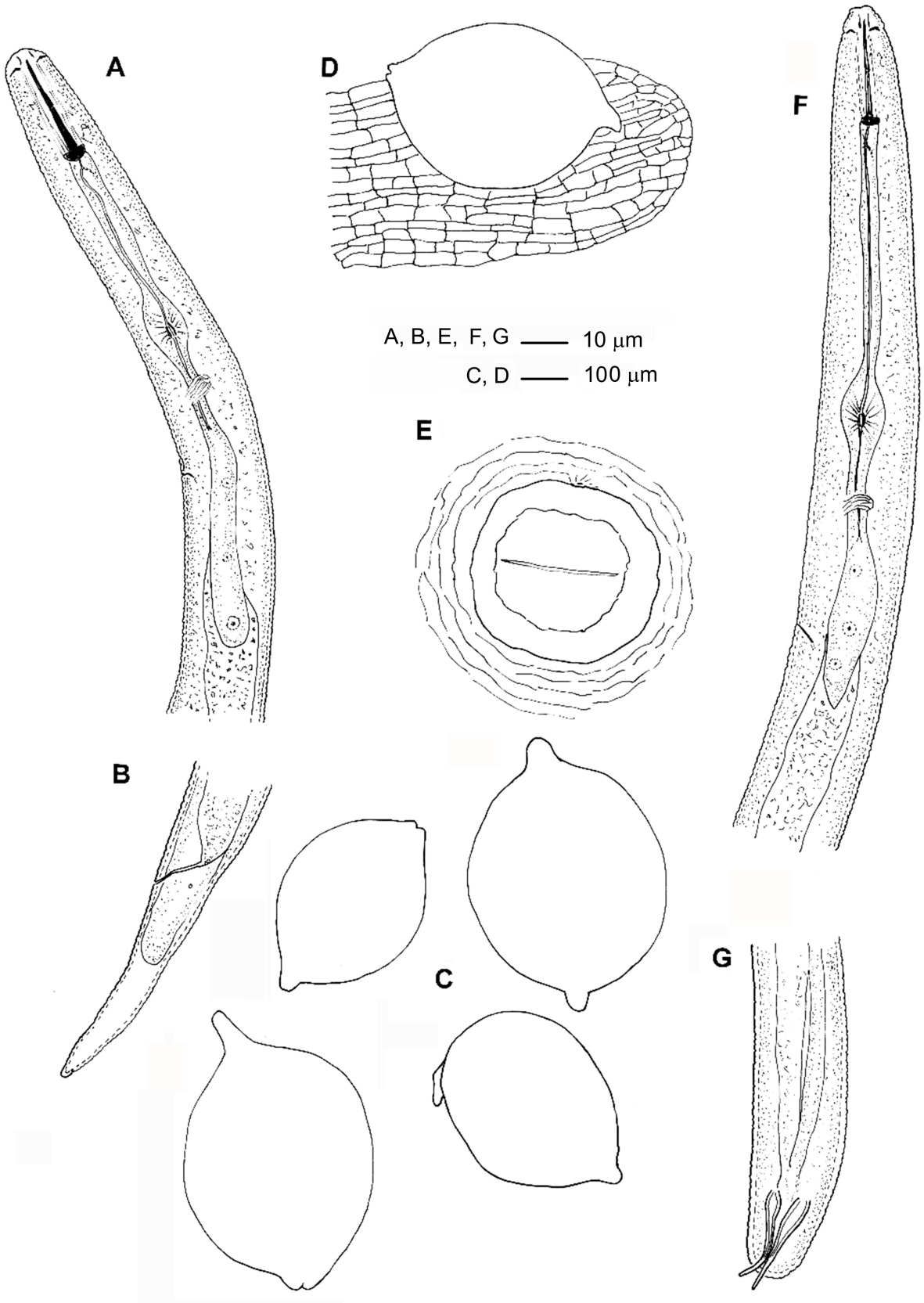

Fig. 1. *Cactodera herba* sp. n. Second-stage juvenile. A: Anterior end body; B: Tail, lateral view. Cysts. C: Cysts; D: Cyst on a root; E: Circumfenestra. Male. F: Anterior end body; G: Posterior end body.

Fig. 2. *Cactodera herba* sp. n. LM photographs. A. Cysts; B, C: Cysts on roots. Scale = 500 µm.

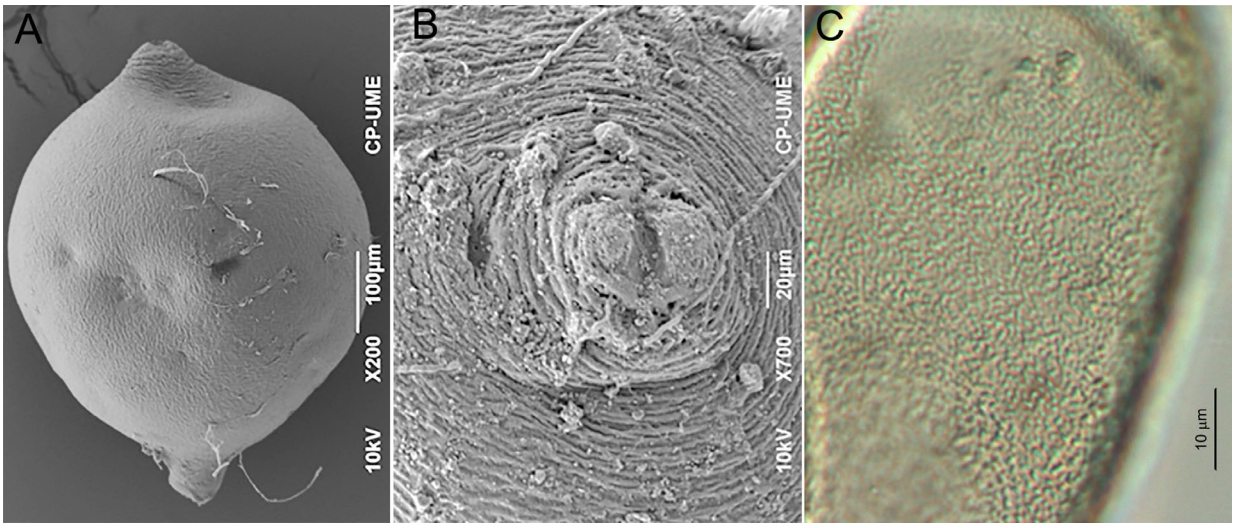

Fig. 3. *Cactodera herba* sp. n. SEM photographs. A: Cyst; B: Fenestral area of cyst. LM photograph. C: Punctation on eggshell.

Golden, 1983 by shorter average body length for J2 (440 *vs* 554 µm), from *C. eremica* Baldwin & Bell, 1985 by shorter average body length for cysts (555 *vs* 620 µm) and *C. evansi* Cid del Prado & Rowe, 2000 by longer average body length for J2 (440 *vs* 387 µm). *Cactodera herba* sp. n. is clearly differentiated from other molecularly characterised *Cactodera* species by sequences of the partial 28S rRNA, ITS and *COI* genes.

Molecular characterisation. Five new D2-D3 of 28S rRNA, 15 ITS rRNA and 3 *COI* gene sequences from 12 samples of seven species were obtained in this study.

The D2-D3 of the 28S rRNA gene. The alignment included 38 sequences of representatives of the family Heteroderidae and was 671 bp in length. Five new sequences, including two sequences of *C. herba* sp. n., were obtained in this study. Phylogenetic relationships of *C. herba* sp. n. with other of representatives of the family Heteroderidae are given in Figure 6. *Cactodera herba* sp. n. was clustered with *Cactodera* sp.3 (PP=93%) and differed from this species in 1.2%. Sequences of two unidentified cyst nematode species (cyst nematode sp.A and sp.B) belonging to an unknown genus differed in 3.9% from each other

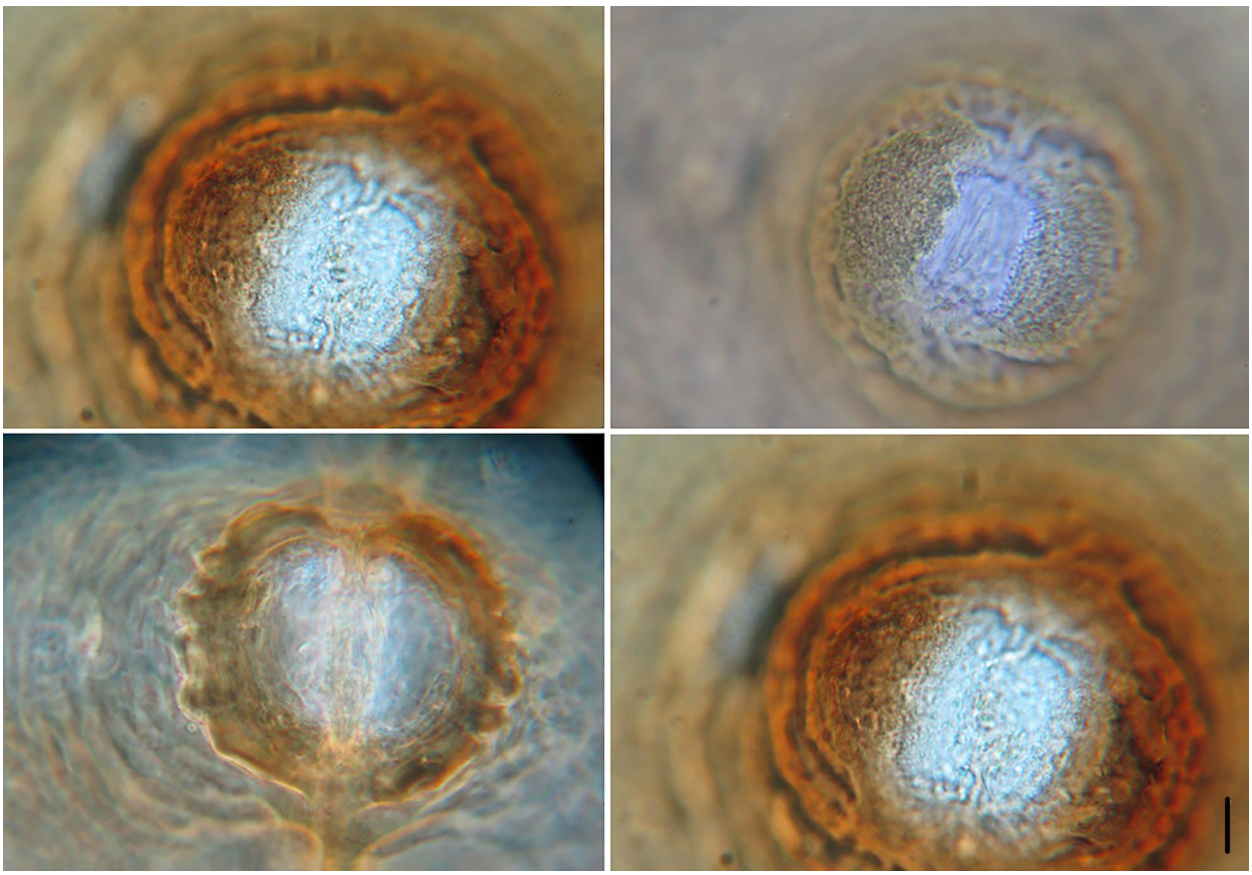

Fig. 4. *Cactodera herba* sp. n. LM photographs. Four vulval plates showing circumfenestral area with vulva slit. Scale = 10 μm.

and this genus relationships with other Punctoderinae were unresolved.

The ITS rRNA gene. The alignment included 57 sequences of representatives of the family Heteroderidae and was 1064 bp in length. Fifteen new sequences, including two sequences of *C. herba* sp. n., were obtained in this study. Phylogenetic relationships of *C. herba* sp. n. with other representatives of the family Heteroderidae are given in Figure 7. *Cactodera herba* sp. n. was clustered with two unidentified *Cactodera* species (PP=100%) and differed from *Cactodera* sp.1 (HQ260420, HQ260421) in 5.7-6.3% and *Cactodera* sp.3 in 4.0%. New sequence of *C. rosae* differed from the known sequence of this species in 0.3%, whereas the new sequence of *C. solani* differed from the known sequence in 0.1%. Sequences of three unidentified cyst nematode species (cyst nematode sp.A, sp.B, sp.C) differed in 12.4-12.5%, 6.7% and 10.5-10.6% from each other, respectively, and formed a separate lineage (PP=100%) within the subfamily Punctoderinae and had sister relationships with the clade of the genus *Globodera* parasitising solanaceous plants.

The *COI* gene. The alignment included 25 sequences of the genus *Cactodera* and two outgroup taxa and was 400 bp in length. Three new sequences, including two sequences of *C. herba* sp. n., were obtained in this study. Phylogenetic relationships of *C. herba* sp. n. with other *Cactodera* are given in Figure 8. *Cactodera herba* sp. n. was clustered with C. *weissi* (PP=72%) and differed from this species in 16%.

DISCUSSION

With this description of *C. herba* sp. n., the total number of valid species of the genus *Cactodera* is 19 (Subbotin *et al*., 2010; Ni *et al.* 2024) and only 12 of those species are presently molecularly characterised. Because of significant overlapping in morphometric diagnostic characters for cysts and J2, molecular characters play a major role in identification and delimiting of species in this genus.

Presently, five valid *Cactodera* species are reported in Mexico. *Cactodera rosae* was described from barley roots. This species was resampled at the

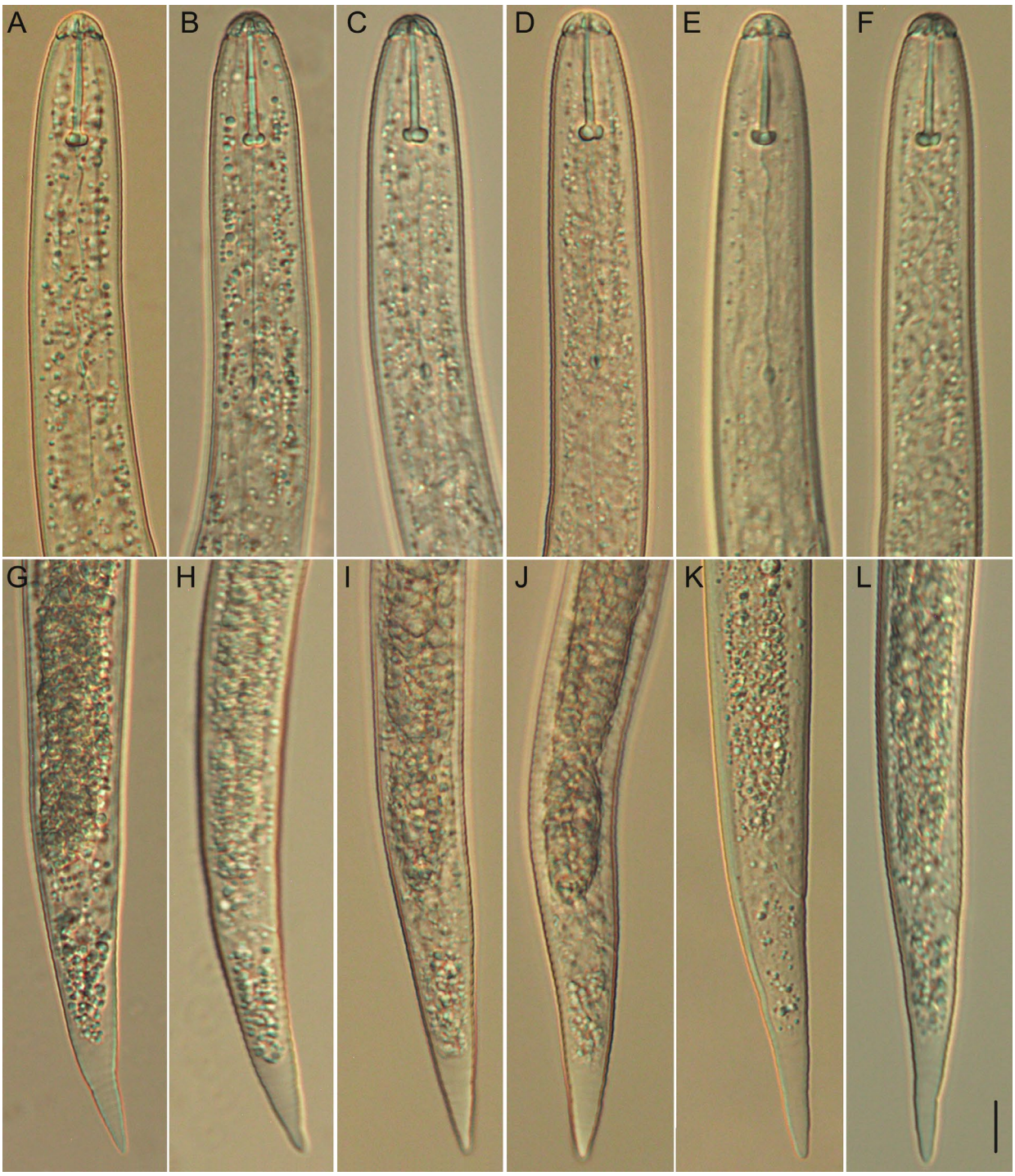

Fig. 5. Second stage juveniles of *Cactodera* spp. LM photographs. A-F: Anterior region; G-L: Posterior region. A, B, G, H: *C. herba* sp. n.; C, D, I, J: *C. solani*; E, F, K, L: *Cactodera* sp.3 Scale = 10 µm.

type locality in Hidalgo state and was also found in Morelos State; both of these populations were molecularly characterised (Subbotin *et al.,* 2011). The present sample of *C. rosae* was collected in Tlaxcala State from soil and showed high molecular identity with the published sequence of this species. *Cactodera solani* was described from tomato and common lambsquarter in greenhouse of Escuela Nacional de Ciencias Biológicas-Instituto Politécnico Nacional, Mexico City and in soil from a field of Puebla State (Escobar-Avila *et al.,* 2021). In the present survey, this species was detected in Tlaxcala State.

Phylogenetic and sequence analyses of

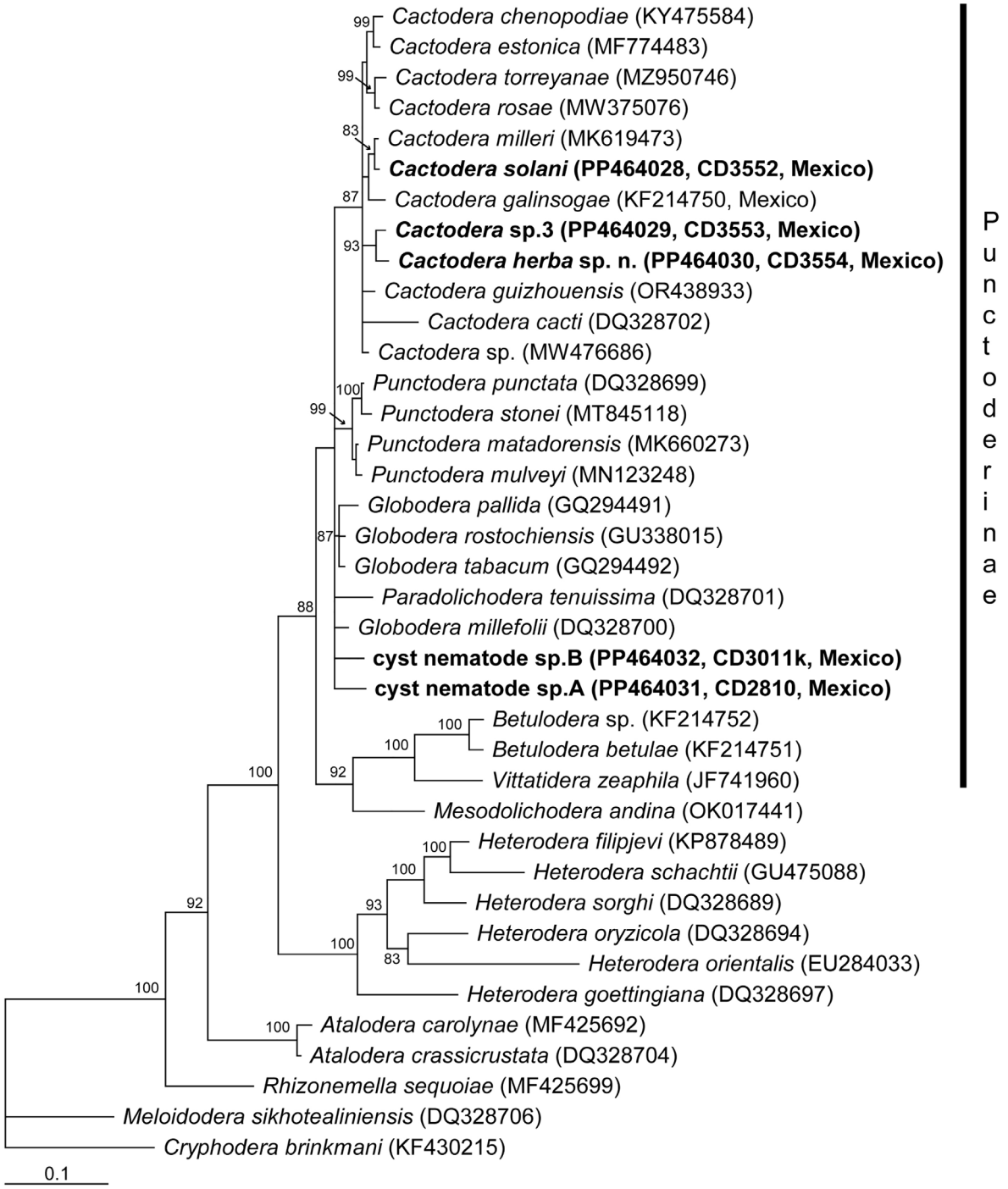

Fig. 6. Phylogenetic relationships of *Cactodera herba* sp. n. with some representatives of the family Heteroderidae: Bayesian 50% majority rule consensus tree from two runs as inferred from analysis of the D2-D3 of 28S rRNA gene sequence alignment under the GTR + I + G model. Posterior probabilities equal to, or more than, 70% are given for appropriate clades. New sequences are indicated in bold.

Cactodera sequences showed that there are several still undescribed species of this genus in Mexico; they include *Cactodera* sp.1 and sp. 2 delimitated by Subbotin *et al.* (2011) from the materials provided by Dr. M. Mundo-Ocampo and from *Cactodera* sp.3 found in the present study.

Cactodera herba sp. n. and *C. rosae* are the only presently known species associated with plants of the family Poaceae. The plant host of the new species described herein is liverseed grass. This

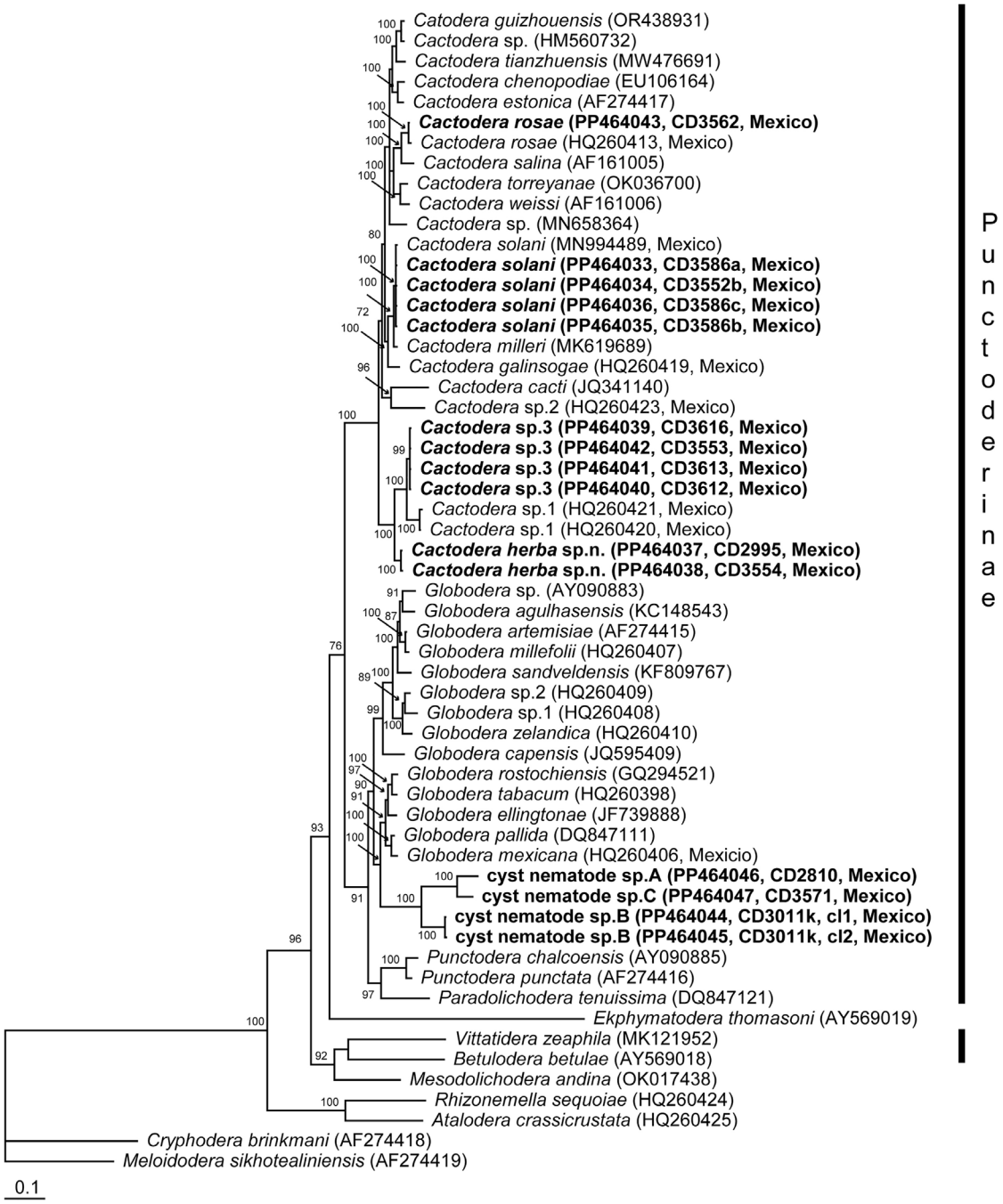

Fig. 7. Phylogenetic relationships of *Cactodera herba* sp. n. with some representatives of the subfamily Punctoderinae: Bayesian 50% majority rule consensus tree from two runs as inferred from analysis of the ITS rRNA gene sequence alignment under the GTR + I + G model. Posterior probabilities equal to, or more than, 70% are given for appropriate clades. New sequences are indicated in bold.

grass is native to Africa and to west and south Asia; it has been introduced to Mexico and other countries. It is likely that *C. herba* sp. n. parasitising native plants growing in Mexico transferred to liverseed grass, and identification of the native hosts will be important in future studies.

During the nematological surveys, several cysts belonging to an unknown genus of the family Heteroderidae were extracted from samples collected from rhizosphere soil of *Solanum rostratum* and grasses. In the phylogenetic tree, ITS rRNA gene sequences of three unidentified cyst

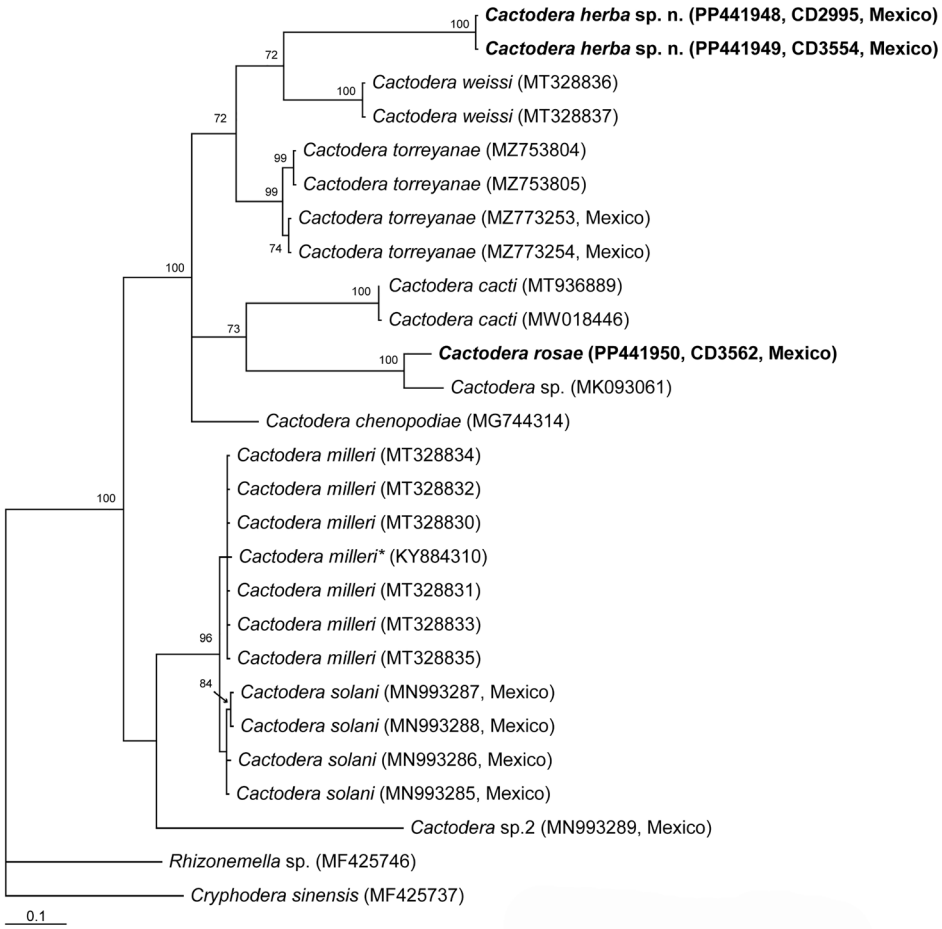

Fig. 8. Phylogenetic relationships of *Cactodera herba* sp. n. with some representatives of the genus *Cactodera*: Bayesian 50% majority rule consensus tree from two runs as inferred from analysis of the *COI* gene sequence alignment under the GTR + I + G model. Posterior probabilities equal to, or more than, 70% are given for appropriate clades. New sequences are indicated in bold. * - originally identified as *Cactodera torreyanae* in the GenBank.

nematode species (cyst nematode sp.A, sp.B, sp.C) formed a separate lineage within the subfamily Punctoderinae and showed close relation with representatives of the clade of the genus *Globodera* parasitising solanaceous plants. The analysis also revealed significant differences in sequences and suggested that three species occurred in the studied samples. In one location, in Hidalgo State, two species were delimited in same soil sample and these cysts were found in a mixture with cysts of an unidentified *Heterodera* sp.2 characterised by Subbotin *et al.* (2021).

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I. Cid del Prado Vera, H. Ferris and S.A. Subbotin. Новый вид *Cactodera herba* sp. n. (Nematoda: Heteroderidae) с молекулярной характеристикой некоторых цистообразующих нематод из Мексики.

Резюме. В 2018-2021 гг. в результате нематологических обследованиях в ряде регионов Мексики были обнаружены и выделены из образцов почвы и корней: *Cactodera solani, C. rosae, Cactodera* sp., новый вид *C. herba* sp. n. и три неизвестных вида цистообразующих нематод, принадлежащих к неизвестному роду. *Cactodera herba* sp. n. описан из почвы и корней травы *Urochloa panicoides*, растущей вокруг персиковых деревьев на кампусе Монтесильо, Колледжа последипломного образования, штат Мехико, Мексика. Для нового вида были характерны: цисты длиной 444–752 мкм, цвета от светлого до темно-коричневого, небольшой вульварный конус и средний диаметр окна 36,4 мкм. Личинки второго возраста нового вида имели длину тела 400-490 мкм, длину стилета 22-28 мкм. Поверхность яиц была с отчетливыми неровностями. Пять новых последовательностей D2-D3 28S рРНК, 15 ITS рРНК и 3 COI генов были получены из 12 образцов семи видов цистообразующих нематод. Эти последовательности генов дифференцировали *C. herba* sp. n. от других молекулярно охарактеризованных видов *Cactodera*. Филогенетические взаимоотношения внутри видов *Cactodera* и других гетеродерид приведены на основе анализа последовательностей D2-D3 28S рРНК, ITS рРНК и COI генов. На ITS рРНК генном древе, последовательности трех цистообразующих нематод (sp.A, sp.B, sp.C), принадлежащих к неизвестному роду, образовали отдельную линию внутри Punctoderinae и эта клада имела сестринские отношения с кладой рода *Globodera*, паразитирующей на пасленовых растениях.