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Vittatidera zeaphila (Nematoda: Heteroderidae), a new genus and species of cyst nematode parasitic on corn (*Zea mays*)

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Abstract: A new genus and species of cyst nematode, *Vittatidera zeaphila*, is described from Tennessee. The new genus is superficially similar to *Cactodera* but is distinguished from other cyst-forming taxa in having a persistent lateral field in females and cysts, persistent vulval lips covering a circumfenestrate vulva, and subventral gland nuclei of the female contained in a separate small lobe. Infective juveniles (J2) are distinguished from all previously described *Cactodera* spp. by the short stylet in the second-stage juvenile (14-17 μm); J2 of *Cactodera* spp. have stylets at least 18 μm long. The new species also is unusual in that the females produce large egg masses. Known hosts are corn and goosegrass. DNA analysis suggests that *Vittatidera* forms a separate group apart from other cyst-forming genera within Heteroderinae.

Key words: cyst nematode, *Eleusine indica*, goosegrass, maize, molecular analysis, new genus, taxonomy, *Vittatidera zeaphila*, *Zea mays*.

Cyst nematodes are widespread but with the exceptions of *Heterodera avenae* Wollenweber and *H. zeae* Koshi, Swarup & Sethi (Baldwin & Mundo-Ocampo 1991) are not significant parasites of Poaceae. In the late 1970s the first author collected specimens of a cyst nematode from goosegrass (*Eleusine indica* (L.) Gaertn.) growing in a tomato field in west Tennessee. These specimens were sent to the late A. Morgan Golden, who was of the opinion (in litt.) that they represented an undescribed species. The species was not described at that time, but in 2006 the fourth author collected a similar nematode from corn (*Zea mays*) in the same general region of the state. The second author compared specimens of the two collections and confirmed that they were the same species. Further examination revealed that this taxon has features unlike any other genus of cyst-forming nematodes.

The objective of this paper is the description of a new genus and species of Heteroderidae, *Vittatidera zeaphila*, which parasitizes corn and goosegrass. A companion paper (Donald et al., in prep.) provides information on host range studies and environmental requirements.

Materials and Methods

Specimens used to prepare the description were obtained from greenhouse corn cultures derived from the collection of an isolate from Obion County, Tennessee, in 2006, and from specimens collected in the late 1970s from Lauderdale County, Tennessee. Specimens were fixed in either warm (40°C) or hot (80°C) 4% formalin, processed to glycerin with a rapid method (Seinhorst 1959), and mounted in anhydrous glycerin on microscope slides.

DNA analyses Juvenile nematodes of *Vittatidera zeaphila* were obtained from culture. Because of an initial concern that *V. zeaphila* might be conspecific with *H. zeae*, frozen individuals of *H. zeae* were sequenced for each of the genetic markers and included in sequence comparisons.

Specimens of *V. zeaphila* were individually selected and manually disrupted to provide template for DNA amplifications (Powers & Harris 1993). Small (18S) and large (28S) subunit rDNA and the internal transcribed spacer 1 (ITS1) regions were amplified in 50-μl reactions, each containing: 31.5 μl distilled water, 5 μl 10x PCR buffer (100 mM Tris-HCl (pH 8.3), 500 mM KCl, 15 mM $MgCl_2$, 0.01% gelatin), 1 μl dNTP mixture (2.5 mM each of dATP, dCTP, dGTP, and dTTP), 3.0 μl 25 mM $MgCl_2$, 1.0 μl of each primer (20 μM), 2.5 μl of JumpStart REDTaq polymerase (Sigma, St. Louis, MO; 1.0 u/μl), and 5 μl of DNA template. All PCR reactions were performed on a DNA Engine PTC-200 Peltier thermal cycler (MJ Research, Watertown, MA) with the following run parameters: one initial denaturation cycle at 95°C for 3 min, followed by 50 cycles at 95°C for 15 sec, 55 or 50°C for 15 sec, ramped increase at 0.5°C per sec to 72°C for 1 min. A final elongation step was run at 72°C for 5 min. Negative controls were included in each amplification series. The following primer sets were used in this study.

18S ribosomal DNA: Near full-length 18S sequence of 1,565 bases was obtained in three separate amplifications. The primer sets were:

5′-first primer pair:
G18S4 (5′ to 3′) –> GCTTGTCTCAAAGATTAAGCC
18s721R (5′ to 3′) –> AGCACTCTAATTTTTTCAAAG
Middle third primer pair:
18s550a(5′ to 3′) –> AGCCGCGGTAATTCCAG
18s977R (5′ to 3′) –> TTTACGGTTAGAACTAGGGCGG
3′-third primer pair:
18s1.2a(5′ to 3′) –> CGATCAGATACCGCCCTAG
18sr2b (5′ to 3′) –> TACAAAGGGCAGGGACGTAAT

Primer 18Sr2b (positions 1567 to 1547) is the reverse complement of primer rDNA2 from Vrain *et al.* (1992).

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28S -D2/3 primer set: This primer set produces a product of 741 bases in *V. zeaphila*.

D2A (5′ to 3′) –> ACAAGTACCGTGAGGGAAAGTTG
D3B (5′ to 3′) –> TCGGAAGGAACCAGCTACTA
ITS1 primer set:
rDNA2: 5′-TTGATTACGTCCCTGCCCTTT-3′
rDNA1.58Sa: 5′-ACGAGCCGAGTGATCCACC-3′

Primer rDNA2 is a modified version of the reverse complement of 18Sr2b (above) and is paired with primer rDNA1.58Sa, which is located in the 5' region of the 5.8S rDNA gene. This primer set produces a fragment of 582 nucleotides in *V. zeaphila*, excluding primers, of which 176 nucleotides are the 3' end of 18S rDNA. Sequence comparisons of the ITS1 region were confined to the spacer region only. Annealing temperature for this ITS1 primer set was 55°C.

PCR products were purified and concentrated with Microcon-100 centrifugal filter units (Millipore Inc., Bedford, Massachusetts) and sent to the DNA Sequencing Lab (University of Arkansas for Medical Sciences) for direct sequencing in both directions. Amplification primers were used as sequencing primers. DNA sequences were edited and assembled using CodonCode Aligner (CodonCode Corp, Dedham, Massachusetts). DNA alignment was by MUSCLE 3.7 (Edgar, 2004). Maximum likelihood analysis (100 bootstrap replicates for estimation of branch support) was carried out with PHYML 3.0 (http://www.phylogeny.fr) using the HKY85 substitution model, with gamma parameters and proportion of invariant sites estimated, and a transition/tranversion ratio of 4.

Description

Vittatidera new genus

Cysts orange-brown to brown, lemon-shaped, necks short; secretions around neck persistent. Cyst surface with weak longitudinal ridges between neck and cone and with underlying transverse rows of punctations; zigzag pattern absent. Lateral field in both females and cysts arched or sinuous, represented by short, transverse lines between neck and cone area. Cuticle in neck region granulated. Vulval cone slightly protuberant, membranous vulval lips persistent; vulval aperture circular to rhomboid, circumfenestrate, with irregular denticle-like protuberances around the periphery of orifice. Bullae, vulval bridge, vulval underbridge, and internal denticles absent. Cone tip encircled with short, wavy ridges extending to vicinity of anus. Cone region with numerous minute, irregularly distributed duct-like tubes extending to pores on cuticle surface. Cuticle around anus thinner than rest of cyst but not developed as a fenestra. Anus subterminal. Subventral gland nuclei of female contained in a discrete lobe extending from the large dorsal gland. Phasmid apertures present on white females, approximately at level of anus. Males of variable length; stylet knobs rounded. Second-stage juveniles heteroderiform, tail conoid with narrowly rounded tip, phasmid apertures pore-like. Egg shell smooth.

Type species: *Vittatidera zeaphila* n. sp.

Etymology: Combined Latin *vittatus* (striped) and Greek *dera* (skin or hide), referring to the presence of a lateral field in the females and cysts.

Differential diagnosis Superficially *Vittatidera* n. g. resembles *Cactodera* Krall & Krall in having a circumfenestrate vulva in the cyst stage. It differs from all cyst-forming genera in possessing a distinct lateral field in the adult female and cyst stages. The new genus also has unusually persistent vulva lips that remain intact well after the cyst has formed. Unlike *Cactodera*, females of the new genus produce large egg masses with numerous eggs, a character shared with a few *Heterodera* spp. The unique characters of this new genus require modification of the diagnosis of Heteroderinae Filipjev & Schuurmans Stekhoven, the subfamily of Heteroderidae that contains the cyst-forming taxa.

Emended diagnosis of Heteroderinae (After Siddiqi, 2000): Heteroderidae. Mature females spherical, oval, pear or lemon-shaped with a short neck, turning into a tough, hard-walled, yellowish, light to dark brown, or blackish cyst containing eggs and juveniles, eggs sometimes laid in a large gelatinous matrix (egg mass). Cuticle surface with zigzag or lace-like pattern, or with elongated, fusiform ridges, overlaying fine pattern of annulations, or annulations absent in mature females and cysts. Lateral field on females and cysts rarely present; if present (*Vittatidera*), lateral field arching or sinuate, extending from neck to cone base. Vulva and anus close together, almost terminal, on raised vulval cone or in flat to concave vulval basin. Clear hyaline single (circumfenestrate) or doubled (ambifenestrate, bifenestrate) vulval fenestrae present; anal fenestra present in *Punctodera*, vulva lips rarely persistent (*Vittatidera*). Esophageal gland nuclei contained in single lobe or subventral gland nuclei of the female contained in separate small lobe (*Vittatidera*) Male developing through metamorphosis with labial region annulated, four incisures in lateral field, tail short, hemispherical, without bursa. Second-stage juvenile stylet over 14 um long; with three to four incisures in lateral field.

Type genus: *Heterodera* Schmidt, 1871

Other genera (after Wouts & Baldwin 1998, Sturhan 2002, Sturhan et al. 2007, Mundo-Ocampo et al. 2008):

Betulodera Sturhan, 2002
Cactodera Krall & Krall, 1978
Dolichodera Mulvey & Ebsary, 1980
Globodera Skarbilovich, 1959
Paradolichodera Sturhan, Wouts & Subbotin, 2007
Punctodera Mulvey & Stone, 1976
Vittatidera n. gen.

Vittatidera zeaphila n. sp.
(Figs. 1-7)

Females Female measurements: length 389-534 µm (mean 455 ± 17.3 SE, CV = 11.4, n = 20), neck length 71-117 µm

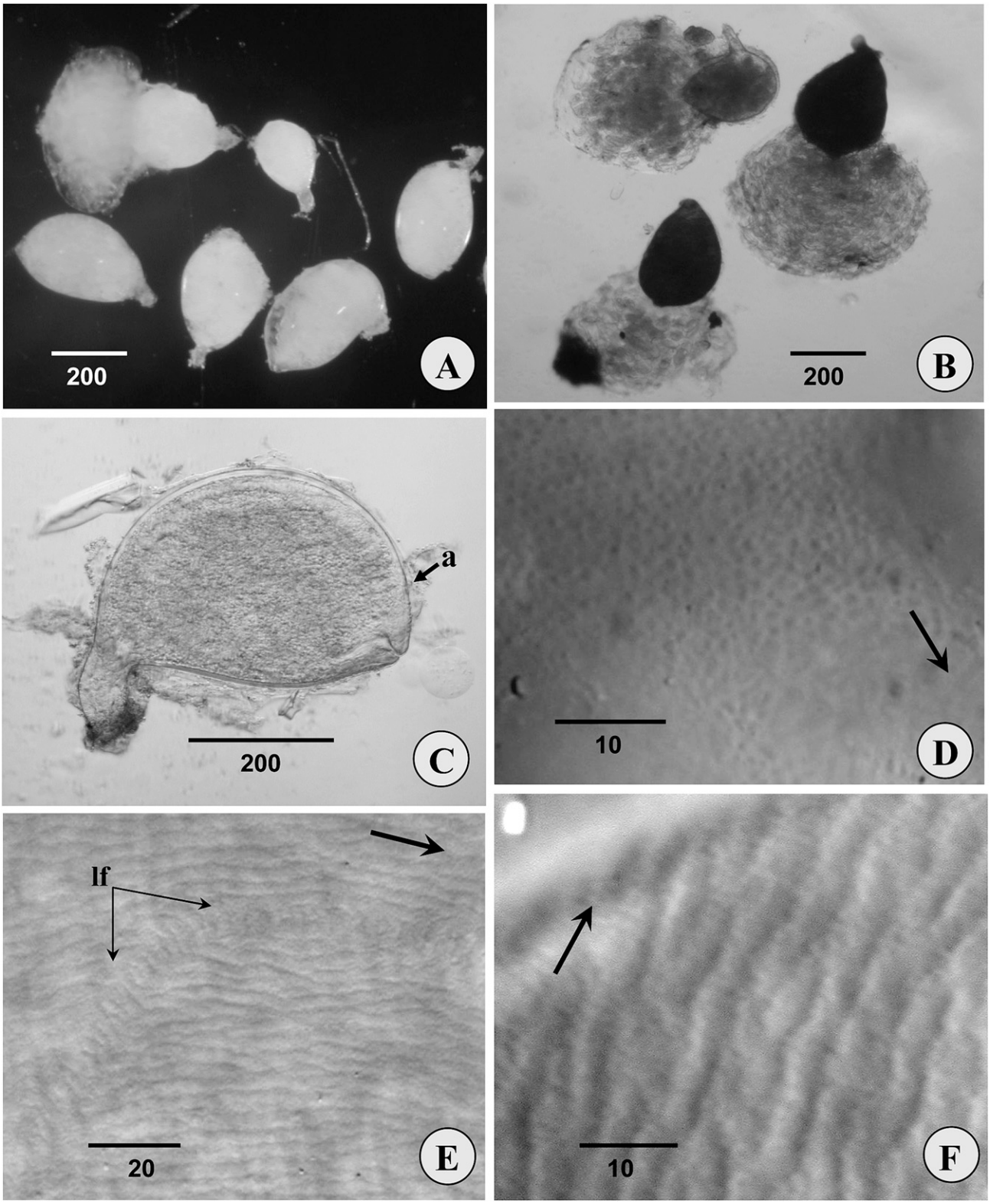

FIG. 1. Light micrographs of *Vittatidera zeaphila* n. sp. A. White females, one with attached egg mass; B. Cysts with attached egg masses; C. Female lateral view (a: anus); D. Cuticular granulation in neck region (arrow points anteriorly); E. Longitudinal striations and lateral field (lf) on white female (large arrow points toward neck); F. Longitudinal striations on senescent female (arrow points toward neck).

(92.7 ± 5.1, CV = 16.5), anterior end to metacorpal valve 50.0-88.6 µm (66.0 ± 5.3, CV = 8.1), *a* ratio 1.5-2.1 (1.8 ± 0.07, CV = 11.4). Holotype female: length 434 µm, neck length 100 µm, anterior end to metacorpal valve 61.2 µm, *a* ratio 1.6.

Female oval (Figs. 1A,C, 2A), white in reflected light, often with large, attached egg mass (Figs. 1A,B). Cuticle of neck region with annulations consisting of fine rows of granules (Fig. 1D). Cuticle of swollen part of body

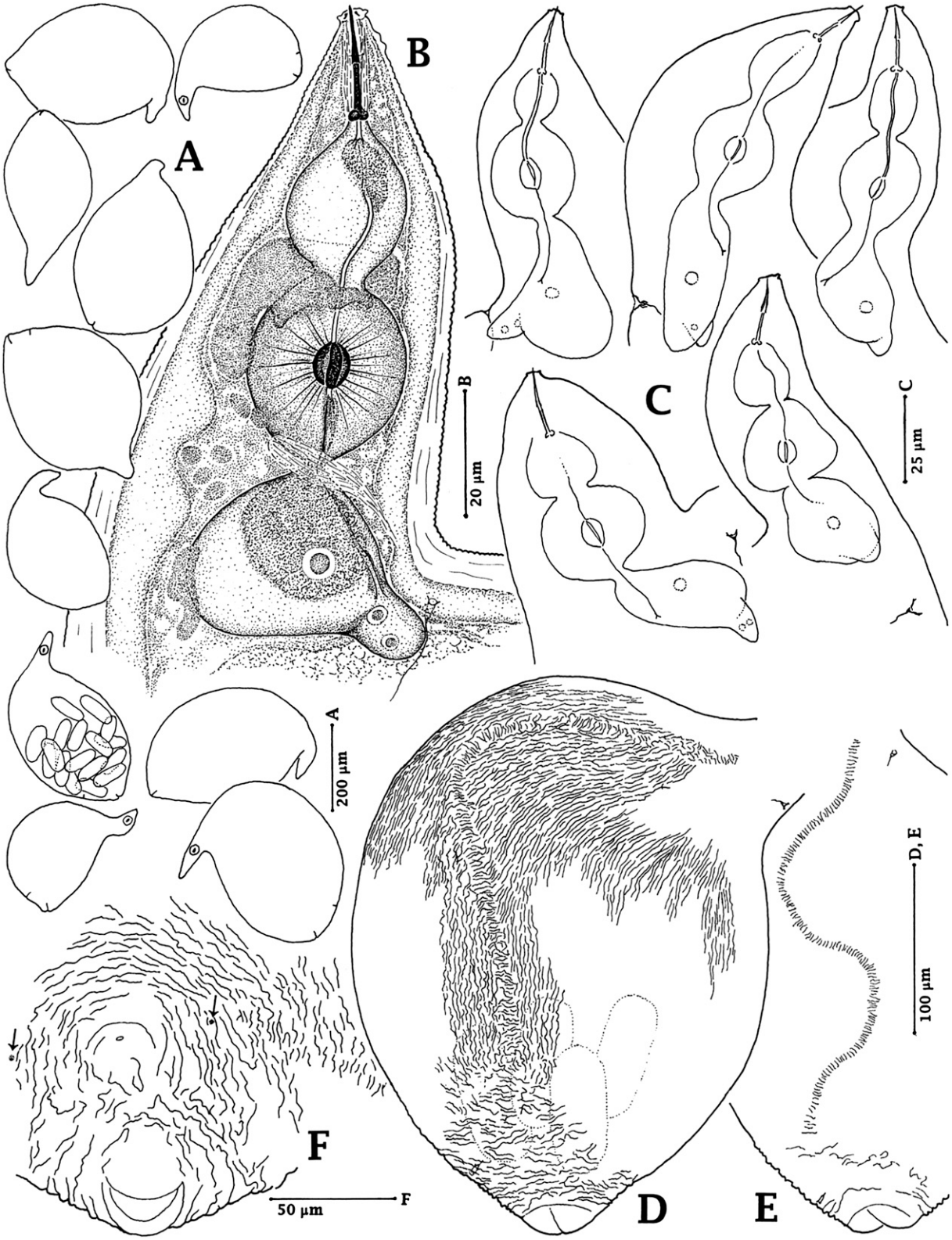

FIG. 2. Females of *Vittatidera zeaphila* n. sp. A. Outlines of females and cysts; B. Anterior end; C. Esophageal variation; D. Cuticular sculpturing and arched lateral field, some sculpturing omitted; E. Sinuous lateral field; F. Perineal region, arrows indicate phasmids.

anterior to vulval cone with elongated, fusiform ridges overlaying fine pattern of annulations (Figs. 1E,F, 2D). Lateral field arching or sinuate, extending from neck to cone base (Figs. 2D,E), indicated by short, fine transverse striae (Figs. 1E, 2D,E). Phasmid apertures minute, pore-like, level with anus (Fig. 2F). Cone area delimited by shallow, transverse, wavy striae; vulval lip region smooth (Figs. 2D,F; 3A). Anterior end with one prominent head

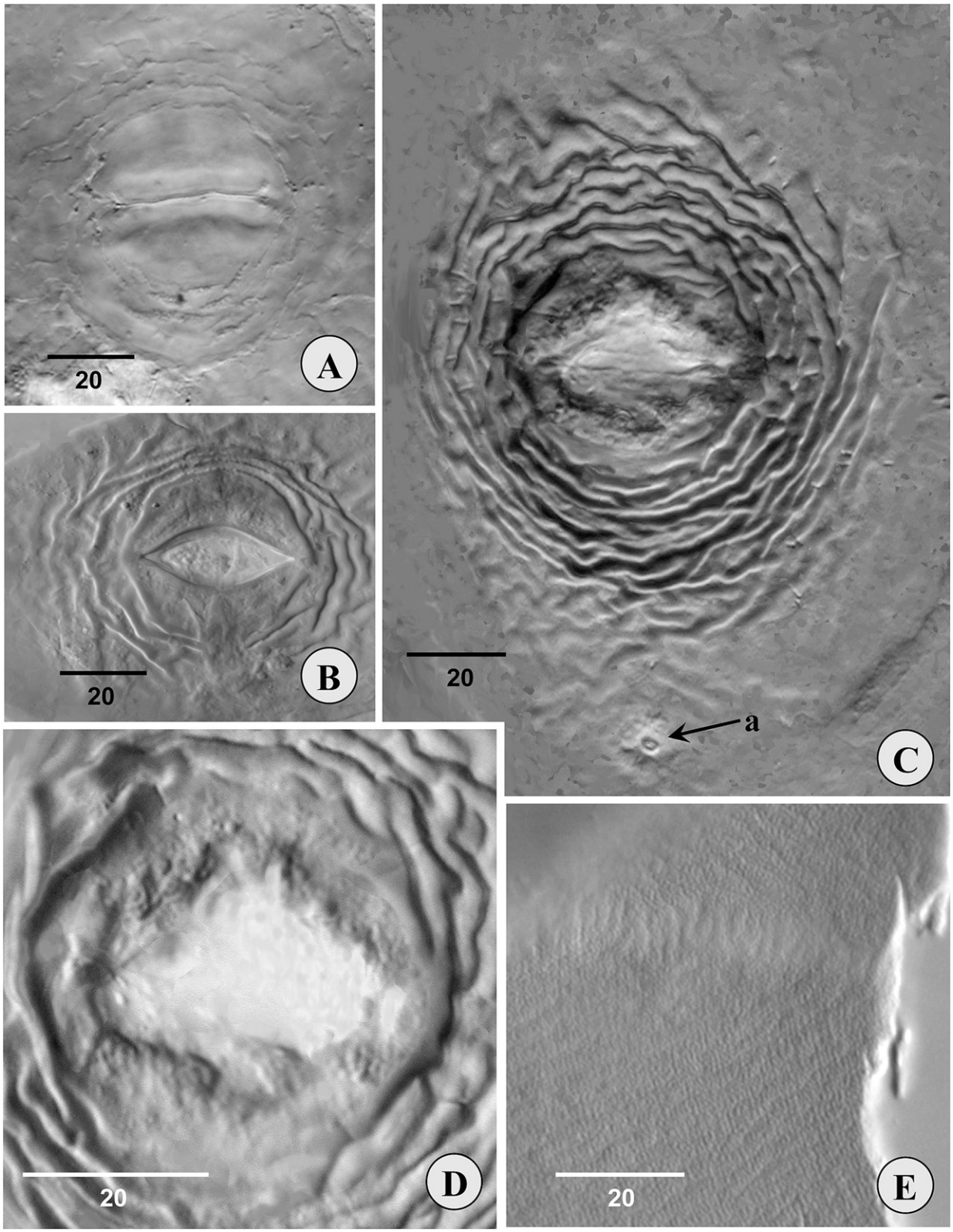

Fig. 3. Cyst features of *Vittatidera zeaphila* n. sp. A. Vulval cone of old female; B. Vulval cone of young cyst; C. Posterior end of mature cyst (a: anus); D. Vulval opening of mature cyst; E. Lateral field, cuticular striations, and annular punctations.

annule (Fig. 2B); anterior structure usually obscured by hardened mucilage-like secretion. Stylet slender, with rounded or slightly posteriorly inclined knobs. Dorsal gland orifice 3-4 μm from stylet base. Subventral gland nuclei in small lobe extending ventro-posteriorly from dorsal gland lobe. Excretory pore usually level with

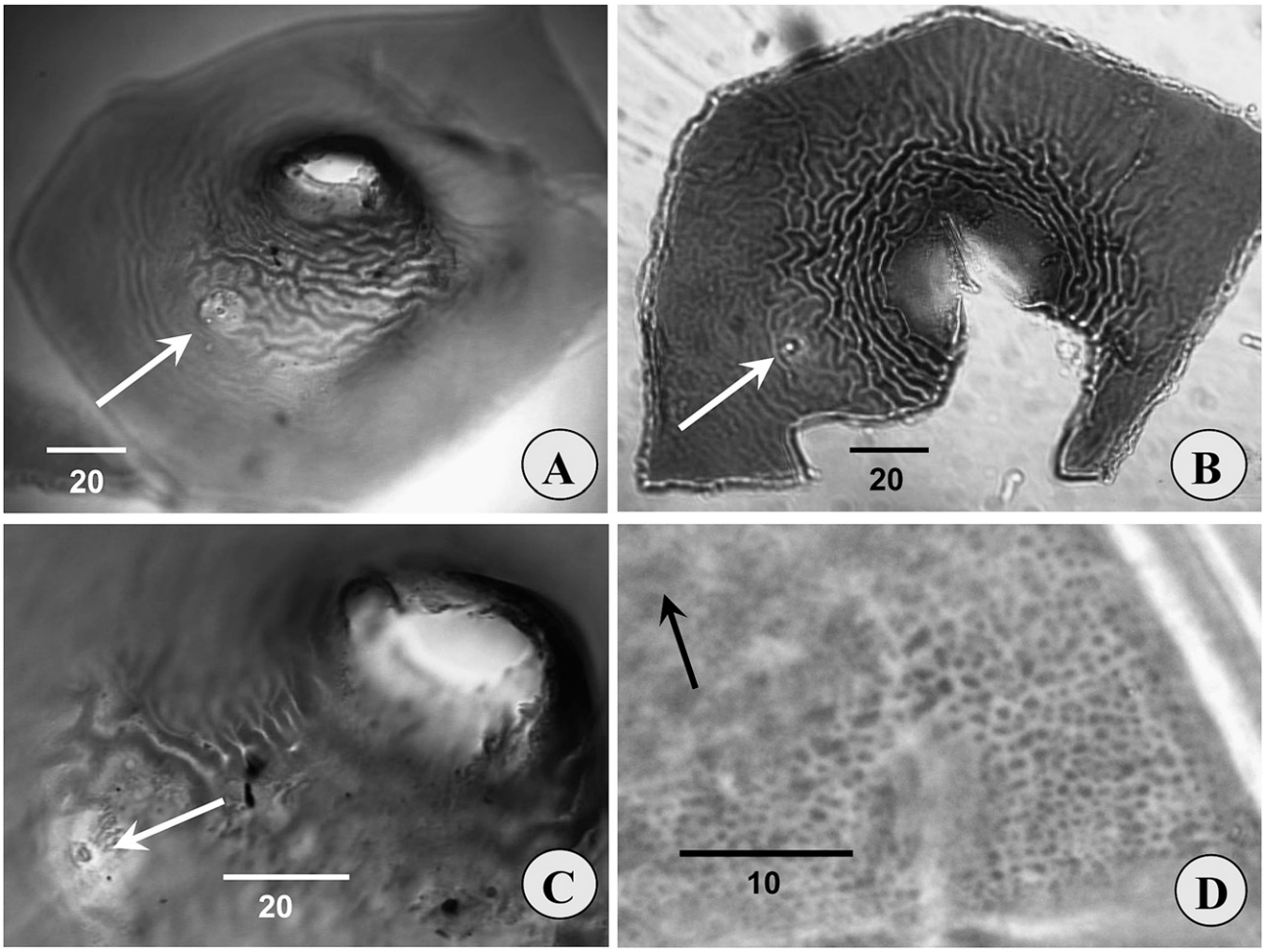

FIG. 4A-C. Vulval cones and surface sculpture of *Vittatidera zeaphila* n. sp. (arrows point to anus); D. Surface granulation in neck region (arrow points toward anterior end)

midregion of esophageal glands, occasionally posterior to glands (Figs. 2B,C).

Cysts Cyst measurements: length 385-608 µm (mean 470 ± 14.6 SE, CV = 13.1, n = 18), width 179-391 µm, (287 ± 11.9, CV = 17.6), neck length 60-119 µm (79.5 ± 4.4, CV = 21.6), *a* ratio 1.4-2.2 (1.67 ± 0.05, CV = 12.9); vulva width 28-48 µm (36); vulva-anus distance 52-74 µm (63).

Cysts lemon-shaped, vulval cone not protuberant, large egg masses persistent (Fig. 1B). Vulval aperture in developing cysts fusiform, lips smooth, surrounding striae long and sinuous, prominent only near cone apex (Fig. 3B). In mature cysts vulval aperture circumfenestrate, circular to rhomboidal, membranous lips persistent on mature cysts (Figs. 3C, 4A-C); underbridge, bullae, and other internal thickenings absent. Old cysts with irregular denticles around the inner edge of aperture (Figs. 3D, 4C). Cuticle around anus thinned but not fenestrate (Figs. 3C, 4A-C). Cuticular sculpturing on mature cyst similar but less pronounced than that of female, with faint longitudinal fusiform figures, underlying transverse annulations, and ladder-like lateral field (Fig. 3E); cuticle of neck region granulated (Fig. 4D).

Males Male length highly variable (Table 1, Fig. 6E). Lip region rounded, slightly set off, with three annules (Figs. 5D, 6F). Lateral field with four incisures, outer bands with scattered incomplete areolation. Tail tip rounded or bluntly conoid (Figs. 6G-I). Stylet knobs rounded, dorsal gland orifice near base of stylet. Esophageal glands in single lobe, subventral nuclei posterior to dorsal nucleus. Excretory pore just posterior to hemizonid, at anterior half of glands (Fig. 6F). Male gonad about 40-60% of body length. Spicules slightly curved, tips bifid (Fig. 6H). In ventral view, gubernaculum star-shaped, with two small anterior processes, two broad, lateral wings, and posterior process (Fig. 6I)

Juveniles Second-stage juveniles heteroderiform (Fig. 6A); stylet length less than 18 µm (Table 2). Lip region rounded, slightly offset, with three annules (Figs. 5A,B, 6B). Stylet knobs rounded (Fig. 5B). Lateral field with four incisures, outer incisures weakly crenulated. Phasmid apertures minute, in middle of lateral field midway between anus and tail tip. Anus without cuticular flap. Esophageal glands in single long lobe, subventral gland nuclei posterior to dorsal nucleus (Fig. 6B). Tail elongate-conoid, tip narrowly rounded (Figs, 5C, 6C,D), regularly

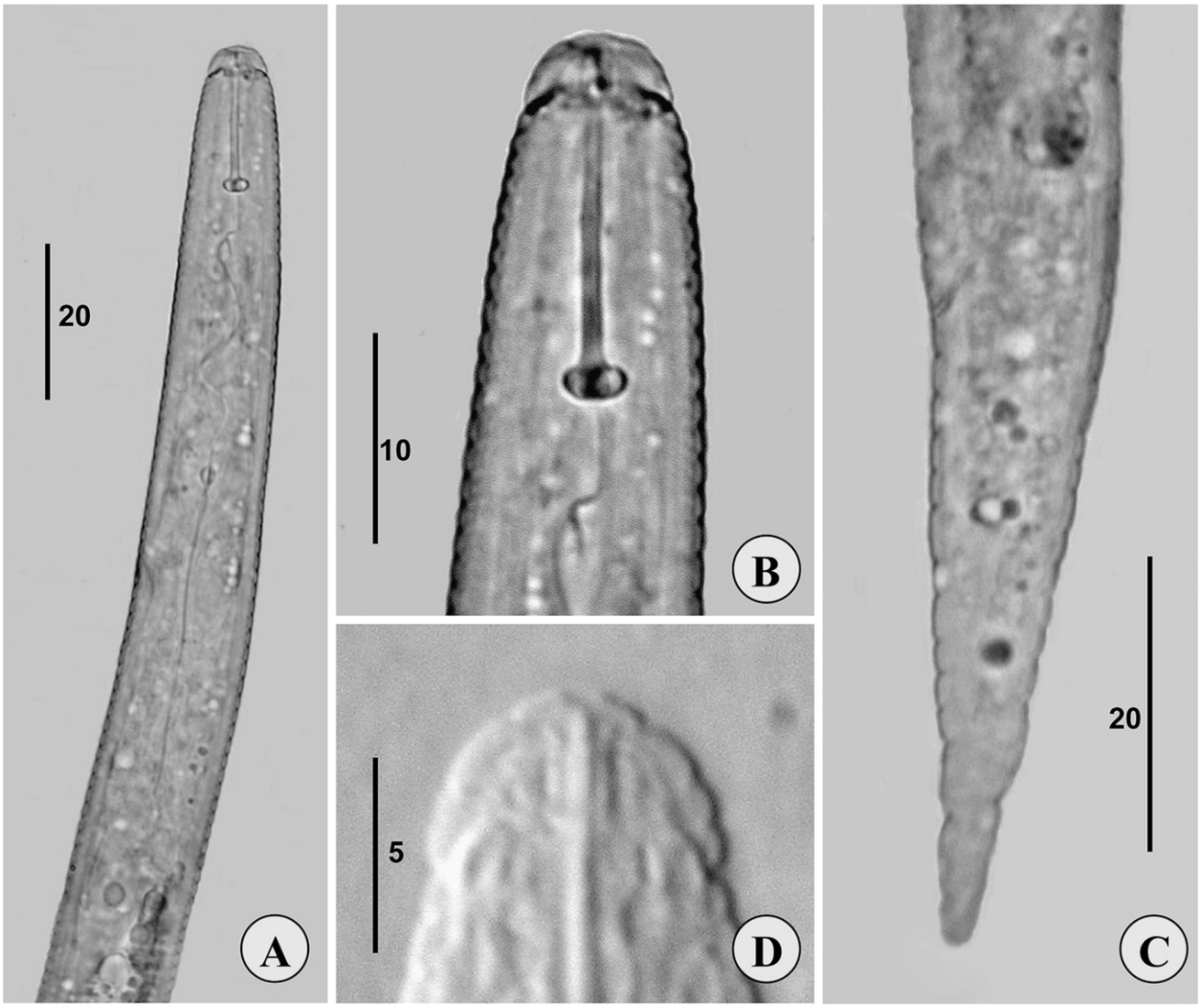

Fig. 5A-C. Second-stage juveniles of *Vittatidera zeaphila* n. sp. A. Anterior end; B. Head end; C. Tail; D. Male lip region.

annulated in anterior two-thirds, posterior third with irregular or incomplete annulation; proximal margin of hyaline region irregular, usually with two invasive lobes of the body lumen (Figs. 6C,D).

Etymology The specific name *zeaphila* means "corn-loving," a reference to its economically important host.

Type locality and host USA, Tennessee, Obion County, Troy, 36°21.35'N, 89°11.1'W, maize (corn) field, 6 October 2006, Patricia Donald, collector.

Type designation and deposition Type specimens deposited in USDA Nematode Collection, Beltsville, MD: Holotype female (**T641t**) selected from greenhouse culture originating from type locality collection. Paratypes on slides, same data: **T-5929p to T-5942p**, cysts (anterior and posterior halves on alternating slides); **T-5943p**, white female; **T-5944p to T-5947p**, second-stage juveniles. Paratypes in vials, same data: **T-553p**, mixed stages. **T-554p**: juveniles. Paratypes on slides, cultured from same field collection by R. D. Heinz and harvested December 2006: **T-5479p**, males; **T-5480p to T-5482p**, second-stage juveniles; **T-5815p to T-5817p**: cyst parts. Paratypes in vials, same origin: **T-502p**, cysts and juveniles; **T-526p**, eggs. Other paratype females, males, juveniles, and cysts maintained in the University of Tennessee Nematode Collection, Knoxville, TN.

Other specimens examined: USA, Tennessee, Lauderdale County, 35°45'N, 89°35'W, on roots of goosegrass, August 1978, E. C. Bernard, coll.: on slides, **T-5908p to T-5916p**, vulval cones; **T-5917p to T-5919p**, anterior halves of cysts; **T-5920p**, cyst wall; **T-5921p to T-5927p**, one cyst (ant. + post.) on each slide; **T-5928p**, second-stage juveniles. In vials: **T-551p**, second-stage juveniles; **T-552p**, females.

Discussion

The placement of this taxon in the classification of Heteroderidae is problematical. Wouts (1985) proposed

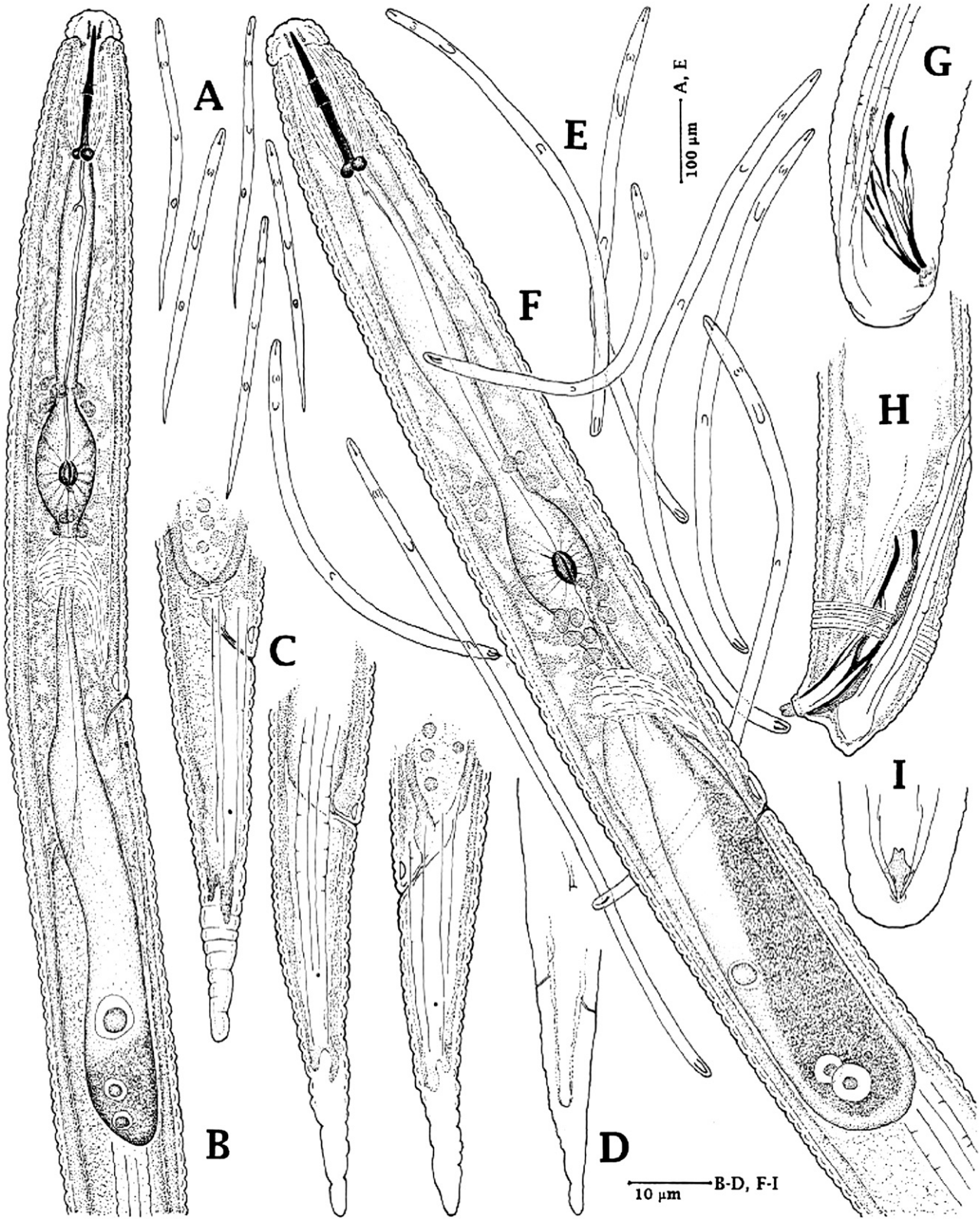

FIG. 6A-D. Second-stage juveniles of *Vittatidera zeaphila* n. sp. A. Outlines; B. Anterior end; C. Tails in lateral view; D. Tail in ventral view. E-I. Males. E. Outlines; F. Anterior end; G. Posterior region, subventral view; H. Posterior region, lateral view; I. Tail region, ventral view showing shape of gubernaculum.

six subfamilies within Heteroderidae. Baldwin and Schouest (1990) rearranged the family, reducing it to subfamily Heteroderinae and proposing six monophyletic tribes within Heteroderinae, but Siddiqi (2000) elevated Heteroderinae back to family rank, a proposal accepted by Subbotin et al. (2006). Of the six taxa proposed by

Baldwin and Schouest (1990), only Heteroderini (now Heteroderinae) possesses the cyst-forming character. Therefore, *V. zeaphila* should be placed in Heteroderinae. However, *V. zeaphila* possesses some unique features that do not appear to fit the confines of this group. The basic cuticular pattern consists of longitudinally oriented and elongated fusiform ridges, similar to the cuticle of *Ekphymatodera thomasoni*, a non-cyst former (Baldwin et al. 1989). Unlike all other described heteroderids, *V. zeaphila* has a distinct lateral field consisting of a dense ladder-like row of short, transverse lines running from the neck base to the cone base. Finally, females appear to have phasmid apertures, a feature not before reported in heteroderid females. Previous

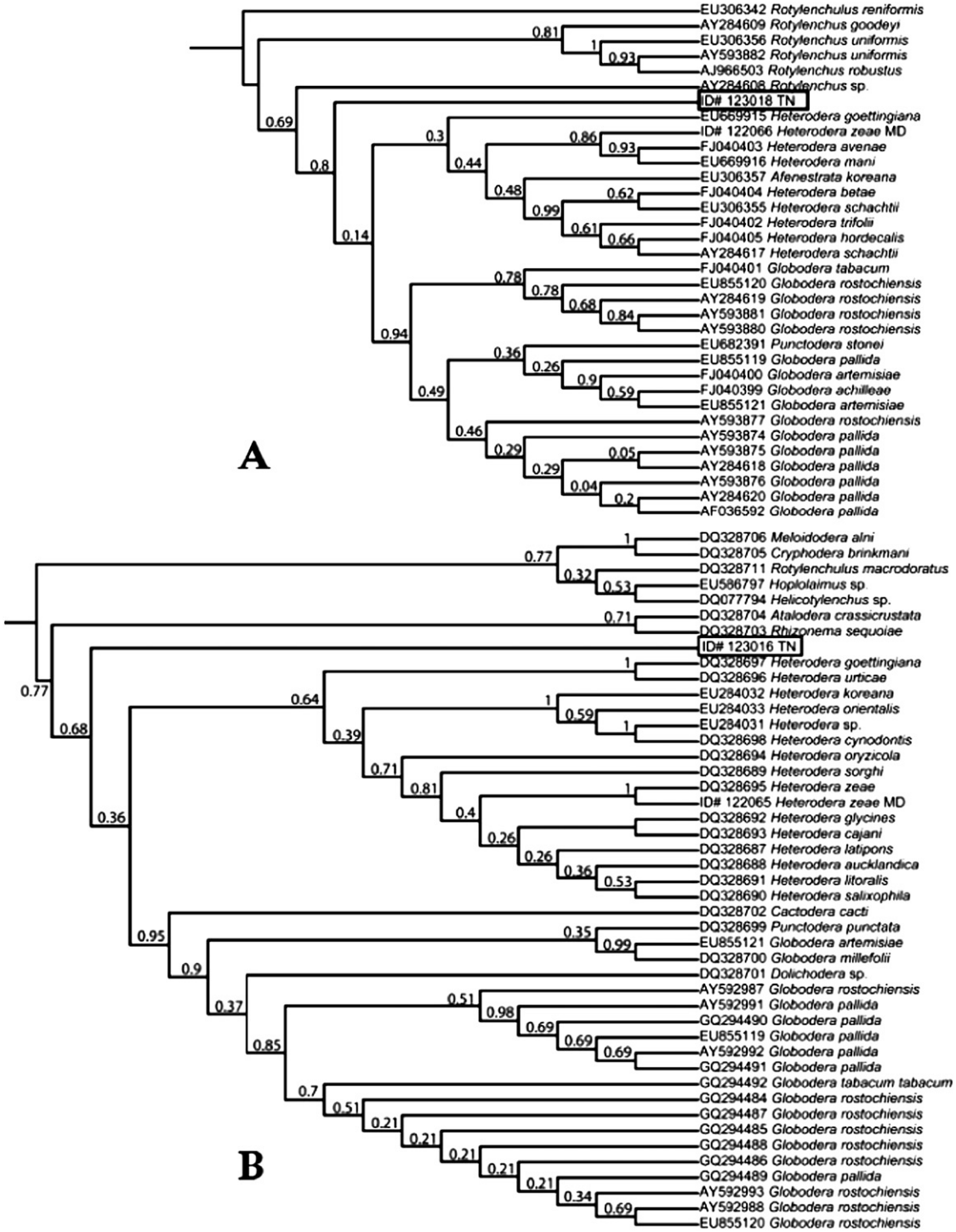

FIG. 7A-C. Molecular relationships of *Vittatidera zeaphila* to other Heteroderidae. A. 18S; B. D2D3; C. ITS. Within each tree the terminal node with *V. zeaphila* is surrounded by a bold box. Bootstrap values are presented for all internal nodes.

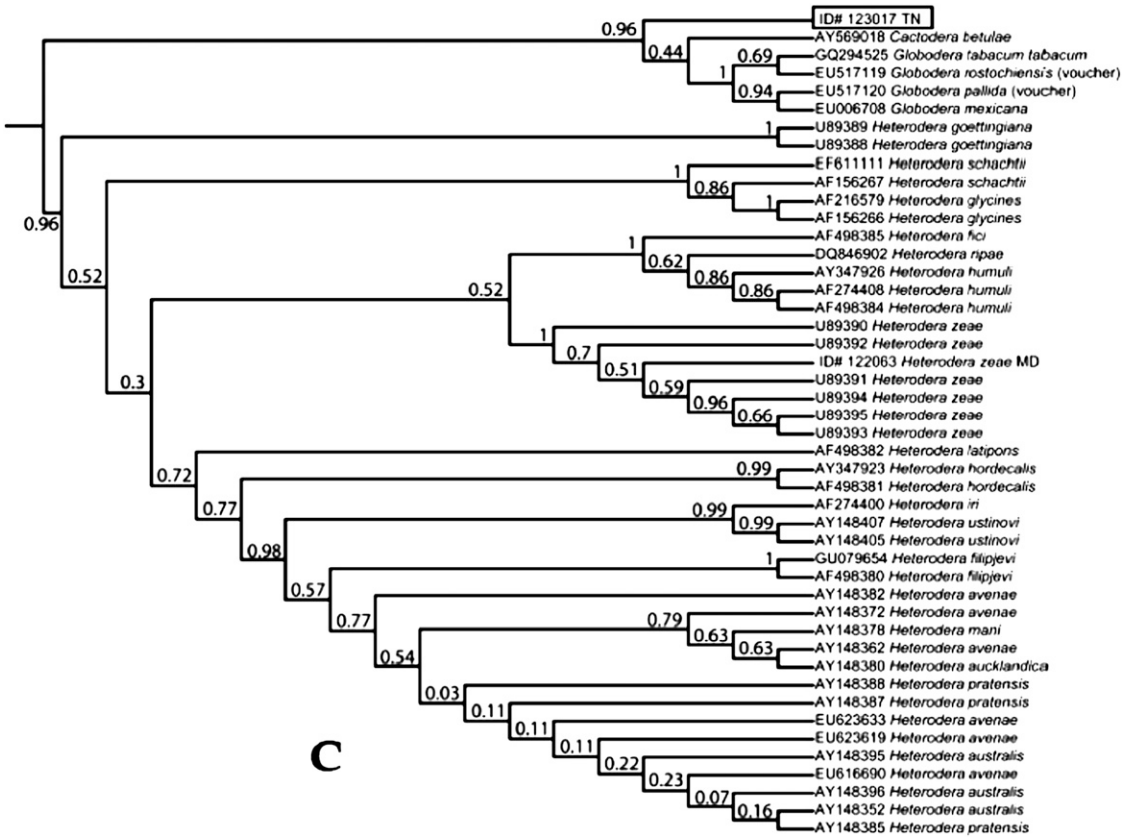

FIG. 7A-C. Continued.

examination of female cyst nematode cuticle generally has been focused on the anterior and posterior regions (Othman et al., 1988; Wouts & Baldwin, 1998).

The presence of a separate small esophageal lobe containing the subventral gland nuclei is a feature that *V. zeaphila* n. sp. has in common with many non-cyst-forming Heteroderinae, such as *Atalodera festucae*, *A. trilineata*, *Ekphymatodera thomasoni*, and *Verutus californicus* (Baldwin et al. 1989). It is tempting to hypothesize that *V. zeaphila* is a cyst-forming member of one of the non-cyst-forming heteroderid tribes on the basis of this apparently unusual gland nuclei arrangement. However, beside the incontrovertible fact of a cyst stage, the J2 has pore-like phasmids, which are typical of the Heteroderini. Second-stage juveniles of most of the non-cyst-forming species have large phasmidial ampullae and the phasmids appear scutelliform. Finally, the sequestration of the subventral gland nuclei may not be unusual and could be the norm in Heteroderidae. We were unable to find detailed illustrations of the esophagus of any *Heterodera* or *Globodera* spp. comparable to those in Baldwin et al. (1989). A similar lobe was illustrated for *H. orientalis* (Kazachenko) (Mundo-Ocampo et al. 2008) but the subventral gland nuclei were reported to be in the anterior third of the esophageal lobe. *Heterodera pakistanensis* was described as having esophageal glands in a single lobe (Maqbool & Shahina 1986), and Stone & Rowe (1976) stated that the esophageal gland lobe of *H. cruciferae* was large and not differentiated into dorsal and subventral sections. Whether this character is of supraspecific value cannot be determined until more typical cyst nematodes have been carefully examined.

TABLE 1. Morphometrics of males of *Vittatidera zeaphila* n. sp. (n = 10).

	Mean ± SE	Range	CV[a]
Measurements (μm)			
Length	691 ± 50.3	478-912	21.8
Stylet length	18.9 ± 0.36	17.3-21.0	5.8
Stylet knobs to dorsal gland orifice	3.3 ± 0.31	2.2-4.8	26.6
Head end to excretory pore	94 ± 6.4	68-115	19.2
Spicule length	24.0 ± 0.59	22.1-26.5	6.9
Ratios			
a	35.7 ± 1.45	29.2-42.7	12.2
S (stylet length/body width at knobs)	1.5 ± 0.06	1.3-1.8	10.3

[a] CV: coefficient of variation.

Molecular comparison of *V. zeaphila* with available heteroderid sequences in GenBank suggests this species is basal to the other cyst-forming Heteroderidae, and is concordant with the unusual morphology of this nematode. Phylogenetic analysis firmly established that *Vittatidera zeaphila* is genetically distant from the other cyst nematode on corn, *Heterodera zeae*. Phylogenetic trees constructed from the three genetic markers

TABLE 2. Morphometrics of second-stage juveniles of *Vittatidera zeaphila* n. sp. (n = 20).

	Mean ± SE	Range	CV[a]
Measurements (μm)			
Length	365 ± 5.20	346-400	4.7
Stylet length[b]	16.7 ± 0.20	15.7-17.5	3.9
Stylet length[c]	15.1 ± 0.09	14.4-16.0	3.8
Stylet knobs to dorsal gland orifice	2.9 ± 0.04	2.2-3.2	3.3
Head end to excretory pore	148 ± 1.8	142-161	4.1
Metacorpal valve to excretory pore	26.1 ± 0.62	23.2-30.0	7.9
Tail length	41.5 ± 1.30	33.0-48.0	10.4
Hyaline terminus length	15.3 ± 0.57	12.4-17.8	12.3
Ratios			
a	24.0 ± 0.44	22.3-26.9	5.9
b	2.5 ± 0.10	2.4-2.7	4.2
c	8.9 ± 0.23	7.8-10.5	8.6
c'	4.2 ± 0.07	3.9-4.6	5.3
S (stylet length/body width at knobs)	1.5 ± 0.04	1.3-1.8	10.0
H (%) (hyaline terminus length/tail length × 100)	37.0 ± 1.14	30.7-43.7	10.2

[a] CV: coefficient of variation.
[b] Live juveniles fixed with hot (80°C) 4% formalin (E. C. Bernard).
[c] Live juveniles fixed with warm (40°C) 3% formalin (Z. A. Handoo).

consistently grouped *H. zeae* within a large *Heterodera* clade that does not include *V. zeaphila* (Fig. 7). The 18S tree (Fig. 7A) places *V. zeaphila* outside a *Heterodera/Globodera* clade that is relatively well-supported (0.8 bootstrap value) in the data set. The limited number of full-length 18S sequences in GenBank of cyst species other than *Heterodera* and *Globodera* precludes a more definitive statement of the position of *Vittatidera* within Heteroderidae based on 18S. The D2/D3 sequence included a wider set of available heteroderid genera for comparison, including *Dolichodera*, *Cactodera*, *Rhizonema*, *Atalodera*, *Cryphodera* and *Meloidodera* (Fig 7B). *Vittatidera* was weakly supported (0.68 bootstrap value) as a member of a clade that includes the genera *Heterodera*, *Cactodera*, *Punctodera*, *Globodera* and *Dolichodera*, excluding *Atalodera* and *Rhizonema*. *Vittatidera* may occupy a sister taxon relationship with the aforementioned ingroup genera; however, additional DNA sequence is necessary to confirm that relationship. A recent phylogenetic tree based on D2/D3 sequence indicates that *Vittatidera* may group with *Betulodera* apart from the other cyst-forming nematodes in Heteroderinae (S. Subbotin pers. comm.). ITS1 sequence could provide insight into the systematic position of *Vittatidera*, but comparative sequences of cyst genera other than *Heterodera* are lacking (Fig. 7C).

It should be kept in mind that the molecular portion of this study was an attempt to determine where *V. zeaphila* fits among the Heteroderidae based on available sequences, but was not intended to be a phylogenetic study of Heteroderidae. The DNA analyses, as well as the morphology of this nematode, support a position outside of the *Heterodera/Globodera* clade. Available sequences for the minor cyst and non-cyst-forming species are limited, and phylogenetic refinement of the placement of *V. zeaphila* in Heteroderidae awaits further study.

The first and second authors measured separate groups of juveniles and obtained similar measurements except for stylet length. Specimens fixed in hot formalin had stylets averaging about 1.5 μm longer than specimens fixed in warm formalin (Table 2). The first author confirmed these figures by measuring both groups of juveniles with the same equipment and methods. Separate measurements of the conus and shaft indicated that both the conus and shaft were slightly shorter in warm-fixed specimens than in hot-fixed specimens. Other factors in processing, such as the temperature of the alcohol-evaporation oven, could have contributed to this difference, which could be either shrinkage or swelling. Regardless of the processing steps that may have led to this discrepancy, this finding points out the variability that can occur in specimen preparation.

LITERATURE CITED

Baldwin, J. G., Bernard, E. C., and Mundo-Ocampo, M. 1989. Four new species of Heteroderidae including *Ekphymatodera* n. gen. from California. Journal of Nematology 21:48–68.

Baldwin, J. G., and Mundo-Ocampo, M. 1991. Heteroderinae, cyst- and non-cyst-forming nematodes. Pp. 273–362 *in* W. R. Nickle, ed. Manual of agricultural nematology. Marcel Dekker, New York.

Baldwin, J. G., and Schouest, L. P., Jr. 1990. Comparative detailed morphology of the Heteroderinae Filip'ev & Schuurmans Stekhoven, 1941, *sensu* Luc et al. (1988): Phylogenetic systematics and revised classification. Systematic Parasitology 15:81–106.

Edgar, R. C. 2007. MUSCLE protein multiple sequence alignment software, version 3.7. http://www.drive5.com/muscle/download3.6.html. Accessed 22 April 2010.

Maqbool, M. A., and Shahina, F. 1986. New species of cyst nematode *Heterodera pakistanensis* (Nematoda: Heteroderidae) attacking wheat in Pakistan. Journal of Nematology 18:541–548.

Mundo-Ocampo, M., Troccoli, A., Subbotin, S. A., Del Cid, J., Baldwin, J. G., and Inserra, R. N. 2008. Synonymy of *Afenestrata* with *Heterodera* supported by phylogenetics with molecular and morphological characterization of *H. koreana* comb. n. and *H. orientalis* comb. n. (Tylenchida: Heteroderidae). Nematology 10:611–632.

Othman, A. B., Baldwin, J. G., and Mundo-Ocampo, M. 1988. Comparative morphology of *Globodera, Cactodera*, and *Punctodera* spp. (Heteroderidae) with scanning electron microscopy. Revue de Nématologie 11:53–63.

Powers, T. O., and Harris, T. S. 1993. A polymerase chain method for identification of five major *Meloidogyne* species. Journal of Nematology 25:1–6.

Seinhorst, J. W. 1959. A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. Nematologica 4:67–69.

Siddiqi, M. R. 2000. Tylenchida: Parasites of plants and insects. 2nd edition. CABI Publishing, Wallingford, UK.

Stone, A. R., and Rowe, J. A. 1976. *Heterodera cruciferae*. C.I.H. Descriptions of Plant-parasitic Nematodes, Set 6, No. 90. Commonweath Institute of Helminthology, St. Albans, UK. 4 pp.

Sturhan, D. 2002. Notes on the genus *Cactodera* Krall & Krall, 1978 and proposal of *Betulodera betulae* gen. nov., comb. nov. (Nematoda: Heteroderidae). Nematology 4:875–882.

Sturhan, D., Wouts, W. M., and Subbotin, S. A. 2007. An unusual cyst nematode from New Zealand, *Paradolichodera tenuissima* gen. n., sp. n. (Tylenchida: Heteroderidae) Nematology 9:561–571.

Subbotin, S. A., Sturhan, D., Chizhov, V. N., Vovlas, N., and 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.

Vrain, T. C., Wakarchuk, D. A., Levesque, A. C., and Hamilton, R. I. 1992. Intraspecific rDNA restriction-fragment-length-polymorphism in the *Xiphinema-americanum* group. Fundamental and Applied Nematology 15:563–573.

Wouts, W. M. 1985. Phylogenetic classification of the family Heteroderidae (Nematoda: Tylenchida). Systematic Parasitology 7:295–328.

Wouts, W. M., and Baldwin, J. G. 1998. Taxonomy and identification. In: Sharma, S. B. (Ed.). The cyst nematodes. Kluwer Acadamic Publishers, Dordrecht, The Netherlands, pp. 83–122.