Zootaxa 4407 (3): 361–375
http://www.mapress.com/j/zt/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

https://doi.org/10.11646/zootaxa.4407.3.4
http://zoobank.org/urn:lsid:zoobank.org:pub:F95D4394-8A44-4331-9D2D-ED8ABBE8AF17

Cactodera chenopodiae (Nematoda: Heteroderidae), a new species of cyst nematode parasitizing common lambsquarter (*Chenopodium album*) in Liaoning, China

YAXING FENG[1], DONG WANG[1], DONGXUE XIAO[1], TIAGO JOSÉ PEREIRA[2], YUANHU XUAN[1], YUANYUAN WANG[1], XIAOYU LIU[1], LIJIE CHEN[1], YUXI DUAN[1] & XIAOFENG ZHU[1,3]

[1]*College of Plant Protection, Nematology Institute of Northern China, Shenyang Agricultural University, Shenyang, China 110866*
[2]*Department of Nematology, University of California, Riverside, 900 University Avenue, Riverside, CA 92521, USA*
[3]*Corresponding author. E-mail: syxf2000@163.com*

Abstract

A new species of cyst nematode, *Cactodera chenopodiae* **n. sp.**, parasitizing common lambsquarter, *Chenopodium album* L., is described from native vegetation in Liaoning, China. *Cactodera chenopodiae* **n. sp.** has a circumfenestrate pattern typical of the genus and is morphologically similar to *C. cacti* Krall & Krall, 1978. However, in the new species, females and cysts show a larger L/W ratio whereas second-stage juveniles (J2s) have a longer hyaline region. The new species is also morphologically similar to *C. milleri* Graney & Bird, 1990, but the J2s differ by a larger b ratio and longer tail. Based on DNA sequences of the 28S and ITS rRNA, *C. chenopodiae* **n. sp.** comes close to *C. estonica* Krall & Krall, 1978, although it is distinct from the latter with respect to the presence of a punctate eggshell and larger b ratio in the J2s. Although morphometric comparisons with additional *Cactodera* species show the overlapping of diagnostic morphological characters, our phylogenetic analyses based on both rRNA genes support *C. chenopodiae* **n. sp.** as a unique lineage.

Key words: genus *Cactodera*, ribosomal genes, morphology, phylogeny, plant-parasitic nematode, taxonomy

Introduction

During the summer of 2015, second-stage juveniles (J2s) of a cyst-forming nematode were detected from soil around common lambsquarter, *Chenopodium album* L., a plant widely distributed in China. In the US, Graney & Bird (1990) also reported cyst nematodes (i.e., *Cactodera milleri* Graney & Bird, 1990) parasitizing weeds including *C. album*, *C. amaranticolor* Coste & Reyn, and *C. quinoa* Willd. Since 2015, additional soil sampling has been performed in China revealing the presence of cysts and a few white females attached to roots of the plant host (i.e., *C. album*). A detailed study of these nematodes indicated a small vulval cone and circumfenestrate pattern typical of *Cactodera* Krall & Krall, 1978. Morphological and molecular analyses of the material were performed and compared with the 14-other valid *Cactodera* species (Subbotin *et al.*, 2010; Cid Del Prado Vera & Subbotin, 2014), thus revealing the nematode to be a new species. Herein, we describe this cyst nematode as *Cactodera chenopodiae* **n. sp.** using morphological and molecular characters.

Material and methods

Nematode isolation. Females, cysts and J2s of *C. chenopodiae* **n. sp.** were collected from the natural habitat of the host plant, *C. album*, in Beiling Park, Shenyang, Liaoning Province, China (41°50’38” N, 123°25’44” E, 51 m a.s.l.). Although soil samples were collected seven times (monthly from May to October, 2015), males were not found. Cysts, J2s–J4s and females were extracted from soil samples using standard centrifugal-flotation and Fenwick methods (Fenwick, 1940). Immature stages and females were dissected directly from the roots under a stereomicroscope (Nikon SMZ800) for further morphological characterization.

Accepted by N. Kanzaki: 7 Mar. 2018; published: 11 Apr. 2018

Morphological study. Nematodes in water were killed, fixed and dehydrate according to Cid Del Prado Vera & Subbotin (2012). Nematodes were then processed to glycerin using a modified Seinhorst (1959) method as described by Cid Del Prado Vera & Subbotin (2012). The specimens were hand-picked from the dish and then mounted on glass slides using a paraffin wax ring method (de Maeseneer & d'Herde, 1963). Permanent slides they were then examined under light microscopy (LM) for morphological characterization. Measurements and drawings were made using a drawing tube mounted on an Olympus BX53 compound microscope. For scanning electron microscopy (SEM), specimens were post fixed in an aqueous solution of 4% osmium tetroxide for 12 hours and then dehydrated in an series of ethanol solutions (20–100%) for 20 min at each concentration (Cid Del Prado Vera *et al.*, 2012). The specimens were critical point-dried and coated with gold-palladium and then observed under a field emission SEM (Zeiss Ultra Plus) at 5 kV.

Nematode-infected plant tissues were treated for observation using a modified acid-fuchsin staining-destaining procedure (Bybd Jr *et al.*, 1983). Washed roots were placed in a 150 ml beaker with 50 ml tap water and 20 ml of chlorine bleach (5.25 % NaClO) resulting in a solution of 1.5% NaClO. After occasional agitation during 4 min in the NaClO, the roots were rinsed in flowing water (30–45 sec) and then allowed to soak in tap water for another15 min to remove residual NaClO. The material was then drained and transferred to a beaker containing 30 ml of water to which was added 1 ml of stain (3.5 g acid fuchsin, 250 ml acetic acid, and 750 ml distilled water). This solution was then heated to boiling for about 30 sec. After cooling to room temperature, excess stain was removed by rinsing in flowing water. The root material was then placed in 20–30 ml of glycerin acidified with 2–3 drops of 5N HCl, heated to boiling, and cooled. For microscopic examination the roots were pressed between glass plates or microscope slides.

Molecular characterization. DNA was extracted from 5 specimens (single cyst containing J2s and eggs) using a Worm lysis buffer [Subbotin *et al*., 2010, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 2.5 mM $MgCl_2$, 0.045% Tween 20, and 0.045% Nonidet P 40] in conjunction with Proteinase K (20 mg/ml, Takara Bio Inc.). Three ribosomal RNA (rRNA) genes (18S, 28S and ITS) and one mitochondrial DNA fragment (COI) were partially amplified via PCR. Detailed primer sequences are summarized in Supplement TABLE 1. Detailed protocols for the molecular procedures (i.e., DNA extraction, and PCR conditions) used in this study are as described in Subbotin *et al.* (2010). All PCR reactions included negative controls.

Cloning and sequencing. Initial DNA sequences of *C. chenopodiae* **n. sp.** obtained through direct sequencing, showed some ambiguous sites in upstream and downstream of the sequence, positive PCR products were cloned and re-sequenced. Briefly, DNA was excised from 1.2% TAE buffered agarose gels using the Tiangen Gel Extraction Kit (Tiangen Biotech Co., Ltd.), cloned into the pMD-T vector (Takara Bio Inc.) and transformed into Top10 Competent Cells. For each specimen, 5 clones were isolated using the blue/white selection and submitted to PCR with vector primers. One clone of each specimen was sequenced using the universal primer of pMD-T vector (Takara Bio Inc.). No intraspecific/genomic variation was observed.

Phylogenetic analyses. New DNA sequences obtained in the present study were submitted to the GenBank database under the accession numbers: ITS (KY475583), 28S (KY475584), 18S (MG566084) and COI (MG744314). Since there were no COI sequences and only 3 18S sequences available in NCBI database. Only ITS and 28S sequences phylogenetic tree were built. The sequences were separately aligned using MEGA 7 (http://www.megasoftware.net) with default parameters. Additional previously published DNA sequences of *Cactodera* species and representatives of other circumfenestrate cyst nematodes were also included in the alignment for phylogenetic context (Bernard *et al.*, 2010; Cid Del Prado Vera *et al.*, 2014; Duan *et al.*, 2012; Ferris *et al.*, 1999, 2004; Maafi *et al.*, 2003; Qin *et al.*, 2004; Sabo *et al.*, 2010; Subbotin *et al.*, 2001, 2006, 2011; Uehara *et al.*, 2005). Outgroup taxa for phylogenetic analyses were chosen according to previous studies of cyst nematodes (Subbotin *et al.*, 2006, 2011).

Phylogenetic relationships among sequences were estimated with maximum likelihood (ML), maximum parsimony (MP), and Bayesian inference (BI). MP analyses were performed in MEGA 7 using heuristic searches and SPR branch swapping to seek for the most parsimonious trees (max. tree number = 100). Gaps in the alignment were treated as missing data. Nonparametric bootstrap analysis (BS), using 1000 pseudo replicates, was used to assess branch support. For ML and BI analyses, MrModelTest 2 (Nylander, 2004.) was used to determine the model of DNA evolution that best fit the data. The model inferred base on the Akaike Information Criterion (AIC) values were GTR + I + G for ITS and GTR + G for 28S. ML analyses were performed in MEGA 7 (Bootstrap=1000). BI analyses were run on MrBayes 3.1.2 (Huelsenbeck & Ronquist, 2001) with a random seed,

and four Metropolis-coupled Markov chain Monte Carlo (MCMC) for 2,000,000 generations with a subsampling frequency of 100. The log-likelihood values stabilized after approximately 5,000 generations; sample points obtained prior to convergence were discarded as burn-in. BI trees were edited using FigTree 1.3.1 (Rambaut, 2009) and Adobe Illustrator® CS4 (Adobe Systems).

Results

Cactodera chenopodiae* **n. sp.**
(Figs. 1–5, Tables 1)
*specific epithet after the host, *Chenopodium album* L.,

Measurements. See Table 1.

Description. Females. Body ovate to rounded or subspherical in shape with small vulval cone (Fig. 1C, 1D), female body pearly-white (Fig. 1G, 1H). Females with small vulval cones having slightly protruding lips (Fig. 1C, 1D). Female full of eggs and gelatinous egg sac not observed (Fig. 1I). Outer cuticular layer marked by a rugose pattern. Head slightly set off from the elongate and protruding neck, stylet and stylet knobs well developed (Fig. 1A). Excretory pore located at same level as end of isthmus (Fig. 1B). Anus distinct (Fig.1E, 1F).

Cysts. Rounded to lemon-shaped, from light to dark brown with small vulval cone (Figs. 1K, 1L, 4A, 5F). Cyst surface with zigzag pattern at mid-body and not prominent on surface of the vulval cone (Fig. 4C, 4D). Cone is circumfenestrate and lacks an underbridge, bullae and vulval denticles (Fig. 1C, 1D, 4D). Anus distinct and encircled within a disc-like cuticular region (Fig. 4B).

Males. Not found.

J2s. Vermiform, tapering anteriorly and posteriorly (Fig. 2A). Stylet knobs rounded to slightly projecting anteriorly (Fig. 2B). Lip region slightly set off with four annuli. In *en face* view, an elongated labial disc surrounded by four submedial and two lateral lips (Fig. 4E, 5G). Excretory pore near level of gland lobe, hemizonid was one and a half annulus above excretory pore (Fig. 2B, 2E, 2F 5B). Lateral field with four lines with outer two ridges partially areolated along the body. (Figs. 2G, 4F, 5C). Tail tapering, with hyaline region; the hyaline region is often shorter than the stylet. Transition to hyaline region usually clearly demarcated by an outline that is V-shaped, U-shaped, or rarely sloping ventrally (Fig. 2C, 2D, 5D).

Eggs. Surface with heavy punctations visible both under LM and SEM (Figs. 1J, 4G–I). Only found inside cysts.

Type host and locality. Common lambsquarter, *C. album* in the Beiling Park, Shenyang, Liaoning province, China. Coordinates: 41°50’38” N, 123°25’44” E, 51 m a.s.l.

Type material. Holotype female, seven paratype females, twenty paratype cysts and twenty paratype J2s were deposited for curation in the collection of the Nematology Institute of Northern China (NINC), Shenyang Agricultural University, Shenyang, China.

Biology. *Cactodera chenopodiae* **n. sp.** was found on roots of *C. album* among other native vegetation of China. On the type host, the cyst nematode ranges from endoparasitic to semi-endoparasitic. Juveniles were detected on the roots of the host plant by acid fuchsin staining(Fig.3A). Moreover, some maturing juveniles (presumed to be sedentary J2s, J3s and J4s) were found with the anterior portion of their body penetrating into the roots (Fig. 3B–C).

Diagnosis and relationships. *Cactodera chenopodiae* **n. sp.** belongs to the genus *Cactodera* which includes species characterized by a small vulval cone and a circumfenestrate terminal pattern. The new species can be differentiated from the fourteen other *Cactodera* species by a combination of morphological and molecular characters.

The ranges of many morphological characters of *C. chenopodiae* overlap with those of other *Cactodera* species and isolates, including with certain *C. cacti* isolates from different regions of the US [e.g., Michigan, Graney & Bird (1990)]; yet, morphometric analyses of *C. chenopodiae* **n. sp.** demonstrate that the means for these morphological features are distinctive relative to *C. cacti*. For example, females of *C. chenopodiae* **n. sp.** have a larger L/W ratio [1.6 (1.4–1.7) vs. 1.2 (1.0–1.4)], and J2s have a smaller b ratio [3.8 (3.6–4.1) vs. 6 (5.6–6.8)] as well as a longer hyaline region [22.7 µm (17.5–28.4) vs. 17.6 µm (13.7–20.5)]. *Cactodera chenopodiae* **n. sp.** is

also distinguished from *C. milleri*, known to parasitize common lambsquarter in Michigan, US (Graney & Bird, 1990), by the cyst having an anus set off within a distinctive disc-shaped cuticular pattern (Fig. 1E–F, 4B) and by having a greater fenestral diameter [23.5 µm (19.9–26.3) vs. 18.7 µm (14.3–22.0)]. Moreover, J2s stages have a larger b ratio [3.8 (3.6–4.1) vs. 2.9 (2.6–3.1)] and a longer hyaline region [22.7 µm (17.5–28.4) vs. 18.2 µm (14.6–21.2)] when compared to *C. milleri*.

TABLE 1. Morphometric measurements of *Cactodera chenopodiae* **n. sp.**

			Paratype		
Stage	Character	Holotype	Mean ± SE	Range	CV[a]
Female					
	n		8		
	Length	566	632.2 ± 31.8	504.1–713.4	23.1
	Width	361	393.2 ± 13.6	316.8–566.18	21.4
	L/W ratio	1.6	1.61 ± 0.02	1.44–1.72	6.3
	Vulval slit	13.55	13.4± 0.55	12.9–16.7	9.5
	Vulva to anus distance	52.45	52.6± 0.58	50.1–55.36	3.7
Cyst					
	n		20		
	Length		486.1 ± 9.20	423.4–585.4	8.7
	Width		333.8 ± 7.65	283.0–398.1	10.5
	L/W ratio		1.5 ± 0.03	1.2–1.7	8.6
	Fenestral diam		23.5 ± 0.55	19.9–26.3	10.6
J2					
	n		20		
	Length		490.3 ± 5.47	438.2–539.3	5
	Width		22.3 ± 0.46	19.5–27.6	9.2
	Stylet		24.0 ± 0.27	21.9–25.9	5.1
	Stylet shaft and knobs		13.4 ± 0.10	8.3–10.0	3.4
	Stylet knobs to DGO		3.9 ± 0.12	3.1–4.3	13.6
	Head end to excretory pore		112.2± 1.10	103.9–121.3	4.4
	Tail		45.7 ± 0.70	39.1–50.6	6.9
	Hyaline region		22.7 ± 0.53	17.5–28.4	10.7
	a		22.0± 0.42	18.0–24.7	8.6
	b		4.1± 0.02	3.46–4.7	2.9
	c		10.8 ± 0.17	9.8–12.6	6.9
	c′		3.2 ± 0.08	2.7–4.0	11.4
	m		0.4 ± 0.01	0.4–0.5	7
	o		0.2 ± 0.01	0.1–0.2	14.4
	H (%)(Hyaline region /Tail × 100)		49.0± 0.01	39.2–61.5	10.6
egg					
	n		25		
	L		112.9 ± 1.13	107.1–120.9	5
	W		46.8 ± 0.53	42.5–52.5	5.6
	L/W		2.4 ± 0.03	2.0–2.7	5.7

All measurements are in µm.
[a]CV: coefficient of variation.

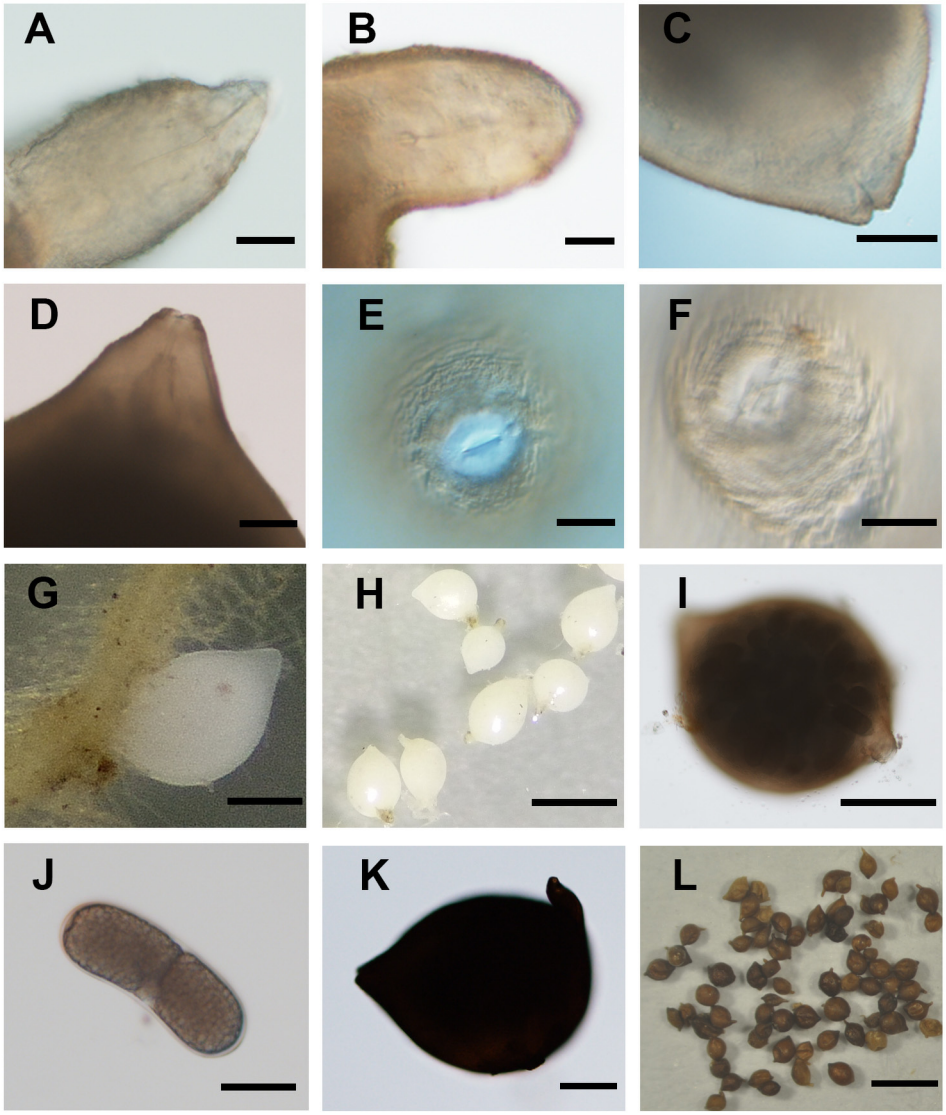

FIGURE 1. Light micrographs of *Cactodera chenopodiae* **n. sp.** (Female and Cyst) A. Stylet of female; B. Neck of female; C–D. Vulva and anus of female; E–F. Posterior ends of female showing vulval slit; G. Immature females on roots; H. Females; I. Mature female (full of eggs); J. Egg; K. Cyst; L. Cysts. (Scale bars: A–B, E, F, J =20 µm, C–D = 50 µm, G, I = 200 µm, H= 500 µm, K = 100 µm, L=1mm.)

As for closely related species (i.e. based on molecular data), *C. chenopodiae* **n. sp.** is distinguished from *C. estonica* (Kirjanova & Krall, 1963) Krall & Krall, 1978, by the eggshell pattern (i.e., punctate in *C. chenopodiae* vs. smooth in *C. estonica*) and larger b ratio in J2s [3.8 (3.6–4.1) vs. 2.8 (2.7–2.9)]. In addition, *C. chenopodiae* **n. sp.** differs from *C. rosae* Cid del Prado & Miranda, 2008 by a longer hyaline region [22.7 µm (17.5–28.4) vs. 6.3 µm (4.0–6.8)]

Molecular profiles and phylogenetic status

The molecular characterization and position of *C. chenopodiae* **n. sp.** within *Cactodera* was evaluated using two ribosomal regions (i.e., ITS and 28S). In the phylogenetic tree inferred from the D2–D3 expansion segments of the 28S rRNA gene, all *Cactodera* species grouped together forming a clade (Fig. 6). Specifically, *C. chenopodiae* **n.**

sp. was a sister taxon of *C. rosae* in subclade A, which also includes *C. torreyanae* Cid Del Prado & Subbotin, 2014. Yet, *C. cacti* and *C. galinsogae* Tovar Soto, Cid Del Prado, Nicol, Evans, Sandoval Islas & Martinez Garza, 2003, were sister to one another in subclade B, and relatively more divergent from species in subclade A (Fig. 6).

In the ITS phylogeny, molecular variation within *Cactodera* was better represented with the inclusion of DNA sequences representing four additional *Cactodera* species in the molecular analyses. All *Cactodera* species were monophyletic in the ITS phylogeny, and although intraspecific variation occurs, this seems to be relatively low as suggested by the short branch lengths (Fig. 7). Indeed, for *C. chenopodiae* **n. sp.** intraspecific variation was not detected as multiple clones representing five different specimens showed identical DNA sequences.

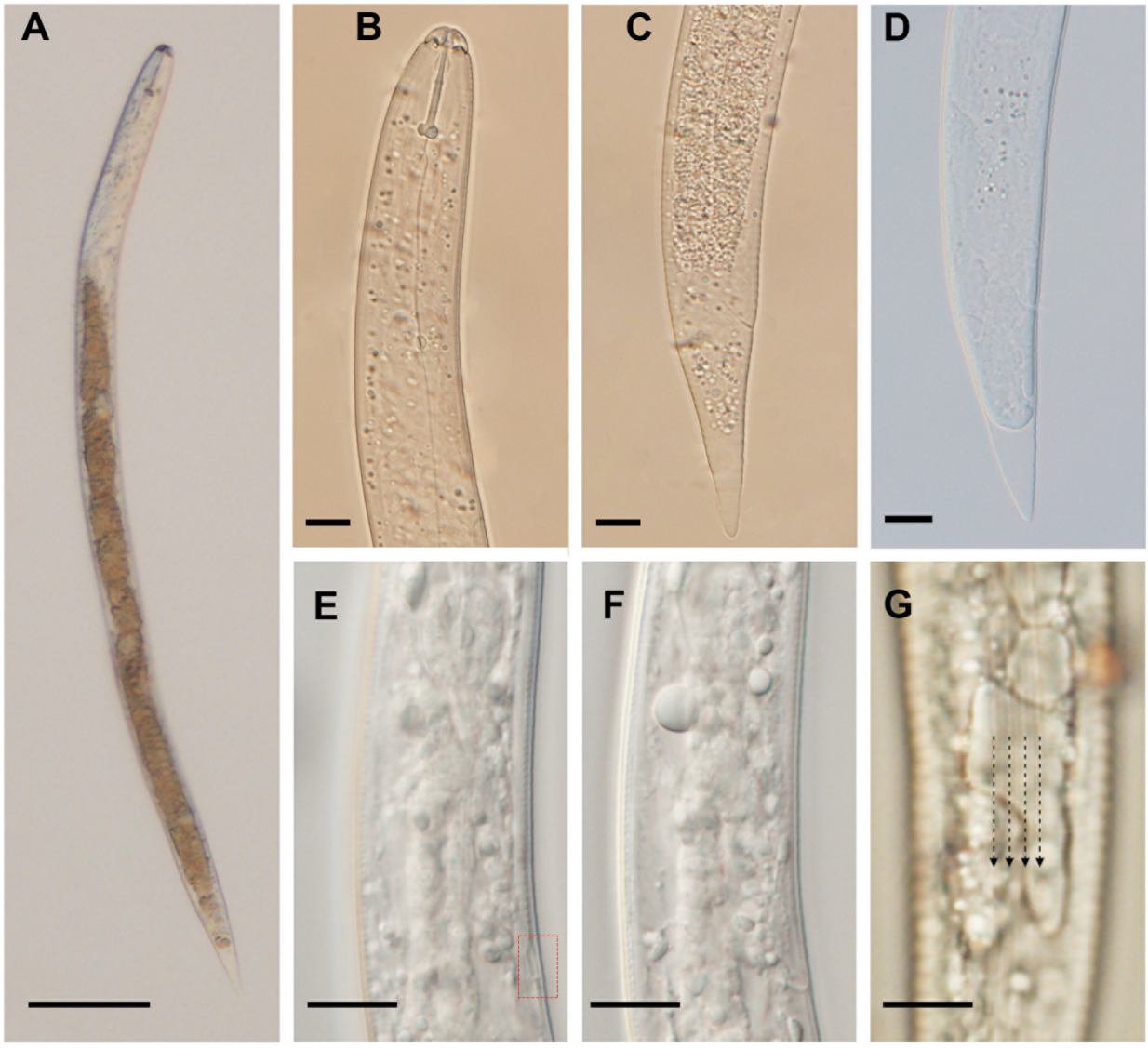

FIGURE 2. Light micrographs of *Cactodera chenopodiae* **n. sp.** (J2) A. Entire body of J2; B: Anterior region of J2; C–D: Tail of J2(U or V); E. Hemizonid of J2; F. Excretory pore of J2; G. Lateral field of J2. (Scale bars: A = 50 µm, B–G = 10 µm)

The phylogenetic tree based on ITS rRNA gene differed slightly from that based on 28S. Four main subclades can be identified in the ITS phylogeny: subclade A includes the same species reported in the 28S phylogeny (without *C. chenopodiae* **n. sp.**), in addition to *C. salina* Baldwin, Mundo-Ocampo & McClure, 1997, and *C. weissi* (Steiner, 1949) Krall & Krall, 1978; subclade B contains *C. estonica* and *C. chenopodiae* **n. sp.**; subclade C bears *C. galinsogae* and *C. milleri*; and subclade D is only represented by sequences of *C. cacti*.

Overall, the 28S and ITS phylogenies were congruent with respect to the monophyly of the different genera

included in the analyses. Within *Cactodera*, however, species relationships differed slightly between the two phylogenies [e.g., *C. chenopodiae* **n. sp.** as sister to *C. rosae* (28S rRNA tree) or sister to *C. estonica* (ITS rRNA tree)]; this difference can be due to the inclusion of more species in the ITS analysis.

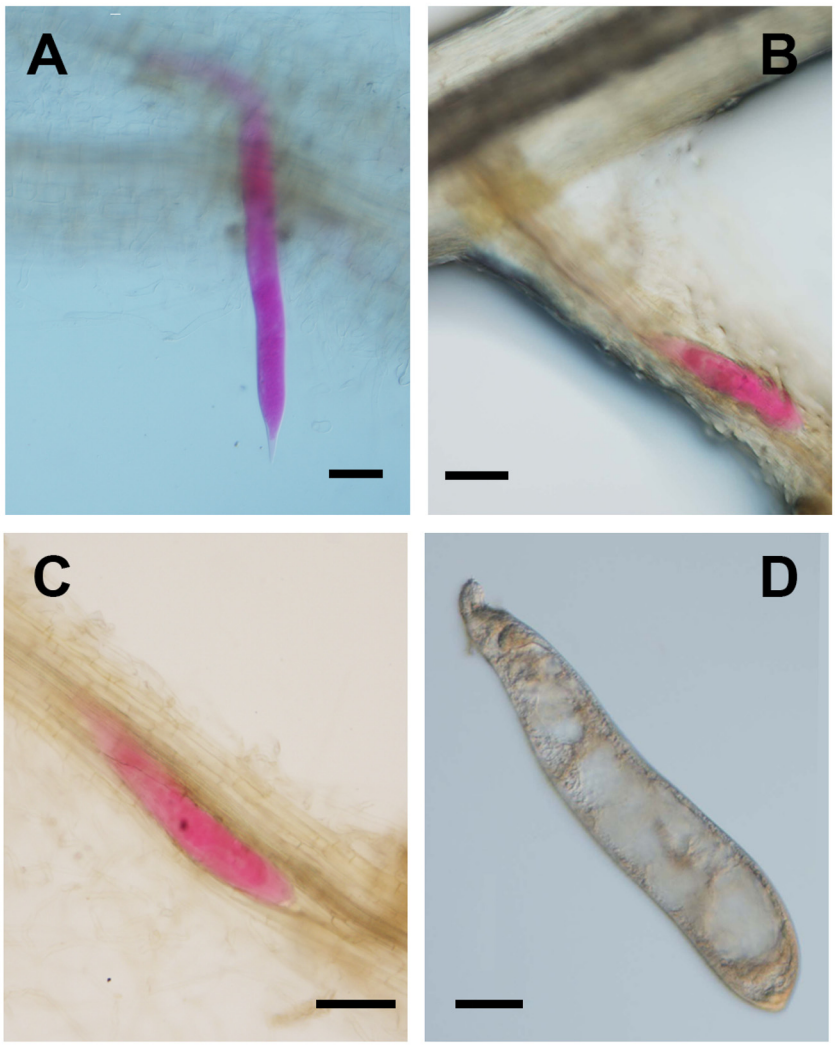

FIGURE 3. Light micrographs of Cactodera chenopodiae **n. sp.** (J3) A–C: J3 in root after root staining; D: J3 off root picked after centrifugal-flotation. (Scale bars: A–C = 100 µm, D = 50 µm)

Cactodera species known to parasitize common lambsquarter also include *C. milleri* (Graney & Bird, 1990). However, phylogenetic analyses based on the ITS rRNA (Fig. 7) suggest that *C. chenopodiae* **n. sp.** is genetically distant from *C. milleri* 4.9 % (44 bp difference). In fact, genetic divergence between *C. chenopodiae* **n. sp.** and species in subclade A for the ITS gene was about 4.9–5.9 % (44–53 bp difference); species in subclade B (*C. estonica*) was 1.6–1.8 % (16–17 bp difference); species in subclade C was about 4.0–4.9 % (36–44 bp difference); species in subclade D (*C. cacti*) was 9.5–9.6 % (86 bp difference). The molecular variation among the different clades supports *C. chenopodiae* **n. sp.** as a unique lineage.

The virtual sequence digestion using Restriction Analysis obtained for *C. chenopodiae* **n. sp.** is presented in Table 2. This RFLP-ITS pattern distinguishes *C. chenopodiae* **n. sp.** from comparable profiles available for other *Cactodera* species, as shown in Subbotin et al. (2010), and especially from species in subclade A, B and D, including *C. estonica*, *C. milleri*, *C. galinsogae*, *C. rosae*, *C. torreyanae*, *C. weissi*, *C. salina*, *C. cacti* (Table 2). A size comparison of fragments indicates that the enzyme *Taq*I is most useful to distinguish the new species from these eight *Cactodera* species.

TABLE 2. Sizes (in bp) * of PCR product and restriction fragments of the ITS-rRNA gene amplified by primers TW81 and AB28 for *Cactodera chenopodiae* n. sp. and eight other *Cactodera* species.

Species	Unrestricted	*AluI*	*Bsh1236I* (*BstUI*)	*BsuRI* (*HaeIII*)	*CfoI* (*HhaI*)	*MvaI* (*BstNI*)	*RsaI*	*TaqI*	References
C. chenopodiae n.sp.	976	895,81	976	525,277,174	348,329,299	459,271,246	534,365,34, 20,14,9	381,272,216, 65,42	KY475583 This study.
C. estonica	898	839,36	898	482,277,139	348,294,256	424,246,228	485,232,112, 51,9,9	562,271,65	AF274417 Subbotin *et al.* (2001)
C. milleri	885	849,36	885	745,140	345,286,254	415,243,164, 63	406.321.75.51 .14.9.9	551,269,65	AF161007 Ferris *et al.* (1999)
C. galinsogae	882	861,21	882	637,139,106	349.294.239	424,247,211	529,344,9	563,254,65	HQ260419 Subbotin *et al.* (2011)
C. rosae	885	864,21	842,43	488,197,125,75	344,196,145,81, 76,43	429,245,211	536,335,14	566,254,65	HQ260415 Subbotin *et al.* (2011)
C. torreyanae	915	878,37	855,60	505,213,197	312,262,195,86, 60	442,246,199, 28	534,362,10,9	580,270,65	KF214755 Cid Del Prado Vera *et al.* (2014)
C. weissi	890	748,106,36	831,59	502,207,181	344,292,195,59	420,244,199, 27	407,326,125, 14,9,9	556,269,65	AF161006 Ferris *et al.* (1999)
C. salina	893	751,106,36	834,59	502,196,101,94	342,195,157,140, 59	423,244,226	428,219,126, 111,9	559,269,65	AF161005 Ferris *et al.* (1999)
C. cacti	900	552,206,142	596.304	458,214,118,40, 24,24,22	343,253,185,112, 7	331,249,198, 95,27	545,346,9	567,268,65	AF498393 Tanha Maafi *et al.* (2003)

*Fragments obtained in results of virtual sequence digestion using Restriction Analysis. (http://www.yeastgenome.org/cgi-bin/PATMATCH/RestrictionMapper).

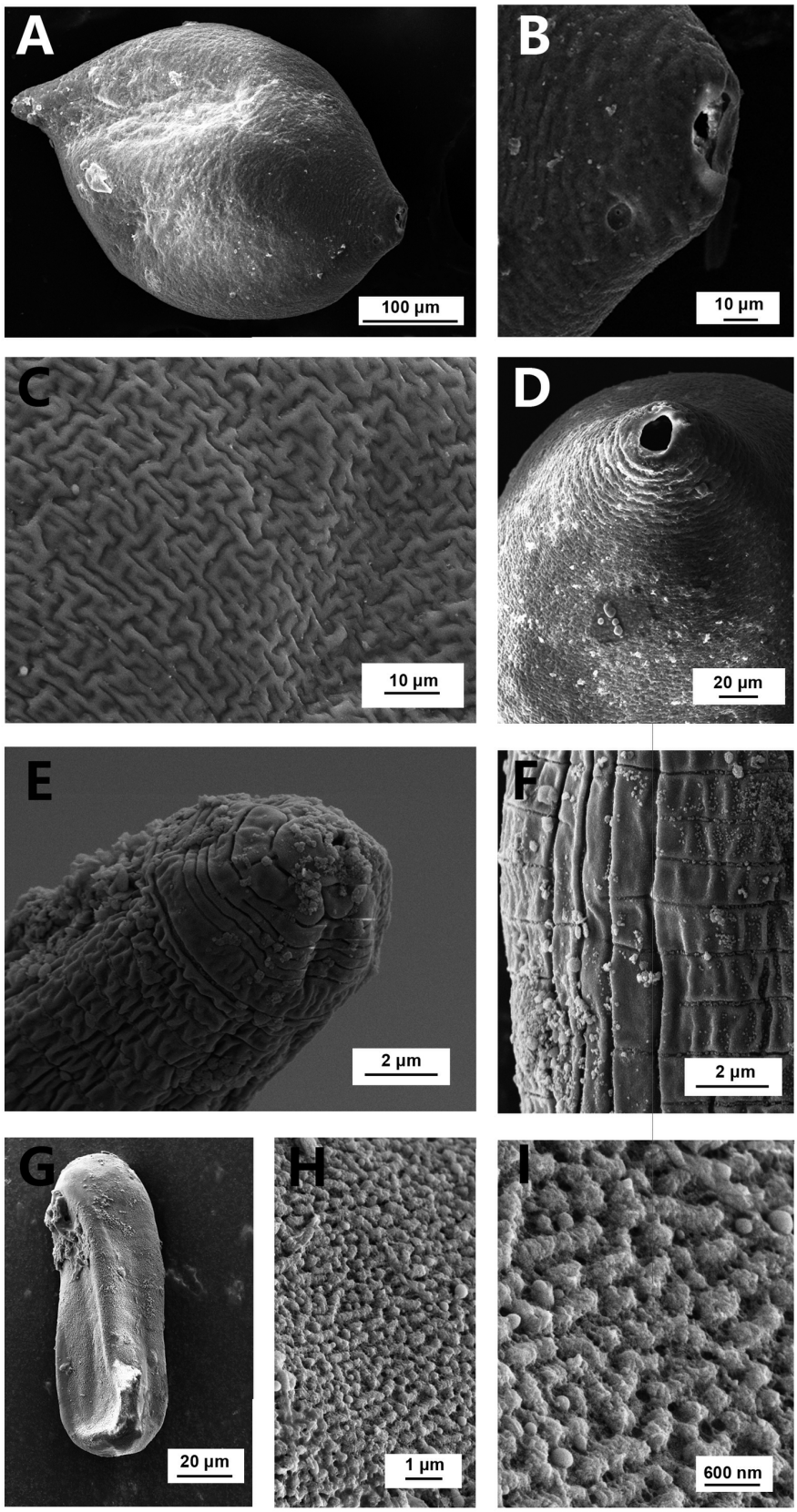

FIGURE 4. SEM micrographs of *Cactodera chenopodiae* **n. sp.** A. Cyst; B. Vulval cone with anus; C. Cuticle surface showing wavy pattern; D. Circumfenestra of cyst; E. Anterior region of J2; F. Lateral field of second-stage juvenile (J2) showing incomplete annulation; G. Egg; H–I. Pattern on surface of egg;

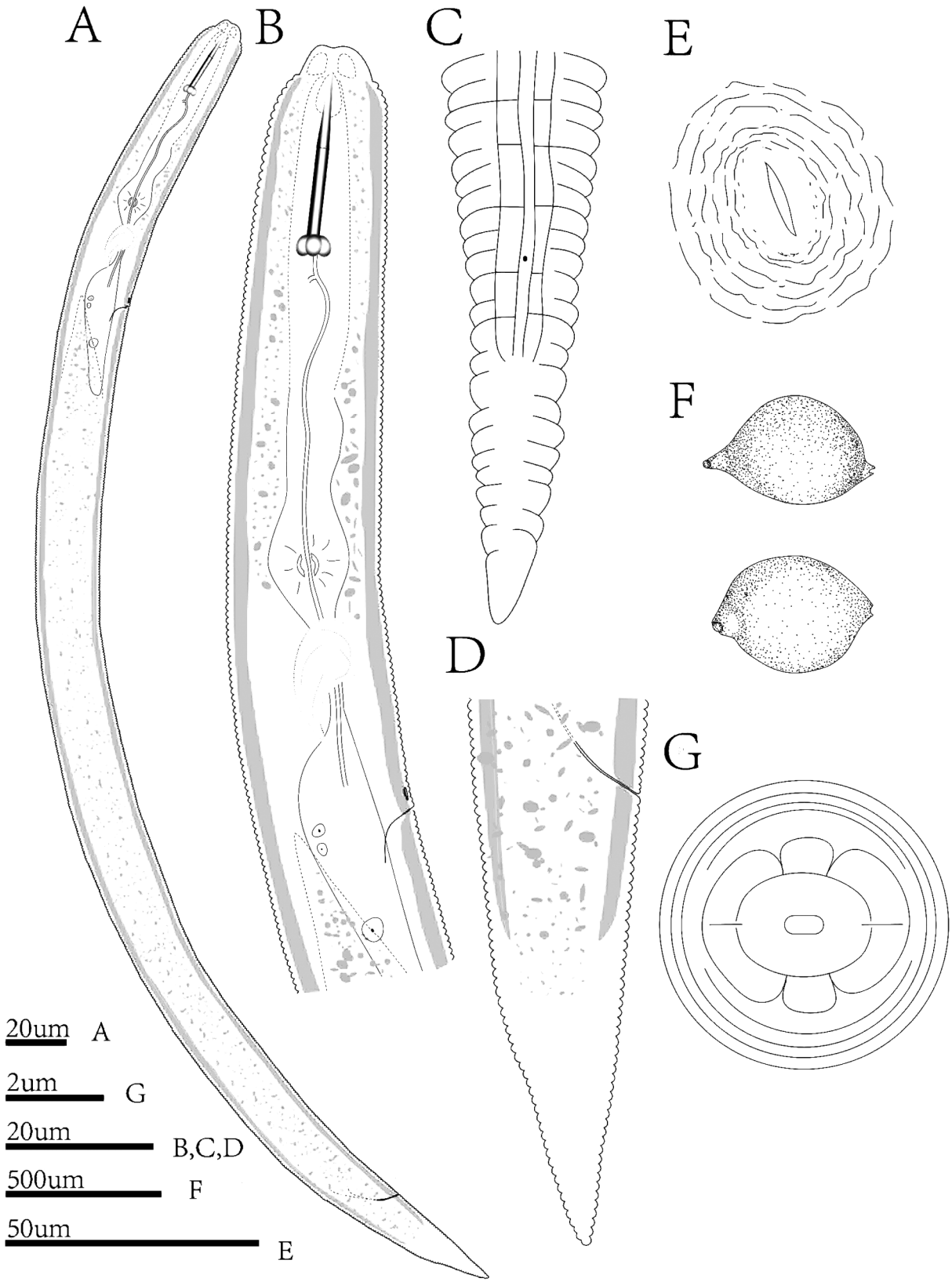

FIGURE 5. Drawing of *Cactodera chenopodiae* **n. sp.** A: Entire body of J2; B: Anterior region of J2; C–D: Tail of J2; E: Terminal view of cone; F: Cyst; G: Face view of J2 as observed with SEM.

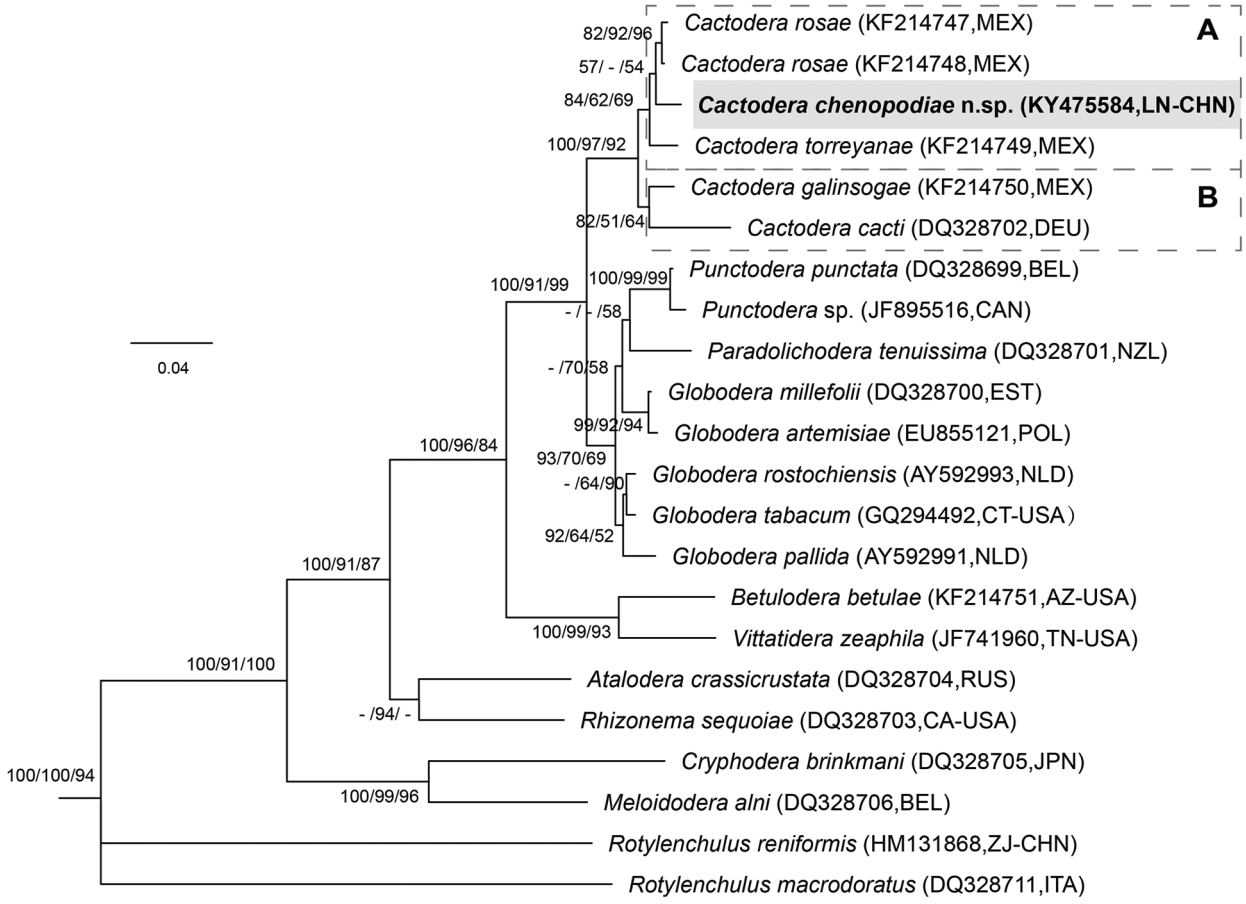

FIGURE 6. Phylogenetic relationships within populations and species of *Heteroderinae* Filipjev & Schuurmans Stekhoven,1941. The 50% majority rule consensus trees from Bayesian analysis generated from two runs as inferred from the analysis of the D2–D3 of 28S rRNA gene sequences under the GTR + G model. Two clades (A and B) are identified among *Cactodera* sequences. Branch support (only above 50%) is shown on branches as Bayesian inference (BI)/maximum likelihood (ML)/maximum parsimony (MP). A dash (-) indicates branch support below 50% or incongruence between BI and ML/MP analyses. Sequences produced in this study are highlighted in gray.

Discussion

Cactodera, a cyst-forming nematode genus with great economic significance worldwide, currently encompasses 14 valid species (Subbotin *et al.*, 2010; Cid Del Prado Vera & Subbotin, 2014). To date, three species of *Cactodera* have been reported in China: *C. cacti* was reported by Pan *et al.* (1982) and Duan *et al.*, (2012) parasitizing on the roots of *Opuntia dillenii* Haw. (Fujian province) and on the roots of *Hylocereus undatu* (Haw.) Britton & Rose (greenhouse, Liaoning province); also *C. thornei* (Golden & Raski, 1977) Mulvey & Golden, 1983 was found in cereal fields in Qinghai province (Peng *et al.*, 1997).

Clearly, *O. dillenii* and *H. undatus* are likely to be introduced into China as ornamental plants from abroad (i.e. not native), whereas *C. thornei* has been only found in typical agro-ecosystems, rather than from a natural undisturbed environment. Conversely, *C. chenopodiae* **n. sp.** was found on common lambsquarter under an ancient elm tree near a lake in the Beiling Park, thus being the first report of *Cactodera* from native vegetation in China. According to an ongoing broad survey in China (Zhu, unpublished), the occurrence of *Cactodera* species reported in China is restricted and regional, and it is not yet known to cause widespread damage.

In the genus *Cactodera*, most species (11 out of 14) have males which suggests that gonochoristic reproduction is common in the genus. Whereas three *Cactodera* species (*C. estonica*, *C. radicale* and *C. rosae*) lack of males (Chizhov *et al.*, 2008; Cid Del Prado Vera & Miranda, 2008; Kirjanova & Krall, 1963; Krall & Krall, 1978; Golden & Raski, 1977; Sturhan, 2010). Although soil samples were repeatedly collected (monthly from May to October,

2015) in type locality of *C. chenopodiae* **n. sp.**, male specimens were never found, suggesting that the new species may have different mode of reproduction, i.e., parthenogenetic or hermaphroditic. Nevertheless, additional sampling, especially representing other seasons of the year or potted plants in the indoor expansion, might be needed to fully test such hypothesis.

As most species of *Cactodera* were described in the 20th century, their descriptions did not include DNA sequences. Thus, some *Cactodera* species are currently not represented in GenBank for further comparisons [e.g., *C. acnidae* (Schuster & Brezina) Wouts, 1985, *C. amaranthi* (Stoyanov) Krall & Krall, 1978, *C. eremica* Baldwin & Bell, 1985, *C. evansi* Cid Del Prado & Rowe, 2000, *C. radicale* Chizhov, Udalova & Nasonova, 2008, *C. thornei*]. Although *C. chenopodiae* **n. sp.** has been herein molecularly characterized, sequence information with respect to the genus *Cactodera* is still limited in molecular databases, thus limiting inferences on species relationships as well as understanding of intra and interspecific sequence variation.

Notwithstanding the morphometrics (i.e., the ranges for some morphological features) of *C. chenopodiae* **n. sp.** overlapped with several other *Cactodera* species (e.g., *C. cacti* and *C. milleri*) phylogenetic analyses based on two rRNA genes clearly supported the new species as an independent lineage. Moreover, sequence divergence for the ITS rRNA among *C. chenopodiae* **n. sp.**, *C. cacti* and *C. milleri* ranged from about 5–10% (44 to 86 bp difference). Our findings also show that morphological characters commonly used on the diagnostics of *Cactodera* species can confound our ability in identifying potential new species and that molecular data should therefore be used as a common practice to describe new species of *Cactodera* in the future.

Increasing molecular representation of *Cactodera* species will certainly improve the knowledge of the taxonomy, phylogeny, and biogeography of the group. Broader understanding of the genus, including *C. chenopodiae* will also be supported by further studies of comparative biology including host range, development, host parasite relationships and distribution.

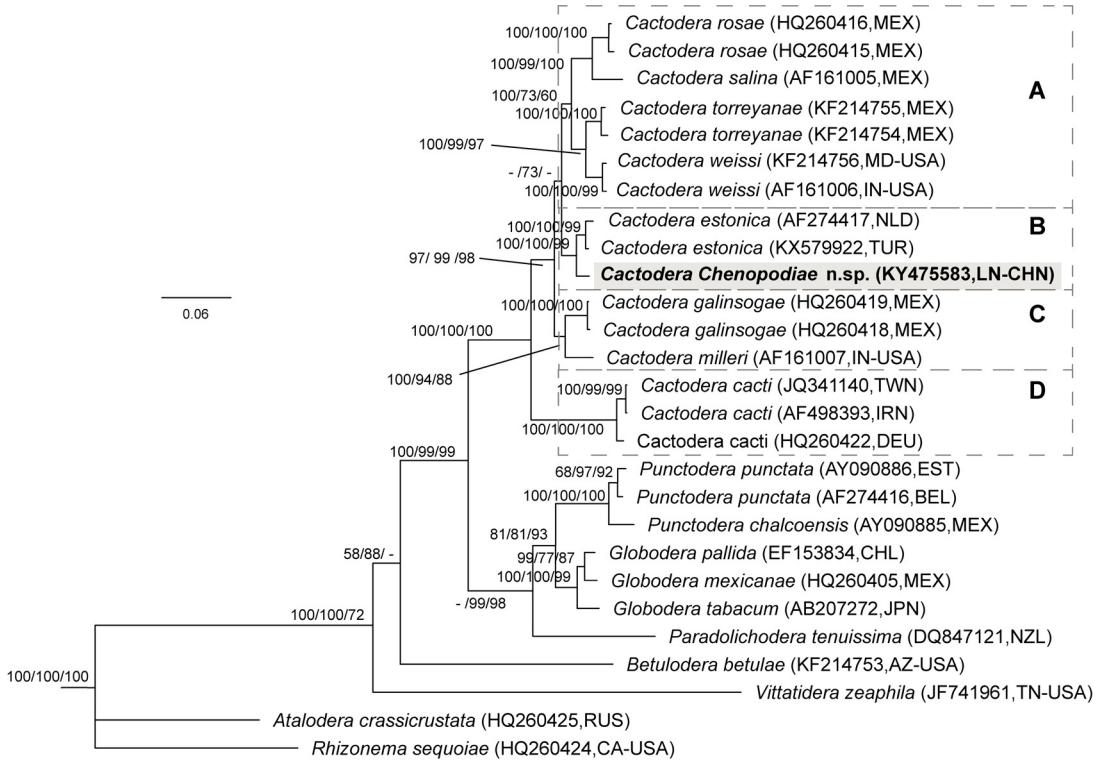

FIGURE 7. Phylogenetic relationships within populations and species of *Heteroderinae* Filipjev & Schuurmans Stekhoven,1941. The 50% majority rule consensus trees from Bayesian analysis generated from two runs as inferred from the analysis of the ITS rRNA gene sequences under the GTR + G + I model. Three clades (A, B, C and D) are identified among *Cactodera* sequences. Branch support (only above 50%) is shown on branches as Bayesian inference (BI)/maximum likelihood (ML)/maximum parsimony (MP). A dash (-) indicates branch support below 50% or incongruence between BI and ML/MP analyses. Sequences produced in this study are highlighted in gray.

Key to species of *Cactodera*
(modified from Subbotin *et al.*, 2010, Cid Del Prado Vera & Subbotin 2014)

1	Cyst generally two times or more longer than wide, mean L/W ratio = 2.3	*C. estonica*
-	Cyst usually less than twice as long as wide, mean L/W ratio = 1.1–1.8.	2
2	Eggshell punctate.	3
-	Eggshell smooth.	10
3	Mean stylet length of J2s ≥ 26 µm.	4
-	Mean stylet length of J2s < 26 µm.	5
4	J2s tail length = 48–64 µm, hyaline region = 23–28 µm, fenestral diam. = 23–41 µm	*C. thornei*
-	J2s tail length = 37–48 µm, hyaline region = 17–24 µm, fenestral diam. = 14–25 µm	*C. eremica*
5	Mean J2s body length ≥ 411 µm, mean tail length ≥ 42 µm.	6
-	Mean J2s body length < 411 µm, mean tail length < 42 µm.	8
6	Mean J2s tail length < 43 µm, b ratio < 3.5	*C. milleri*
-	Mean J2s tail length ≥ 43 µm, b ratio > 3.5.	7
7	Female L/W ratio < 1.4, mean hyaline region of J2s < 22 µm.	*C. cacti*
-	Female L/W ratio ≥ 1.4, mean hyaline region of J2s ≥ 22 µm	*C. chenopodiae* ***sp. n***
8	Fenestral diam. < 25 µm	9
-	Fenestral diam. ≥ 25 µm.	*C. galinsogae*
9	Hyaline region of J2s = 4–8 µm.	*C. rosae*
-	Hyaline region of J2s = 16–23 µm.	*C. evansi*
10	Mean J2s tail length < 40 µm	11
-	Mean J2s tail length ≥ 40 µm.	13
11	Mean J2s body length < 406 µm, mean hyaline region < 16 µm.	*C. amaranthi*
-	Mean J2s body length ≥ 406 µm, mean hyaline region ≥ 16 µm.	12
12	Cyst with distinct vulval cone, J2s stylet length =21.0–23.0 µm.	*C. torreyanae*
-	Cyst without distinct vulval cone, J2s stylet length =23.4–25.0 µm	*C. salina*
13	J2s stylet knobs anterior surface concave, DGO = 4.5–5.6 µm.	14
-	J2s stylet knobs anterior surface convex, DGO = 2.5–3.0 µm	*C. acnidae*
14	Vulval denticles present, J2s tail length = 43–50 µm.	*C. weissi*
-	Vulval denticles absent, J2s tail length = 46–60 µm.	*C. radicale*

Acknowledgements

This work was made possible through the National Natural Science Foundation of China (31571985, 31330063), the Special Fund for Agro-Scientific Research in the public interest 201503114-12, and China Agriculture Research System CARS-04-PS13. The first author would like to also express his sincere gratitude to Dr. James G. Baldwin for the helpful guidance on the identification and description of the new species.

References

Baldwin, J.G. & 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 & Sher, 1971 (Heteroderidae). *Journal of Nematology*, 17, 187–201.

Baldwin, J.G., Mundo-Ocampo, M. & McClure, M.A. (1997) *Cactodera salina* n. sp. from the Estuary plant, *Salicornia bigelovii*, in Sonora, Mexico. *Journal of Nematology*, 29, 465–473.

Bernard, E.C., Handoo, Z.A., Powers, T.O., Donald, P.A. & Heinz, R.D. (2010) *Vittatidera zeaphila* (Nematoda: Heteroderidae), a new genus and species of cyst nematode parasitic on corn (*Zea mays*). *Journal of Nematology*, 42, 139–150.

Bybd Jr, D., Kirkpatrick, T. & Barker, K. (1983) An improved technique for clearing and staining plant tissues for detection of nematodes. *Journal of nematology*, 15, 142–143.

Chizhov, V.N., Udalova, Zh. V. & Nasonova, L.V. (2008) *Globodera arenaria* n. sp. and *Cactodera radicale* n. sp. (Nematoda: Tylenchida) from rizosphere of meadows in Mid-Volga region. *Russian Parasitological Journal*, 2, 109–116.

Cid Del Prado Vera, I. & Miranda, B.L. (2008) Second cyst-forming nematode parasite of barley (Hordeum vulgare L. Var. Esmeralda) from Mexico. *Nematropica*, 38, 105–114.

Cid Del Prado Vera, I. & Rowe, J.A. (2000) *Cactodera evansi* sp. n. and *Meloidodera astonei* sp. n. (Tylenchida: Heteroderidae) from Mexico. *International Journal of Nematology*, 10, 159–168.

Cid Del Prado Vera, I. & Subbotin, S.A. (2012) *Belonolaimus maluceroi* n. sp. (Tylenchida: Belonolaimidae) from a tropical forest in Mexico and key to the species of *Belonolaimus*. *Nematropica*, 42, 201–210.

Cid Del Prado Vera, I. & Subbotin, S.A. (2014) A new cyst nematode, *Cactodera torreyanae* n. sp. (Tylenchida: Heteroderidae), parasitising romerito, *Suaeda torreyana*, in Texcoco, Mexico. *Nematology*, 16, 163–174. https://doi.org/10.1163/15685411-00002754

Cid Del Prado Vera, I., Ferris, H., Nadler, S.A. & Argumedo, R.L. (2012) Four new species of *Tripylina* Brzeski, 1963 (Enoplida: Tripylidae) from México, with an emended diagnosis of the genus. *Journal of Nematode Morphology & Systematics*, 15, 71–86.

De Maeseneer, J. & d'herde, J. (1963) Méthodes utilisées pour l'étude des anguillules libres du sol. *Revue d'Agriculture*, 16, 441–447.

Duan, Y.X., Wang, D. & Chen, L.J. (2012) First report of the cactus cyst nematode, *Cactodera cacti*, on cactus in northern China. *Plant Disease*, 96, 1385. https://doi.org/10.1094/PDIS-04-12-0374-PDN

Fenwick, D.W. (1940) Methods for the recovery and counting of cysts of *Heterodera schachtii* from soil. *Journal of Helminthology*, 18, 155–172. https://doi.org/10.1017/S0022149X00031485

Ferris, V.R., Krall, E., Faghihi, J. & Ferris, J.M. (1999) Phylogenetic relationships of *Globodera millefolii*, *G. artemisiae*, and *Cactodera salina* based on ITS region of ribosomal DNA. *Journal of Nematology*, 31, 498–507.

Ferris, V.R., Sabo, A., Baldwin, J.G., Mundo-Ocampo, M., Inserra, R.N. & Sharma, S. (2004) Phylogenetic relationships among selected Heteroderoidea based on 18S and ITS ribosomal DNA. *Journal of Nematology*, 36, 202–206.

Filipjev, I.N. & Schuurmans-Stekhoven, J.H. Jr. (1941) A manual of agricultural helminthology. *European Journal of Plant Pathology*, 47, 234–236.

Graney, L.S.O. & 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, 457–480.

Golden, A.M. & Raski, D.J. (1977) *Heterodera thornei* n. sp. (Nematoda: Heteroderidae) and a review of related species. *Journal of Nematology*, 9, 93–112.

Huelsenbeck, J.P. & Ronquist, F. (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, 17, 754–755. https://doi.org/10.1093/bioinformatics/17.8.754

Kirjanova, E.S. & Krall, E. (1963) *Heterodera estonica* n. sp. (Nematodes: Heteroderidae), Estonian cyst forming nematode. *Izvestija Akademii Nauk Estonskoj SSR*, *Biologicheskaja Seria*, 12, 219–223.

Krall, E.L. & 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. *In: Fitogel'mintologicheskie Issledovaniya.* USSR, Nauka, Moscow, pp. 39–56.

Maafi, Z.T., Subbotin, S.A. & Moens, M. (2003) Molecular identification of cyst-forming nematodes (Heteroderidae) from Iran and a phylogeny based on ITS-rDNA sequences. *Nematology*, 5, 99–111. https://doi.org/10.1163/156854102765216731

Mulvey, R.H. & Golden, A.M. (1983) An illustrated key to the cystforming genera and species of Heteroderidae in the western hemisphere with species morphometrics and distribution. *Journal of Nematology*, 15, 1–59.

Nylander, J.A.A. (2004) MrModeltest v2. Program distributed by the author. *Bioinformatics*, 24, 581–583.

Pan, C., Lin, J. & Xue, R. (1997) Description of *Cactodera cacti* and their observation by scanning electron microscope. *Acta Parasitologica et Medica Entomologica Sinica*, 4, 214–217.

Peng, D.L. & Vovlas, N. (1994) Occurrence of the cyst-forming nematode *Cactodera thornei* in China. *Nematologia Mediterranea*, 22, 75–78.

Qin, L., Kudla, U., Roze, E.H.A., Goverse, A., Popeijus, H., Nieuwland, J., Overmars, H., Jones, J.T., Schots, A. & Smant, G. (2004) Plant degradation: a nematode expansin acting on plants. *Nature*, 427, 30. https://doi.org/10.1038/427030a

Rambaut, A. (2009) FigTree v1.3.1. Available from: http://tree.bio.ed.ac.uk (accessed 14 March 2018)

Sabo, A., Reis, L.G.L., Krall, E., Mundoocampo, M. & Ferris, V.R. (2002) Phylogenetic relationships of a distinct species of *Globodera* from Portugal and two *Punctodera* species. *Journal of Nematology*, 34, 263–266.

Seinhorst, J.W. (1959) A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. *Nematologica*, 4, 67–69. https://doi.org/10.1163/187529259X00381

Steiner, G. (1949) Plant nematodes the grower should know. *Proceedings Soil Science Society of Florida*, 4B, 72–117.

Subbotin, S.A., Cid Del Prado Vera, I., Mundo-Ocampo, M. & Baldwin, J.G. (2011) Identification, phylogeny and phylogeography of circumfenestrate cyst nematodes (Nematoda: Heteroderidae) as inferred from analysis of ITS-rDNA. *Nematology*, 13, 805–824. https://doi.org/10.1163/138855410X552661

Subbotin, S.A., Mundo-Ocampo, M. & Baldwin, J.G. (2010) *Systematics of cyst nematodes (Nematodes: Heteroderinae)*. *Vol. 8*. *Part A*. Brill Academic Publishers, Leiden, 351 pp.

Subbotin, S.A., Sturhan, D., Chizhov, V.N., Vovlas, N. & Baldwin, J.G. (2006) Phylogenetic analysis of Tylenchida Thorne, 1949 as inferred from D2 and D3 expansion fragments of the 28S rRNA gene sequences. *Nematology*, 8, 455–474. https://doi.org/10.1163/156854106778493420

Subbotin, S.A., Vierstraete, A., De, L.P., Rowe, J., Waeyenberge, L., Moens, M. & 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 & Evolution*, 21, 1–16.
https://doi.org/10.1006/mpev.2001.0998

Sturhan, D. (2010) Notes on morphological characteristics of 25 cyst nematodes and related Heteroderidae. *Russian Journal of Nematology*, 18, 1–8.

Tovar Soto, A., Cid Del Prado Vera, I., Nicol, J.M., Evans, K., Sandoval Islas, J.S. & Martinez Garza, A. (2003) *Cactodera galinsogae* n. sp. (Tylenchida: Heteroderinae) on barley (*Hordeum vulgare* L.) in the high valleys of Mexico. *Nematropica*, 33, 41–54.

Uehara, T., Kushida, A., Itou, K., Narabu, T. & Momota, Y. (2005) Discrimination of three cyst-forming nematodes of the genus *Globodera* (Nematode: Heteroderidae) from Japan based on PCR-RFLP of ribosomal DNA. *Applied Entomology & Zoology*, 40, 537–543.
https://doi.org/10.1303/aez.2005.537

Wouts, W.M. (1985) Phylogenetic classification of the family Heteroderidae (Nematoda: Tylenchida). *Systematic Parasitology*, 7, 295–328.
https://doi.org/10.1007/BF00009997

SUPPLEMENTARY TABLE 1. Detail primer used in this study.

Primer Code	Sequence (5' → 3')	Amplified gene	References
998F	CTCAAAGATTAAGCCATGC	18S rDNA	Holterman *et al.,* 2006
1212R	TTTACGGTCAGAACTAGGG		
1813F	CTGCGTGAGAGGTGAAAT	18S rDNA	Holterman *et al.,* 2006
2646R	GCTACCTTGTTACGACTTTT		
D2A	ACAAGTACCGTGAGGGAAAGTTG	28S rDNA	De Ley *et al.,* 1999
D3B	TCGGAAGGAACCAGCTACTA		
TW81	GTTTCCGTAGGTGAACCTGC	ITS rDNA	Joyce et.al., 1994
AB28	ATATGCTTAAGTTCAGCGGGT		
JB3	TTTTTTGGGCATCCTGAGGTTTAT	COI mtDNA	Bowles *et al.,* 1992
JB4.5	TAAAGAAAGAACATAATGAAAATG		