

Research Paper

**Cite this article:** Ni C-H, Li Q-Y, Yang Z-F, Xu C-L and Xie H (2024). *Cactodera xinanensis* n. sp. (Nematoda: Heteroderinae), a new species of cyst-forming nematode from Southwest China, with a key to the Genus *Cactodera*. *Journal of Helminthology*, **98**, e69, 1–12  
<https://doi.org/10.1017/S0022149X24000415>.

Received: 29 April 2024  
Revised: 22 May 2024  
Accepted: 22 May 2024

**Keywords:**

*cactodera*; morphology; molecular; phylogeny; plant-parasitic nematode; new species

**Corresponding author:**

H. Xie;  
Email: [xiehui@scau.edu.cn](mailto:xiehui@scau.edu.cn)

# *Cactodera xinanensis* n. sp. (Nematoda: Heteroderinae), a new species of cyst-forming nematode from Southwest China, with a key to the Genus *Cactodera*

C.-H. Ni<sup>1</sup>, Q.-Y. Li<sup>1</sup>, Z.-F. Yang<sup>2</sup>, C.-L. Xu<sup>1</sup> and H. Xie<sup>1</sup> 

<sup>1</sup>Laboratory of Plant Nematology and Research Center of Nematodes of Plant Quarantine, Department of Plant Pathology, College of Plant Protection, South China Agricultural University, Guangzhou, People's Republic of China and <sup>2</sup>Department of Plant Pathology, College of Agriculture, Guizhou University, Guiyang, 550025, People's Republic of China

## Abstract

The cyst nematodes, subfamily Heteroderinae, are plant pathogens of worldwide economic significance. A new cyst nematode of the genus *Cactodera* within the Heteroderinae, *Cactodera xinanensis* n. sp., was isolated from rhizospheres of crops in the Guizhou and Sichuan provinces of southwest China. The new species was characterized by having the cyst with a length/width =  $1.3 \pm 0.1$  (1.1–1.6), a fenestral diameter of  $28.1 \pm 4.3$  (21.3–38.7)  $\mu\text{m}$ , vulval denticles present; second-stage juvenile with stylet  $21.5 \pm 0.5$  (20.3–22.6)  $\mu\text{m}$  long, tail  $59.4 \pm 2.0$  (55.9–63.8)  $\mu\text{m}$  long and hyaline region  $28.7 \pm 2.7$  (25.0–36.3)  $\mu\text{m}$  long, lateral field with four incisures; the eggshell with punctations. The new species can be differentiated from other species of *Cactodera* by a longer tail and hyaline region of second-stage juveniles. Phylogenetic relationships within populations and species of *Cactodera* are given based on the analysis of the internal transcribed spacer (ITS-rRNA), the large subunit of the nuclear ribosomal RNA (28S-rRNA) D2-D3 region and the partial cytochrome oxidase subunit I (COI) gene sequences here. The ITS-rRNA, 28S-rRNA and COI gene sequences clearly differentiated *Cactodera xinanensis* n. sp. from other species of *Cactodera*. A key and a morphological identification characteristic table for the species of *Cactodera* are included in the study.

## Introduction

Guizhou and Sichuan provinces, both situated in the southwestern region of China, boast diverse and complex terrain featuring mountains, hills, and basins. This geographical diversity contributes to distinct vertical climatic characteristics, fostering various ecological types and a rich array of species. Moreover, the land in this region is fragmented, with a significant portion consisting of sloping farmland (Chen & Liu, 2022). The unique ecoclimatic conditions allow for a diversity in the types and cultivation methods of crops in this region. The main crops include rice, potato, maize, buckwheat, and tobacco, among others (Lu & Jia, 2007). Cyst nematodes, subfamily Heteroderinae, are plant pathogens of worldwide economic significance. Crops parasitized by these nematodes exhibit symptoms similar with physiological diseases such as nutrient deficiency, which are difficult to identify (Jones *et al.*, 2013). Cyst nematodes parasitize plant roots and commonly spread through soil in farmland. The characteristics of farmland and crop distribution in the southwestern region of China are favorable for the spread of these nematodes.

During a survey of plant nematode species in Guizhou and Sichuan provinces in 2023, roots and rhizosphere soil samples were collected from a variety of crops. Among these, 18 rhizosphere soil samples taken from crops such as potatoes, buckwheat, maize, cabbage, and tea were found to contain cyst nematodes in the laboratory. Through the comparison of morphological characteristics and sequences of the internal transcribed spacer (ITS-rRNA), the large subunit of the nuclear ribosomal RNA (28S-rRNA) D2-D3 region, as well as the *the partial cytochrome oxidase subunit I (COI) gene*, it was determined that these 18 populations belong to the same species within the genus *Cactodera* of the subfamily Heteroderinae. They are distinct from any known species of the genus *Cactodera* and represent a new species of this genus, named as *Cactodera xinanensis* n. sp.. In this paper, the morphological and molecular characteristics of the new species were described, and the key and the morphological diagnostic feature table for species within *Cactodera* were revised and supplemented.

## Material and methods

### *Nematode isolates and morphological identification*

The cysts were isolated using the Cobb's sieving method (Cobb, 1918). The eggs inside the cysts were extracted and then hatched in sterile water to obtain J2s (second-stage juveniles).

Subsequently, the J2s were heat killed, fixed in FG fixative, and dehydrated using a glycerol-ethanol dehydration process according to Seinhorst's method (Seinhorst, 1959), and then mounted on slides. Vulva cones were dissected and mounted in neutral balsam according to Subbotin *et al.* (2010). The nematodes were photographed, measured, and observed using an AxioCam MRm Zeiss digital camera attached to a Zeiss Scope A1 microscope (Zeiss, Jena, Germany) equipped with differential interference contrast (Zeiss Scope A1 ZEN light 2012 software). For scanning electron microscopy, J2s, cysts and eggs were processed according to the method of Wang *et al.* (2013), then observed and photographed with a Zeiss EVO MA15 at 10 kV (Zeiss).

### Molecular and phylogenetic analyses

DNA was extracted from J2 individuals using extraction buffer containing Proteinase K, three replicates (Xu *et al.*, 2016). The ITS-rRNA gene was amplified using primers TW81 (5'-GTTTCCGTAGGTGAACCTGC-3') and AB28 (5'-ATATGCTTAAGTTCAGCGGGT-3') (Maafi *et al.*, 2003). The D2-D3 region of the 28S-rRNA gene was amplified with the D2A (5'-ACAAGTACCGTGAGGGAA AGTTG-3') and D3B (5'-TCGGAAGGAACCAGCTAC-TA-3) (De Ley *et al.*, 1999). The partial COI gene was amplified with Het-coxiF (5'-TAGTTGATCGTAA TTTAATGG-3') and Het-coxiR (5'-CCTAAAACATAATGAAAATGWGC-3') (Subbotin, 2015). The polymerase 2×Phanta Flash Master Mix (Vazyme) was used for polymerase chain reaction (PCR). The PCR products were purified using a gel extraction kit (Genesand Biotech Co., Ltd; Beijing, China), ligated into pEASY-blunt cloning vector (TransGen Biotech; Beijing, China) and sequenced by Sangon Biotech Co. Ltd. (Shanghai, China). The sequences were aligned by BLAST in the GenBank database and deposited in GenBank.

The newly obtained sequences for the ITS-rRNA, D2-D3 region of the 28S-rRNA and partial COI gene were aligned using MAFFT v7.149b (Katoh & Standley, 2013) with the corresponding gene sequences for *Cactodera* and edited in Gblock (Castresana, 2000). The best-fit model of DNA evolution for Bayesian inference was obtained using the program MrModeltest2.3 (Nylander, 2004) according to the Akaike Information Criterion. Sequence datasets for each gene fragment were analysed separately with Bayesian inference using MrBayes 3.1.1 (Huelsenbeck & Ronquist, 2001). The phylogenetic consensus trees were visualised using the software FigTree v.1.4.3 (Rambaut, 2014). Outgroup taxa for each dataset were chosen according to the results of previously published data (Escobar-Avila *et al.*, 2020; Ni *et al.*, 2024).

## Results

*Cactodera xinanensis* n. sp. (Figs 1-3). For measurements, see Table 1.

### Description

**Cyst.** Subspherical to lemon-shaped, brown to tan, with protruding neck and vulvar cone (Figs 1 A-B, 2 A, 3 A). Cyst surface with reticulated ridge patterns, punctations usually present (Fig 2 B). Vulval cone surface with wavy ridge patterns and often broken by short oblique or vertical lines (Figs 1C-D, 2 C-D). Cone top concave, circumfenestrate, with vulval denticles, without under-bridge and bullae (Figs 1 C-F, 2 C-D, 3 B). Anus round, dot-like, without fenestration (Figs 1 C-D, 3 B).

**Second-stage juvenile.** Body vermiform, slightly curved ventrally (Figs 1 G, 3 C). Head region slightly offset with four to five annuli, lip disc oval dorsoventrally elongated, four submedial lips distinct, two lateral submedial lips greatly reduced. Amphidial apertures conspicuous (Fig 2 E-F). Stylet well developed, cone about half the length of the stylet, knobs slightly rounded, anterior surface sloping posteriorly (Figs 1 I-J, 3 E). Median bulb oval with distinct valvular apparatus (Fig 1 H). Hemizonid and excretory pore located at the level of the between isthmus and esophageal gland. Excretory pore immediately behind hemizonid (Figs 1 H, 3D). Pharyngeal glands filling body cavity. Lateral field with four lines, the middle two lines merging at the posterior part of phasmid and becoming three lines (Figs 1 L, 2 H, 3 G). Phasmid pore-like openings located in anterior to hyaline region (Figs 1 O, 2 H G, 3 G). Tail conoid, with thin rounded terminus. Hyaline region approximately 50% of the tail length, often longer than stylet (Figs 1 M-N, 2 G, 3 E).

**Egg.** Eggshells with fine punctates visible with both light microscope and scanning electron microscopy (Figs 1 P-Q, 2 I-J).

**Male.** Not found.

### Type habitat and locality

Roots and rhizospheric soil of potato, *Solanum tuberosum* (Solanaceae, Solanales), in Ertang Town, Weining Yi Hui Miao Autonomous County, Bijie City, Guizhou Province (E: 104.665496, N: 26.675777).

### Other habitats

The information for the other habitats are provided in Table 2.

### Etymology

This new species is named after xinan, the pinyin for southwest China, where it was collected.

### Type material

The holotype cyst, 30 paratype cysts, 26 paratype J2s and 17 paratype eggs were deposited in the Laboratory of Plant Nematology and Research Center of Nematodes of Plant Quarantine, South China Agriculture University, Guangzhou, Guangdong, China.

### Diagnosis and relationships

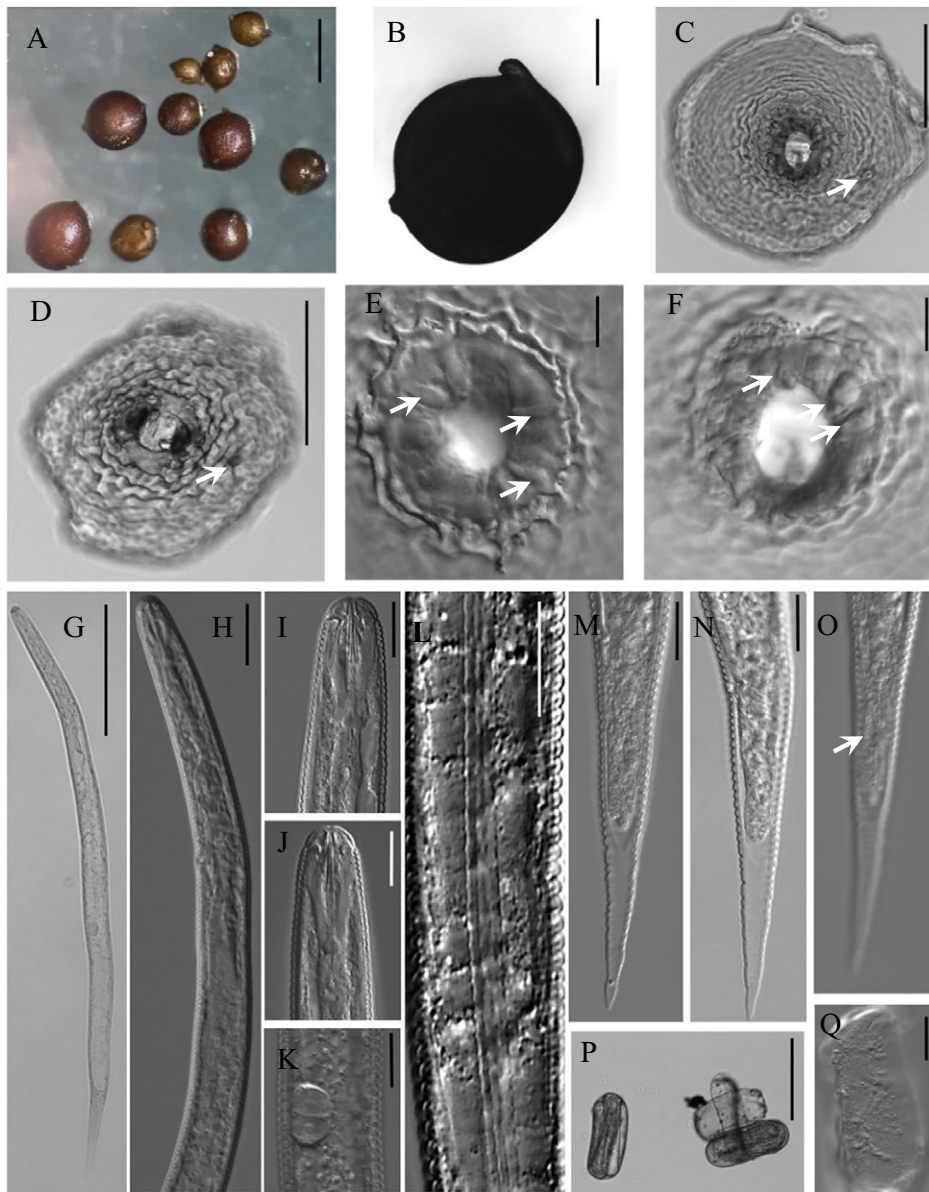
*C. xinanensis* n. sp. is characterized by the following features: length/width (L/W) ratio  $1.3 \pm 0.1$  (1.1–1.6) (including neck), surface with punctations, vulval denticles present, bullae and underbridge absent, fenestral diameter  $28.1 \pm 4.3$  (21.3–38.7)  $\mu\text{m}$  in cysts; stylet  $21.5 \pm 0.5$  (20.3–22.6)  $\mu\text{m}$ , tail  $59.4 \pm 2.0$  (55.9–63.8)  $\mu\text{m}$ , hyaline region  $28.7 \pm 2.7$  (25.0–36.3)  $\mu\text{m}$ , lateral field with four lines in J2s; eggshell with punctations.

*C. xinanensis* n. sp. can be differentiated from known species of *Cactodera* by longer mean tail (59  $\mu\text{m}$ ) and hyaline region (29  $\mu\text{m}$ ) of J2s. The new species is close to *C. cacti* (Krall & Krall, 1978) in having an L/W ratio of 1.1–1.6, fenestral diameter  $28.1 \pm 4.3$  (21.3–38.7)  $\mu\text{m}$ , with vulval denticles in cysts and eggshell with punctations, but differs by J2s having longer mean tail (59  $\mu\text{m}$  vs. 41–55  $\mu\text{m}$ ) and hyaline region (29  $\mu\text{m}$  vs. 16–25  $\mu\text{m}$ ), hyaline region longer than stylet vs. hyaline region shorter than stylet.

The main morphological and morphometric characters of *C. xinanensis* n. sp. and 18 valid species of the genus *Cactodera*, are compared in Table 3.

### Molecular characterization and phylogenetic analysis

The ITS-rRNA sequences from these 18 populations of *C. xinanensis* n. sp. were obtained. All sequence lengths were



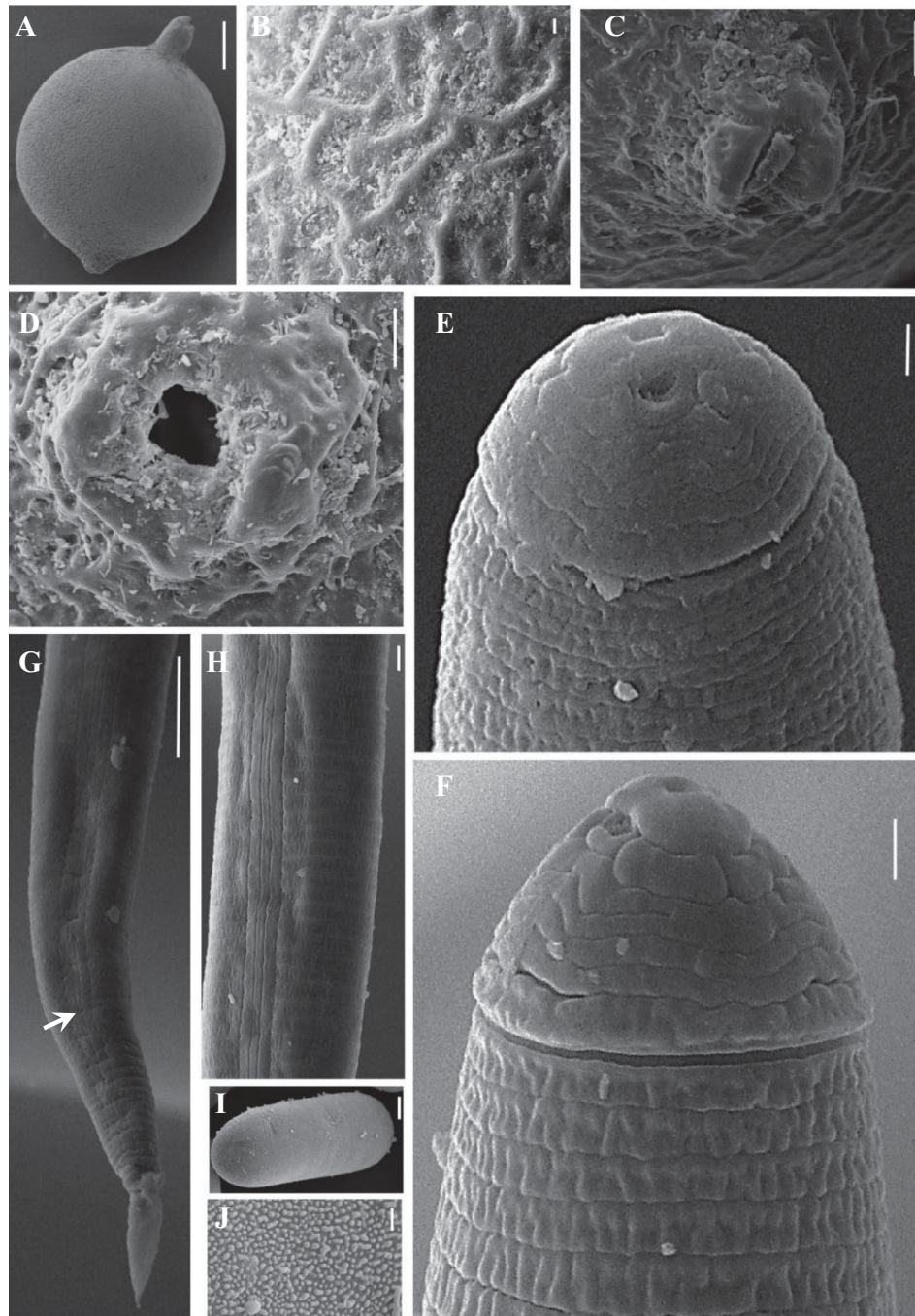
**Figure 1.** Microphotographs of *Cactodera xinanensis* n. sp. Cyst: A, B entire body; C-D vulval cone (anus is indicated by arrow); E-F, vulval denticles (arrowed). Second-stage juvenile: G, entire body; H, anterior end of body; I, J, head part; K, genital primordium; L, lateral field; M, N, tails; O, phasmid; Egg: P, entire; Q, eggshell. Scale bar: A = 600  $\mu$ m; B = 200  $\mu$ m; C, D, G, P = 100  $\mu$ m; E, F, H, Q = 20  $\mu$ m; I-O = 10  $\mu$ m.

966 bp (including primer sequences), without intraspecific sequence variation. The results of BLAST showed that the ITS-rRNA gene sequences from *C. xinanensis* n. sp. were closest to those from *Cactodera* sp. (MW821355), with 98.55% identity and 14-bp variation. Six sequences were randomly selected for phylogenetic analysis. The Bayesian phylogenetic tree generated from ITS dataset under GTR + G model is presented in Fig 4, which shows that the six sequences of *C. xinanensis* n. sp. formed a clade, the posterior probability (PP) = 100, and closest to *Cactodera* sp. (MW821355, MW821356 and MW658364).

The 28S-rRNA sequences from these 18 populations of *C. xinanensis* n. sp. were obtained. All sequences lengths were 782 bp (including primer sequences), without intraspecific sequence variation. The results of BLAST showed that the 28S

rRNA gene sequences from *C. xinanensis* n. sp. were closest to those from *C. guizhouensis* (OR438934), with 98.85% identity and 9-bp variation. Six sequences were randomly selected for phylogenetic analysis. The Bayesian phylogenetic tree generated from 28S-rRNA dataset under GTR + I + G model is presented in Fig 5, which shows that the six sequences of *C. xinanensis* n. sp. formed a clade (PP = 99).

The COI sequences from these 18 populations of *C. xinanensis* n. sp. were obtained. All sequences lengths were 490 bp (including primer sequences), without intraspecific sequence variation. The results of BLAST showed that the COI gene sequences from *C. xinanensis* n. sp. were closest to those from *C. chenopdiae* (MG744314), with 88.19% identity and 49-bp variation. Six sequences were randomly selected for phylogenetic analysis. The



**Figure 2.** Scanning electron microscope microphotographs of *Cactodera xinanensis* n. sp. Cyst: A, entire body; B, surface; C, vulval cone; D, vulval fenestral. Second-stage juvenile: E-F, head part; G, tails (phasmid is indicated by arrow); H, lateral field. Egg: I, entire; J, eggshell. Scale bar: A = 100  $\mu\text{m}$ ; B, H, I = 2  $\mu\text{m}$ ; C = 20  $\mu\text{m}$ ; D, G, I = 10  $\mu\text{m}$ ; E, F, J = 1  $\mu\text{m}$ .

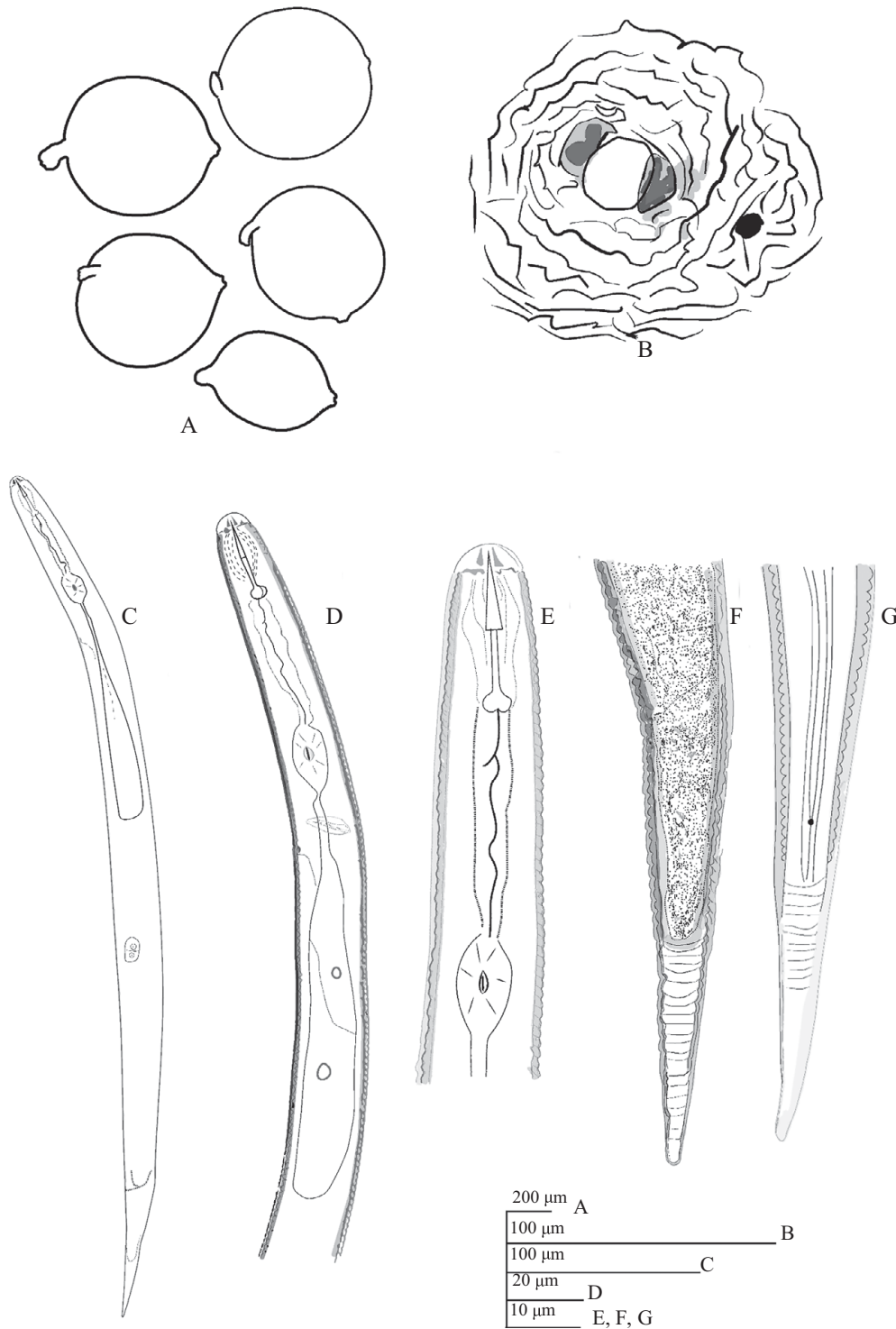
Bayesian phylogenetic tree generated from *COI* dataset under GTR + I + G model is presented in Fig 6, which shows that the six sequences of *C. xinanensis* n. sp. formed a clade (PP = 100).

The accession numbers of all sequences submitted to the GenBank database in this paper are listed in the Table 2.

### Discussion

The genus *Cactodera* was created by Krall and Krall in 1978, with the type species *C. cacti*, which has 18 valid species at present.

Apart from a few species whose type populations were collected from the rhizosphere of crops such as barley (*C. rosae*, *C. galinsogae*), tomato (*C. solani*), and potato (*C. guizhouensis*), the majority inhabit the rhizospheres of weeds from plant families including Amaranthaceae, Polygonaceae, Chenopodiaceae, and Asteraceae (Subbotin *et al.*, 2010; Cid Del Prado Vera & Subbotin, 2014; Escobar-Avila *et al.*, 2020; Li *et al.*, 2021; Ni *et al.*, 2024). In China, five species of genus *Cactodera* have been found, including *C. cacti* (Pan *et al.*, 1997), *C. thornei* (Peng & Vovlas, 1994), *C. chenopodiae* (Feng *et al.*,



**Figure 3.** Line drawing of *Cactodera xinanensis* n. sp. Cyst: A, whole body; B, vulval cone. Second-stage juveniles: C, entire body; D, anterior part of body; E, head and stylet; F, G, tails.

2018), *C. tianzhuensis* (Li *et al.*, 2021), and *C. guizhouensis* (Ni *et al.*, 2024). Among these, *C. chenopodiae*, *C. tianzhuensis*, and *C. guizhouensis* were respectively isolated from the rhizosphere of Chenopodiaceae plants, Polygonum plants, and potato. These three species have only been recorded in China to date. This study describes the 19th species of *Cactodera*,

*C. xinanensis* n. sp., which is also the sixth species of the genus found in China. *C. xinanensis* n. sp. was isolated from the rhizospheric soil of six different plants including potato, wormwood (*Artemisia argyi*), maize (*Zea mays*), buckwheat (*Fagopyrum esculentum*), cabbage (*Brassica oleracea*), and tea (*Camellia sinensis*), and some cysts were isolated from the roots of potato,

**Table 1.** Morphometrics of *Cactodera xinanensis* n. sp

Stage	Character	Holotype	Paratypes	
Cyst	n	–	30	
	Length (including neck)	712.1	672.3±96.3 (508.3–915.9)	
	Length (excluding neck)	610.2	584.7±81.4(429.6–755.9)	
	Width	546.9	524.3±74.2 (402.5–697.9)	
	Neck length	91.6	86.3±23.3 (33.5–166.4)	
	Length/width ratio (including neck)	1.3	1.3±0.1 (1.1–1.6)	
	Length/width ratio (excluding neck)	1.1	1.1±0.1 (1.0–1.3)	
	Fenestral diameter	28.7	28.1±4.3 (21.3–38.7)	
	Anus-fenestra distance	46.4	42.5±6.2 (29.0–51.4)	
	J2	n	–	26
L		–	440.2±17.1 (402.5–461.8)	
a		–	22.1±1.3 (18.8–24.5)	
b		–	4.0±0.2 (3.3–4.2)	
b'		–	2.6±0.2 (2.2–2.9)	
c		–	7.4±0.3 (6.7–8.0)	
c'		–	4.8±0.2 (4.3–5.2)	
Stylet		–	21.5±0.5 (20.3–22.6)	
Length of stylet cone		–	10.6±0.5 (9.8–11.2)	
Stylet knob height		–	2.2±0.2 (1.9–2.6)	
Stylet knob width		–	3.8±0.4 (3.2–4.5)	
Labial region width		–	9.0±0.4 (8.1–10.1)	
Labial region height		–	4.2±0.3 (3.7–4.8)	
Distance of dorsal oesophageal gland orifice to base of stylet (DGO)		–	4.6±0.7 (3.4–6.1)	
Median bulb length		–	13.9±1.3 (11.9–16.5)	
Median bulb width		–	8.8±0.7 (7.4–9.9)	
Anterior end to excretory pore		–	91.3±4.8 (79.2–96.8)	
Anterior end to median bulb valve		–	62.9±4.0 (54.3–71.4)	
Body diam. at mid-body		–	20.0±0.9 (17.9–21.4)	
Body diam. at anus level		–	12.4±0.5 (11.4–13.5)	
Hyaline region length		–	28.7±2.7 (25.0–36.3)	
Tail length		–	59.4±2.0 (55.9–63.8)	
Hyaline region length/tail length		–	0.5±0.0 (0.4–0.6)	
L/anterior end to median bulb valve		–	7.0±0.4 (6.1–7.8)	
Hyaline region length/stylet length		–	1.3±0.1 (1.2–1.6)	
Egg		n	–	17
		Length	–	104.7±3.4 (100.8–114.8)
		Width	–	42.6±2.7 (38.8–50.1)
		Length/width ratio	–	2.5±0.2 (2.1–2.7)

All measurements are in  $\mu\text{m}$ , and in the form: mean±standard deviation (range).

buckwheat and wormwood. Of the 18 populations obtained, 10 were from the potato rhizospheres, and during field sampling, we discovered cysts in the potato roots, so we designated potato as the putative host plant of the new species. Because the cysts of

some samples were obtained not from the roots but from the rhizosphere soil, there may be other host plants in the field for this new species. In addition, we found that that the eggs of the new species can hatch in water. This finding suggests that the

**Table 2.** Samples and sequences information

Sample number	Habitats	Location	GPS	Sequence number			Note
				ITS rRNA	28S rRNA	COI	
GZ39	Potato roots and rhizospheric soil	Ertang Town, Weining Yi Hui Miao Autonomous County, Bijie City, Guizhou Province	E: 104.665496 N: 26.675777	PP801271	PP809742	PP803413	Type habitat and locality
GZ57	Potato roots and rhizospheric soil	Ertang Town, Weining Yi Hui Miao Autonomous County, Bijie City, Guizhou Province	E: 104.822575 N: 26.106609	PP728282	PP728288	PP734312	
GZ24	Potato roots and rhizospheric soil	Shunchang Miao Yi Buyi Township, Shuicheng District, Liupanshui City, Guizhou Province	E: 104.290468 N: 26.876131	PP801273	PP809747	PP803414	
YX2	Potato rhizospheric soil	Nanqing Town, Yuexi County, Liangshan Yi Autonomous Prefecture, Sichuan Province	E: 102.462204 N: 28.530421	PP801279	PP809745	PP803421	
YX4	Potato rhizospheric soil	Banqiao Town, Yuexi County, Liangshan Yi Autonomous Prefecture, Sichuan Province	E: 102.560765 N: 28.756901	PP801280	PP809746	PP803422	
YX10	Potato rhizospheric soil	Dahua Town, Yuexi County, Liangshan Yi Autonomous Prefecture, Sichuan Province	E: 102.478292 N: 28.613910	PP801281	PP809744	PP803423	
YX12	Potato rhizospheric soil	Matuo Town, Yuexi County, Liangshan Yi Autonomous Prefecture, Sichuan Province	E: 102.472729 N: 28.466944	PP801282	PP809743	PP803424	
YX18	Potato rhizospheric soil	Erjue Town, Yuexi County, Liangshan Yi Autonomous Prefecture, Sichuan Province	E: 102.590562 N: 28.635621	PP728285	PP728291	PP734315	
YX28	Potato rhizospheric soil	Zhu'ajue Town, Yuexi County, Liangshan Yi Autonomous Prefecture, Sichuan Province	E: 102.720114 N: 28.435591	PP728286	PP728293	PP734316	
RHX1	Potato rhizospheric soil	Riha Township, Zhaojue County, Liangshan Yi Autonomous Prefecture, Sichuan Province	E: 103.111944 N: 28.026111	PP728287	PP728292	PP734317	
GZ20	Buckwheat roots and rhizospheric soil	Shunchang Miao Yi Buyi Township, Shuicheng District, Liupanshui City, Guizhou Province	E: 104.822529 N: 26.106595	PP801278	PP809753	PP803420	
GZ36	Buckwheat roots and rhizospheric soil	Ertang Town, Weining Yi Hui Miao Autonomous County, Bijie City, Guizhou Province	E: 104.665538 N: 26.675748	PP728283	PP728289	PP734314	
GZ22	Wormwood roots and rhizospheric soil	Shunchang Miao Yi Buyi Township, Shuicheng District, Liupanshui City, Guizhou Province	E: 104.822589 N: 26.106602	PP801277	PP809751	PP803418	
GZ43	Maize rhizospheric soil	Ertang Town, Weining Yi Hui Miao Autonomous County, Bijie City, Guizhou Province	E: 104.665568 N: 26.675614	PP801274	PP809748	PP803415	
GZ44	Maize rhizospheric soil	Ertang Town, Weining Yi Hui Miao Autonomous County, Bijie City, Guizhou Province	E: 104.665399 N: 26.675964	PP801276	PP809750	PP803417	
GZ41	Maize rhizospheric soil	Ertang Town, Weining Yi Hui Miao Autonomous County, Bijie City, Guizhou Province	E: 104.584954 N: 26.736335	PP801275	PP809749	PP803416	
GZ50	Cabbage rhizospheric soil	Ertang Town, Weining Yi Hui Miao Autonomous County, Bijie City, Guizhou Province	E: 104.588088 N: 26.734605	PP801272	PP809752	PP803419	
GZ11	Tea rhizospheric soil	Longchang Miao Bai Yi Township, Shuicheng District, Liupanshui City, Guizhou Province	E: 104.769110 N: 26.087161	PP728284	PP728290	PP734313	

new species may possess a wider range of potential hosts. Therefore, the host range and pathogenicity of the new species need to be further studied.

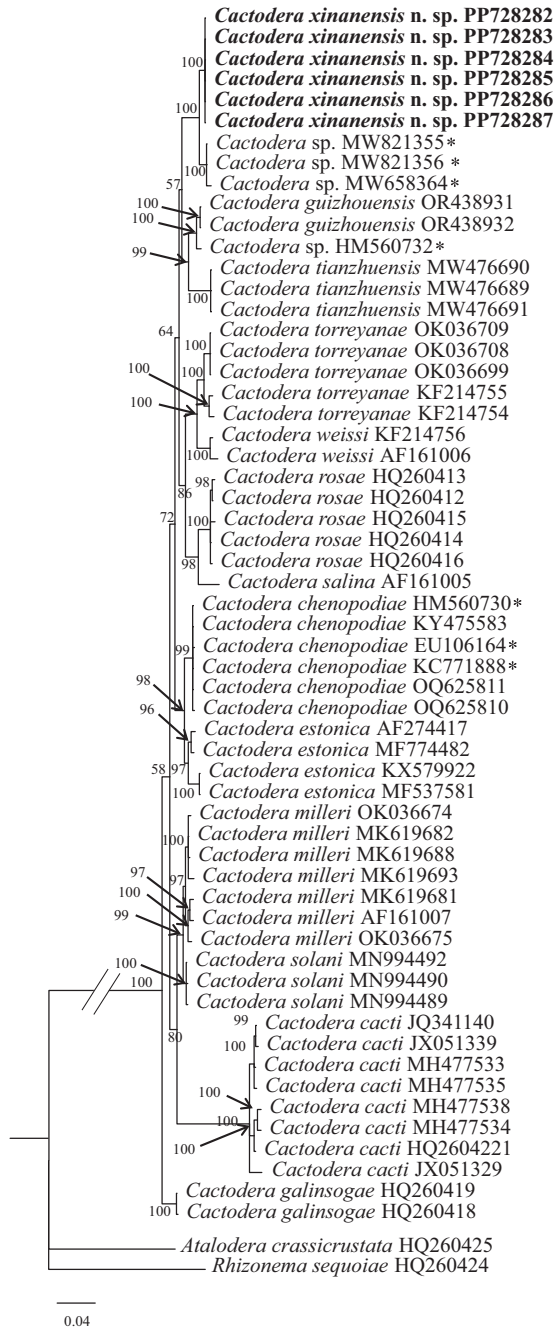
The morphological identification and differentiation of species within the genus *Cactodera* primarily rely on characteristics such as L/W, fenestral diameter, the presence or absence of vulval denticles in the cyst; the presence or absence of punctate on eggshell surface; as well as the length of stylet, tail and hyaline region in J2 (Subbotin *et al.*, 2010; Perry *et al.*, 2018). However, with the continuous description of new species and the increase

in the number of species within *Cactodera*, the overlap of morphological measurements between species began to appear, making it difficult to distinguish similar species based on morphological characteristics. Molecular characteristics provide a new reference for accurate species identification, and sequence alignment and phylogenetic analysis have become important methods to identify the species of the cyst-forming genera (Subbotin *et al.*, 2001; Handoo *et al.*, 2021). However, because of the early descriptions of some species within the *Cactodera* genus, molecular biological characteristic

**Table 3.** Morphological and morphometric characters useful for identification of *Cactodera* species (means are given in  $\mu\text{m}$ )

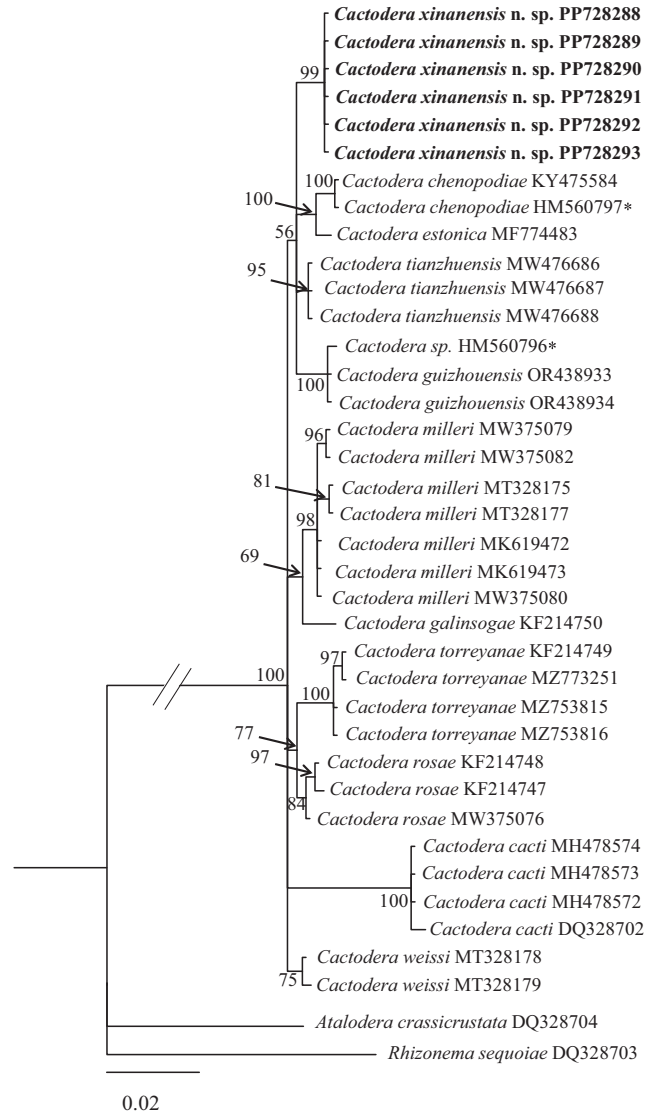
Species	Stage											Reference
	Cyst			Egg		J2			Male			
	Size	L/W ratio (including neck)	Fenestral diameter	Vulval denticles	Shell surface	L	Stylet	Lateral lines	Hyaline region	Tail	Spicule	
<i>C. acnidae</i>	698×417	1.8	33	–	Smooth	411	21	4	22	44	26	Schuster & Brezina, 1979
<i>C. amaranthi</i>	642×472	1.4	31	Present	Smooth	368–374	21	4	15	35	31	Stoyanov, 1973
<i>C. cacti</i>	442–587×328–475	1.1–1.4	26–38	Present	Punctate	368–504	22–26	4	16–25	41–55	34	Subbotin <i>et al.</i> , 2010
<i>C. chenopodiae</i>	486 ×334	1.5	23	Absent	Punctate	490	24	4	23	46	–	Feng <i>et al.</i> , 2018
<i>C. eremica</i>	620×434	1.5	21	Absent	Punctate	480	27	4	18	40	38	Baldwin & Bell, 1985
<i>C. estonica</i>	852–922×383–391	2.3	18–30	Present	Smooth	428–440	23	4	17–19	34–40	–	Subbotin <i>et al.</i> , 2010
<i>C. evansi</i>	459×334	1.4	18–23	–	Punctate	387	20	3	21	40	25	Cid Del Prado Vera & Rowe, 2000
<i>C. galinsogae</i>	523×384	1.4 (excluding neck)	41	Absent	Punctate	401	22	4	18	37	10	Tovar-Soto <i>et al.</i> , 2003
<i>C. guizhouensis</i>	621×438	1.6	25	Absent	Punctate	488	27	4	26	53	–	Ni <i>et al.</i> , 2024
<i>C. milleri</i>	632×506	1.4	18	Present	Punctate	426	22	4	18	43	36	Graney & Bird, 1990
<i>C. radicale</i>	682×438	1.6	24	Absent	Smooth	488	25	–	20	52	–	Chizhov <i>et al.</i> , 2008
<i>C. rosae</i>	654×433	1.5	18	Present	Punctate	397	20	4	6	39	–	Cid Del Prado Vera & Miranda, 2008
<i>C. salina</i>	603×375	1.6	24	Absent	Smooth	458	24	4	20	35	41	Baldwin <i>et al.</i> , 1997
<i>C. solani</i>	417×324	1.3 (excluding neck)	26	Present	Punctate	434	25	4	16	40	35	Escobar-Avila <i>et al.</i> , 2020
<i>C. thornei</i>	548–656×432–448	1.3–1.5	29–34	Present	Punctate	457–554	25–27	4	25–27	52–56	43	Subbotin <i>et al.</i> , 2010
<i>C. tianzhuensis</i>	571×454	1.3 (excluding neck)	23	Present	Smooth	539	25	4	26	54	–	Li <i>et al.</i> , 2021
<i>C. torreyanae</i>	575 ×303	1.9	20	Absent	Smooth	440	22	4	19	38	31	Cid Del Prado Vera & Subbotin, 2014
<i>C. weissi</i>	524–598×350–394	1.7	34	Present	Smooth	407–489	20–21	3	20–24	46	32	Steiner, 1949
<i>C. xinanensis</i> n. sp.	596×545	1.3	28	Present	Punctate	440	22	4	29	59	–	<b>Present study</b>





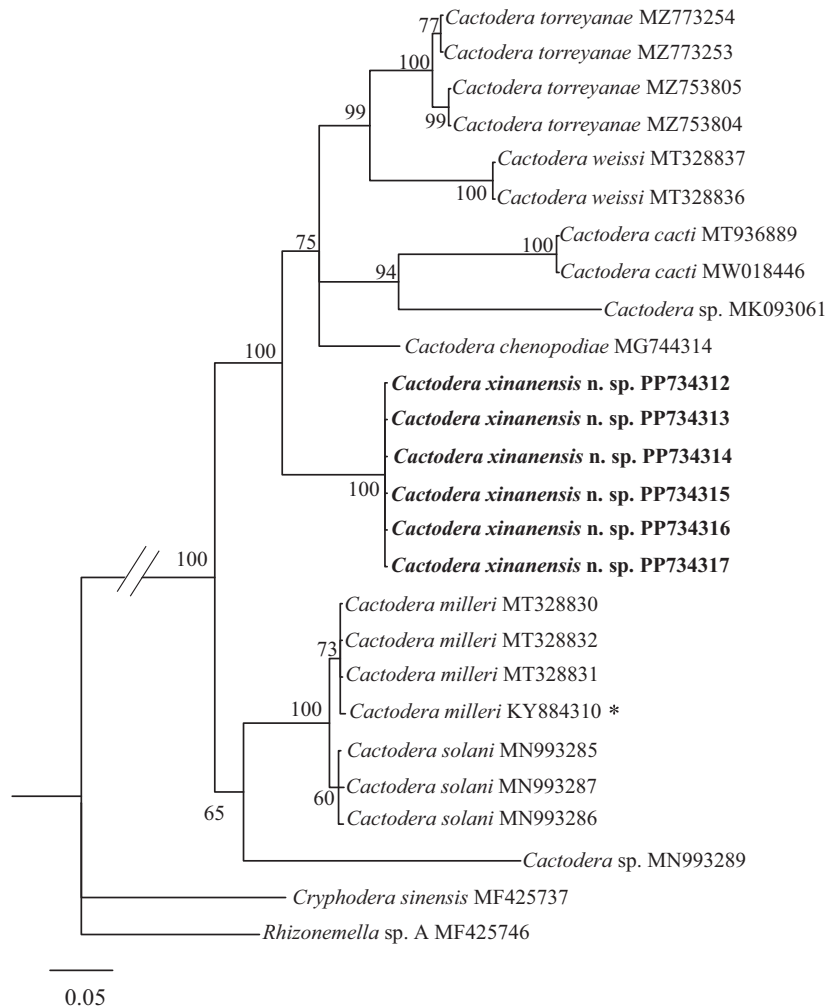
**Figure 4.** Phylogenetic relationships of *Cactodera* species inferred from Bayesian analyses of ITS rRNA sequences under GTR+G model. Posterior probabilities more than 50% are given to appropriate clades. Newly obtained sequences are indicated in bold font. \*Originally identified as *C. estonica* in GenBank.

information is lacking. For instance, in GenBank, *C. acnidae* (Schuster & Brezina, 1979), *C. amaranthi* (Krall & Krall, 1978), *C. eremica* (Baldwin & Bell, 1985), *C. evansi* (Cid Del Prado Vera & Rowe, 2000), *C. radicale* (Chizhov *et al.*, 2008), and *C. thornei* (Mulvey & Golden, 1983) lack both ITS-rRNA and 28S-rRNA sequences information, with only six species having *COI* sequences uploaded. Therefore, the molecular characteristics and phylogenetic analysis of species within the genus are limited. Additionally, there are many incorrect sequences in GenBank, for example, the ITS-rRNA sequences HM560732,



**Figure 5.** Phylogenetic relationships of *Cactodera* species inferred from Bayesian analyses of D2-D3 of 28S rRNA sequences under GTR+I+G model. Posterior probabilities more than 50% are given to appropriate clades. Newly obtained sequences are indicated in bold font. \*Originally identified as *C. estonica* in GenBank.

HM560730, EU106164, KC771888, and the 28S-rRNA sequences HM560979, HM560796 were originally uploaded as *C. estonica*, but these sequences have been repeatedly indicated as not belonging to *C. estonica* (Cid Del Prado Vera *et al.*, 2014; Escobar-Avila *et al.*, 2020; Li *et al.*, 2021; Ni *et al.*, 2024). The results of the phylogenetic analysis in this paper also indicate that these sequences do not belong to *C. estonica*. These erroneous sequences add to the difficulty of identifying species within this genus based on molecular characteristics. Therefore, it is necessary to rely on the combination of morphological and molecular characteristics to accurately identify the species. *C. xinanensis* n. sp. can be differentiated from all known species of the genus *Cactodera* by its longer tail and hyaline region of J2. Furthermore, it forms a separate clade in the phylogenetic trees of ITS-rRNA, 28S-rRNA D2-D3 region, and *COI* sequences, indicating that it is a new species of the genus.



**Figure 6.** Phylogenetic relationships of *Cactodera* species inferred from Bayesian analyses of *COI* sequences under GTR+I+G model. Posterior probabilities more than 50% are given to appropriate clades. Newly obtained sequences are indicated in bold font. \*Originally identified as *C. torreyanae* in GenBank.

**Key to species of the genus *Cactodera***

(This key is mainly based on information taken from Subbotin et al., 2010; Cid Del Prado and Subbotin, 2014; Feng et al., 2018; Li et al., 2021; Ni et al. 2024)

1.	Cyst generally two times or more longer than wide, mean L/W ratio = 2.3	<i>C. estonica</i>
	Cyst usually less than twice as long as wide, mean L/W ratio < 2.0	2
2.	Eggshell punctate	3
	Eggshell smooth	13
3.	Mean fenestral diameter ≥ 26 μm	4
	Mean fenestral diameter < 26 μm	8
4.	Mean tail length of J2 ≥ 41 μm	5
	Mean tail length of J2 < 41 μm	7
5.	Mean hyaline region > 27 μm, tail length > 56 μm for J2	<i>C. xinanensis n. sp.</i>
	Mean hyaline region ≤ 27 μm, tail length ≤ 56 μm for J2	6

(Continued)

(Continued)

6.	Mean hyaline region of J2 = 16–25 μm; female stylet = 33.2 (30.2–35.6) μm, vulval slit = 13 (10–15) μm	<i>C. cacti</i>
	Mean hyaline region of J2 = 25–27 μm; female stylet = 29.4 (28.4–30.7) μm, vulval slit = 17 (16–19) μm	<i>C. thornei</i>
7.	Cyst fenestral diameter = 26 (20–36) μm, vulval denticles present	<i>C. solani</i>
	Cyst fenestral diameter = 41 (33–56) μm, vulval denticles absent	<i>C. galinsogae</i>
8.	Mean stylet length of J2 > 23 μm	9
	Mean stylet length of J2 ≤ 23 μm	11
9.	Mean hyaline region < 20 μm, mean tail length < 43 μm for J2	<i>C. eremica</i>
	Mean hyaline region ≥ 20 μm, mean tail length ≥ 43 μm for J2	10
10.	Stylet length of J2 = 24 (22–26) μm; female vulval slit = 13 (13–17) μm	<i>C. chenopodiae</i>
	Stylet length of J2 = 27 (24–29) μm; female vulval slit = 25 (24–26) μm	<i>C. guizhouensis</i>

(Continued)

(Continued)

11.	Hyaline region of J2 = 6 (4–8) $\mu\text{m}$	<i>C. rosae</i>
	Mean hyaline region of J2 > 10 $\mu\text{m}$	12
12.	Mean cyst size = 459 $\times$ 334 $\mu\text{m}$ , J2 lateral field with three incisures	<i>C. evansi</i>
	Mean cyst size = 632 $\times$ 506 $\mu\text{m}$ , J2 lateral field with four incisures	<i>C. milleri</i>
13.	Mean tail length of J2 $\leq$ 40 $\mu\text{m}$	14
	Mean tail length of J2 > 40 $\mu\text{m}$	16
14.	Cyst vulval denticles present, fenestral diameter = 31 (25–38) $\mu\text{m}$ ; hyaline region of J2 = 15 (11–18) $\mu\text{m}$	<i>C. amaranthi</i>
	Cyst vulval denticles absent, mean fenestral diameter $\leq$ 25 $\mu\text{m}$ ; mean hyaline region $\geq$ 18 $\mu\text{m}$	15
15.	Stylet length = 22 (21–23) $\mu\text{m}$ , pharynx length = 89 (66–122) $\mu\text{m}$ for J2; spicule length of male = 31 (25–40) $\mu\text{m}$	<i>C. torreyanae</i>
	Stylet length = 24 (23–25) $\mu\text{m}$ , pharynx length = 146 (130–163) $\mu\text{m}$ for J2; spicule length of male = 41 $\mu\text{m}$	<i>C. salina</i>
16.	Mean stylet length > 22 $\mu\text{m}$ , mean tail length $\geq$ 50 $\mu\text{m}$ for J2	17
	Mean stylet length $\leq$ 22 $\mu\text{m}$ , mean tail length < 50 $\mu\text{m}$ for J2	18
17.	Cyst vulval denticles absent, J2 a=19 (18–21)	<i>C. radiale</i>
	Cyst vulval denticles present, J2 a=23 (21–27)	<i>C. tianzhuensis</i>
18.	Stylet knob anterior surface convex, lateral field with four incisures for J2; spicule length of male = 26 (25–29) $\mu\text{m}$	<i>C. acnidae</i>
	Stylet knob anterior surface concave, lateral field with three incisures for J2; spicule length of male = 32(31–34) $\mu\text{m}$	<i>C. weissii</i>

**Acknowledgements.** This research was supported by the Plant Quarantine Station of Sichuan Agricultural and Rural Department (No. N5100012022001943) and the Detection and Control of Crop Disease and Pests Project of China (No. 101821301082351011).

## References

- Baldwin, J.G., and Bell, A.H. (1985) *Cactodera eremica* n. sp., *Afenestrata africana* (Luc et al., 1973) n. gen., n. comb., and an emended diagnosis of *Sarisodera* Wouts and Sher, 1971 (Heteroderidae). *Journal of Nematology* 17(2), 187–201.
- Baldwin, J.G., Mundo-Ocampo, M., and McClure, M.A. (1997) *Cactodera salina* n. sp. from the estuary plant, *Salicornia bigelovii*, in Sonora, Mexico. *Journal of Nematology* 29(4), 465–473.
- Castresana, J. (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution* 17(4), 540–552.
- Chen, M. and Liu, S.W. (2022) Study on the optimization of crop planting structure in southwest China. *Acta Agriculturae Universitatis Jiangxiensis*, 44 (01), 12–20.
- Chizhov, V.N., Udalova, Z.V., and Nasonova, L.V. (2008) *Globodera arenaria* n. sp. and *Cactodera radiale* n. sp. (Nematoda: Tylenchida) from rizosphere of meadows in mid-volga region. *Russian Parasitological Journal*, 2, 109–116.
- Cid Del Prado Vera, I., and Miranda, B.L. (2008) A second cyst-forming nematode parasite of barley (*Hordeum vulgare* L. Var. Esmeralda) from Mexico. *Nematropica*, 105–114
- Cid Del Prado Vera, I., and Rowe, J.A. (2000) *Cactodera evansi* sp. n. and *Meloidodera astonei* sp. n. (Tylenchida: Heteroderidae) from Mexico. *International Journal of Nematology* 10(2), 159–168.
- Cid Del Prado Vera, I., and Subbotin, S.A. (2014) A new cyst nematode, *Cactodera torreyanae* sp. n. (Tylenchida: Heteroderidae), parasitising romerito, *Suaeda torreyana*, in Texcoco, Mexico. *Nematology* 16(2), 163–174.
- Cobb, N.A. (1918) Estimating the nema population of soil, with special reference to the sugar-beet and root-gall nemas, *Heterodera schachtii* Schmidt and *Heterodera radicola* (greef) muller and with a description of *Tylencholaimus aequalis* n. sp. *Agricultural Technology Circular* 1, 1–48.
- De Ley, P., Felix, M., Frisse, L., Nadler, S., Sternberg, P., and Thomas, W.K. (1999) Molecular and morphological characterisation of two reproductively isolated species with mirror-image anatomy (Nematoda: Cephalobidae). *Nematology* 1(6), 591–612.
- Escobar-Avila, I.M., Subbotin, S.A., and Tovar-Soto, A. (2020) *Cactodera solani* n. sp. (Nematoda: Heteroderidae), a new species of cyst-forming nematode parasitising tomato in Mexico. *Nematology* 23(1), 1–14.
- Feng, Y.X., Wang, D., Xiao, D.X., Pereira, T.J., Xuan, Y.H., Wang, Y.Y., Liu, X.Y., Chen, L.J., Duan, Y.X., and Zhu, X.F. (2018) *Cactodera chenopodiae* (Nematoda: Heteroderidae), a new species of cyst nematode parasitizing common lambsquarter (*Chenopodium album*) in Liaoning, China. *Zootaxa* 4407(3), 361–375.
- Graney, L.S.O., and Bird, G.W. (1990) Descriptions and comparative morphology of *Cactodera milleri* n. sp. (Nematoda: Heteroderidae) and *Cactodera cacti* with a review and key to the genus *Cactodera*. *Journal of Nematology* 22 (4), 457–480.
- Handoo, Z.A., Skantar, A.M., Subbotin, S.A., Kantor, M.R., Hult, M.N., and Grabowski, M. (2021) Molecular and morphological characterization of a first report of *Cactodera torreyanae* Cid Del Prado Vera & Subbotin, 2014 (Nematoda: Heteroderidae) from Minnesota, the United States of America. *Journal of Nematology* 53(1), 1–5.
- Huelsenbeck, J.P., and Ronquist, F. (2001) MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics* 17(8), 754–755.
- Jones, J., Haegeman, A., Danchin, E., Gaur, H., Helder, J., Jones, M., Kikuchi, T., Manzanilla-Lopez, R., Palomares-Rius, J., and Wesemael, W. (2013). Top 10 plant-parasitic nematodes in molecular plant pathology. *Molecular Plant Pathology* 14(9), 946–961.
- Katoh, K., and Standley, D.M. (2013) Mafft multiple sequence alignment software version 7: improvements in performance and usability *Molecular Biology and Evolution* 30(4), 772–780.
- Krall, E.L., and Krall, K.A. (1978) Revision of the plant nematodes of the family Heteroderidae on the basis of the trophic specialization of these parasites and their co-evolution with their host plants. pp. 39–56 in Krall EL and Krall KA (Ed.) *Fitogel'mintologicheskie Issledovaniya*. Moscow, USSR, Nauka: Institute of Zoology and Botany of the Academy of Science of the Estonian SSR.
- Li, W.H., Li, H.X., Ni, C.H., Shi, M.M., Wei, X.J., Liu, Y.G., Zhang, Y.W., and Peng, D.L. (2021) A new cyst-forming nematode, *Cactodera tianzhuensis* n. sp. (Nematoda: Heteroderinae) from *Polygonum viviparum* in China with a key to the genus *Journal of Nematology* 53(1), 1–15.
- Lu, Y.Q., and Jia, Y.T. (2007) A study on the regional agriculture development and cropper growth comparative advantage basing on the demonstration analysis of Chongqing, Sichuan, Yunnan's data. *Journal of Chongqing University of Arts and Science (Natural Science Edition)* 26(02), 50–56.
- Maafi, Z.T., Subbotin, S.A., and Moens, M. (2003) Molecular identification of cyst-forming nematodes (Heteroderidae) from Iran and a phylogeny based on ITS-rDNA sequences. *Nematology* 5(1), 99–111.
- Mulvey, R.H., and Golden, A.M. (1983) An illustrated key to the cyst-forming genera and species of Heteroderidae in the Western hemisphere with species morphometrics and distribution. *Journal of Nematology* 15(1), 1–59.
- Ni, C.H., Xie, Y.J., Yang, S.H., Yang, Z.F., Xu, C.L., and Xie, H. (2024) *Cactodera guizhouensis* n. sp. (Nematoda: Heteroderinae), a new species of cyst-forming nematode parasitizing potato in Guizhou, China. *European Journal of Plant Pathology*, 1–11.
- Nylander, J.A.A. (2004) Mrmodeltest 2.3. Evolutionary Biology Centre, Uppsala University.
- Pan, C.S., Lin, J., and Xue, R. (1997) Description of *Cactodera cacti* and their observation by scanning electron microscope. *Acta Parasitologica Et Medica Entomologica Sinica* 4(4), 214–217.

- Peng, D.L., and Vovlas, N.** (1994) Occurrence of the cyst-forming nematode *Cactodera thornei* in China. *Nematologia Mediterranea* **22**(1), 75–78.
- Perry, R.N., Moens, M., and Jones, J.T.** (2018) Cyst nematodes: CABI.
- Rambaut, A.** (2014) Figtree v1. 4.2, a Graphical Viewer of Phylogenetic Trees. Available online: <http://tree.bio.ed.ac.uk/software/figtree/>.
- Schuster, M.L., and Brezina, L.** (1979) Association of soil-borne pathogens with soybean platte valley yellows: 1. *Heterodera acnidae* n. sp. (Heteroderidae: Nematoda) a parasite of *Acnida altissima*. *Fitopatologia Brasileira* **4**(3), 379–389.
- Seinhorst, J.W.** (1959) A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. *Nematologica*, **4**(1), 67–69.
- Steiner, G.** (1949) Plant nematodes the grower should know. *Proceedings of the Soil Science Society of Florida* **4**, 82–117.
- Stoyanov, D.** (1973) *Heterodera amaranthi*. Life cycle, hosts and distribution. *Poeyana, Instituto de Zoologia Cuba* **97**, 12
- Subbotin, S.A.** (2015) *Heterodera sturhani* sp. n. from China, a new species of the *Heterodera avenae* species complex (Tylenchida: Heteroderidae). *Russian Journal of Nematology* **23**(2), 145–152.
- Subbotin, S.A., Mundo-Ocampo, M., and Baldwin, J.G.** (2010) Systematics of cyst nematodes (Nematoda: Heteroderinae) in Hunt DJ and Perry RN (Ed.) *Nematology Monographs and Perspectives* 8A. Leiden, The Netherlands, Brill: Brill
- Subbotin, S.A., Vierstraete, A., De Ley, P., Rowe, J., Waeyenberge, L., Moens, M., and Vanfleteren, J.R.** (2001) Phylogenetic relationships within the cyst-forming nematodes (Nematoda, Heteroderidae) based on analysis of sequences from the ITS regions of ribosomal DNA. *Molecular Phylogenetics and Evolution* **21**(1), 1–16.
- Tovar-Soto, A., Cid Del Prado Vera, I., Nicol, J.M., Evans, K., Sandoval-Islas, J.S., and Martinez Garza, A.** (2003) *Cactodera galinsogae* n. sp. (Tylenchida: Heteroderinae) on barley (*Hordeum vulgare* L.) of the high valleys of Mexico. *Nematropica* **33**(1), 41–54.
- Wang, K., Xie, H., Li, Y., Xu, C., Yu, L., and Wang, D.** (2013) *Paratylenchus shenzhenensis* n. sp. (Nematoda: Paratylenchinae) from the rhizosphere soil of *Anthurium andraeanum* in China. *Zootaxa* **3750**(2), 167–175.
- Xu, C., Zhao, C., Ding, S., Zhang, J., and Xie, H.** (2016) A modified crude DNA preparation for direct PCR reaction of single plant-parasitic nematodes. *Nematology* **18**(5), 625–628.