Taxonomy, Morphology and Phylogenetics of Coffee-Associated Root-Lesion Nematodes, Pratylenchus spp

Article · January 2008

DOI: 10.1007/978-1-4020-8720-2_3 · Source: OAI

Zafar Handoo
United States Department of Agriculture

Lynn Carta

Andrea Skantar
United States Department of Agriculture

Chapter 3
Taxonomy, Morphology and Phylogenetics of Coffee-Associated Root-Lesion Nematodes, *Pratylenchus* spp.

Zafar A. Handoo, Lynn K. Carta and Andrea M. Skantar

Abstract This review includes a synthesis of information on eight species of root-lesion nematodes (*Pratylenchus* spp.) that parasitize coffee or inhabit its rhizosphere. It includes a table of important morphological characters, a diagnostic key, photographs of anterior ends and tails of specimens from the USDA nematode collection, and a phylogenetic tree based on ribosomal DNA with drawings of scanning electron microscopic face-patterns. Information sources are evaluated and future research needs are outlined.

Keywords Diagnostic key · phylogeny · taxonomy · phylogenetic tree · evolution

3.1 Introduction

Root-lesion nematodes (*Pratylenchus* spp.) are among the most common and damaging to coffee (*Coffea* sp.) aside from root-knot nematodes and a few other genera. The genus *Pratylenchus* is comprised of 97 valid species of worldwide distribution and economic importance, which parasitize a wide variety of plant species. Members of this genus are called root-lesion nematodes because they produce lesions on feeder roots and occasionally on other underground plant parts as a result of their feeding. They are sometimes referred to as meadow nematodes due to their frequent occurrence in that environment.

The first described root-lesion nematode was *Tylenchus pratensis* De Man (de Man, 1880), which was redescribed and illustrated by De Man (1884). The genus name *Pratylenchus* was established by Filipjev in 1936, with *P. pratensis* (de Man) Filipjev as the type species. Sher and Allen (1953) first put the taxonomy of the genus on a basis familiar to modern taxonomists. Loof (1960; 1978; 1991) reviewed in detail the anatomy, morphology, distribution, systematics, variability and identification of the genus, and presented a key to its species. Key and comprehensive compendia including histories of the morphological work performed

---

Z.A. Handoo
United States Department of Agriculture/ARS, Nematology Laboratory, Beltsville, USA
e-mail: zafar.handoo@ars.usda.gov

R.M. Souza (ed.), *Plant-Parasitic Nematodes of Coffee*,

used to distinguish them are body length, head shape and number of annules, length of stylet, shape of stylet knobs, structure of lateral field, presence/absence and shape of spermatheca, length and structure of posterior uterine branch, shape of female tail terminus, presence or absence of males and shape and length of spicule and gubernaculum. Loof (1991) discussed these characters in detail, with particular emphasis on the intraspecific variation that limits their reliability. Table 3.1 contains updated morphometric data for the most important diagnostic characters of the coffee-associated *Pratylenchus* species. Figures 3.1 through 3.4 contain photomicrographs of female's heads and tails.

Identification of *Pratylenchus* species is hampered by the similarity among species in some cases, and by the significant intraspecific variability of both morphological and morphometric diagnostic characters in other cases. Several authors have described morphological variations within species that make it difficult to separate species accurately using traditional microscopy. Variable features include tail shape, the number of annules in the ventral part of tail, the lateral field throughout the body (Corbett and Clark, 1983), and the presence of one supplementary lip annule in some specimens (Baujard et al., 1990). This situation has prompted researchers to discover alternate methods and features for more accurate identification. Although not considered routine, SEM is a technique sometimes used for morphological analysis (see for example Sher and Bell, 1975; Corbett and Clark, 1983; Trett and Perry, 1985; Baujard et al., 1990; López and Salazar, 1990; Sakwe and Geraert, 1994; Inserra et al., 1998; 2005a; Duncan et al., 1999; Hernández et al., 2001; Carta et al., 2001; 2002). Fortunately, for taxonomic purposes, SEM has shown the stability and reliability of several nematode surface features, with the lip and face region, lateral field, and tail receiving the most attention (see for example Anderson and Townshend, 1980; Inserra et al., 2005a). In an SEM study of the surface features of nine *Pratylenchus* species the lip region has been demonstrated to be a particularly good taxonomic character; species have been separated into three groups according to the pattern of the first lip annule and the oral disc (Hernández et al., 2001).

3.2.2 *The Coffee-Associated Root-Lesion Nematodes,* **Pratylenchus** *spp.*

The eight species listed below have been reported from the roots of coffee (Campos et al., 1990). However, a recently described species from northern Europe, *P. brzeskii* Karssen, Waeyenberge and Moens, is morphologically similar to *P. coffeae* and *P. loosi* (Karssen et al., 2000), species both known to parasitize coffee in more southern climates and difficult to distinguish by morphology (Pourjame et al., 1999). To our knowledge, *P. brzeskii* has not been examined for possible parasitism on coffee. This closely related species is another indication that *P. coffeae* and relatives represent a densely populated species complex (Campos and Villain, 2005). Uncertainty about the identity of some amphimitic *Pratylenchus* populations has been recorded from Brazil (Siciliano-Wilcken et al. 2002a,b; Silva and Inomoto,

Table 3.1 Compendium of eight *Pratylenchus* species known to parasitize coffee. Values represent range, average

Species	Body length (mm)	Stylet (μm)	No. lip annules	a[a]	b	c	V%	No. tail annules	Tail shape[b]	Tail terminus	Spicule length (μm)	Guber-naculum length (μm)	Spermatheca	Males
P. brachyurus	0.39–0.75	17–22	2	15–29	5–10	13–28	82–89	15–21	SCYL, TRC, SHM, SMO	SMO	16–17	5–6	Empty	Very rare
	0.59	20.7		23	8	25	85.6	18.5						
P. coffeae	0.46–0.70	15–18	2	21–30	5–8	17–27	76–82	17–24	HEM-BLP, SMO	IND, TRC, BR, SMO	15–18	4.2–6	Large, broadly oval to round	Common
	0.53	16.5		25	6.5	22	80	20			16.5			
P. panamaensis (= *P. gutierrezi*)	0.43–0.55	16–18	2	15–24.9	3.5–4.5	17–25	74–84	18–23	SCYL	BLR, ANN	15.5–21	3.2–4.4	Oval to round	Common
	0.5	16.8		19.8	3.9	19.9	80	21			16.8	3.7		
P. goodeyi	0.40–0.68	14–17	4	24–37	5.5–7.3	14–18	73–75	21	SC	TP, SMO	15.5–21	5–6	Large, oblong	Common
	0.52	15.7		32	6.5	16.5	74							
P. loosi	0.48–0.64	14–18	2	28–36	5.7–7.1	18–25	79–85	27–34	NAR, SA	NAR, SMO	16–20	4–7	Oval	Common
	0.57	16.5		32	6.4	21	82.5				17.5	5.5		

2002), Guatemala (Villain et al., 1998; Villain, 2000), El Salvador and Costa Rica (Herve, 1997; Duncan et al., 1999) based on host range and genetic information.

Drawings of *Pratylenchus* species other than *P. goodeyi* and *P. pratensis* (see below) may be found at the USDA website (http://ars.usda.gov/Main/docs.htm?docid = 9866) and at the University of Nebraska websites http://nematode.unl.edu/pratkey7.htm#pratkey7, http://nematode.unl.edu/pracoff.htm, http://nematode.unl.edu/ploos.htm and http://nematode.unl.edu/prapse.htm.

3.2.2.1 *Pratylenchus coffeae* (Zimmermann, 1898) Filipjev and Schuurmans Stekhoven, 1941

The taxonomy of *P. coffeae* (Fig. 3.1 A–H) has been the subject of numerous studies (Sher and Allen, 1953; Loof, 1960; 1978; 1991; Roman and Hirschmann, 1969; Siddiqi, 1972; Rashid and Khan, 1978; Bajaj and Bhatti, 1984; Inserra et al., 1996; 1998; 2001; Mizukubo, 1992; Duncan et al., 1999; Ryss, 2002a; Van Den Berg et al., 2005).

This species is the most widespread and damaging on coffee. It occurs in the Dominican Republic, El Salvador, Guatemala, Puerto Rico, Costa Rica, Brazil, India, Southeast Asia, Barbados, Martinique, Tanzania, Madagascar, Indochina, Java, Indonesia and Venezuela. On other hosts this species is found throughout the tropics and in many subtropical regions. Specific locations include Japan, Australia, South Africa, Brazil, Oman (Campos and Villain, 2005) and southern parts of the United States (Norton et al., 1984).

3.2.2.2 *Pratylenchus brachyurus* (Godfrey, 1929) Filipjev and Schuurmans Stekhoven, 1941

The taxonomy of *P. brachyurus* (Fig. 3.1 I–P) has been advanced by authors in Europe and the Americas (Sher and Allen, 1953; Loof, 1960; 1978; 1991; Roman and Hirschmann, 1969; Corbett, 1976; Corbett and Clark, 1983; López and Salazar, 1990; Hernández et al., 2001; Ryss, 2002a).

In South America, this was one of the first root-lesion nematodes known on coffee (Campos and Villain, 2005). It is found primarily in the tropics and subtropics, and specifically in Australia, Brazil, Peru, USA, Turkey, West Africa, South Africa and Japan. In some areas of Brazil it may be more widespread than *P. coffeae* (Campos and Villain, 2005).

3.2.2.3 *Pratylenchus loosi* Loof, 1960

Taxonomic and morphological studies of *P. loosi* (Fig. 3.2 A–H) have been published in various review papers (Seinhorst, 1977; Loof, 1978; 1991; Inserra et al., 1996; 2001).

This species has been reported on coffee in Sri Lanka (Hutchinson, 1963 cited by Whitehead, 1968). On other hosts its geographic distribution includes Sri Lanka, India, Japan (Seinhorst, 1977; Campos and Villain, 2005), Korea (Park et al., 2002),

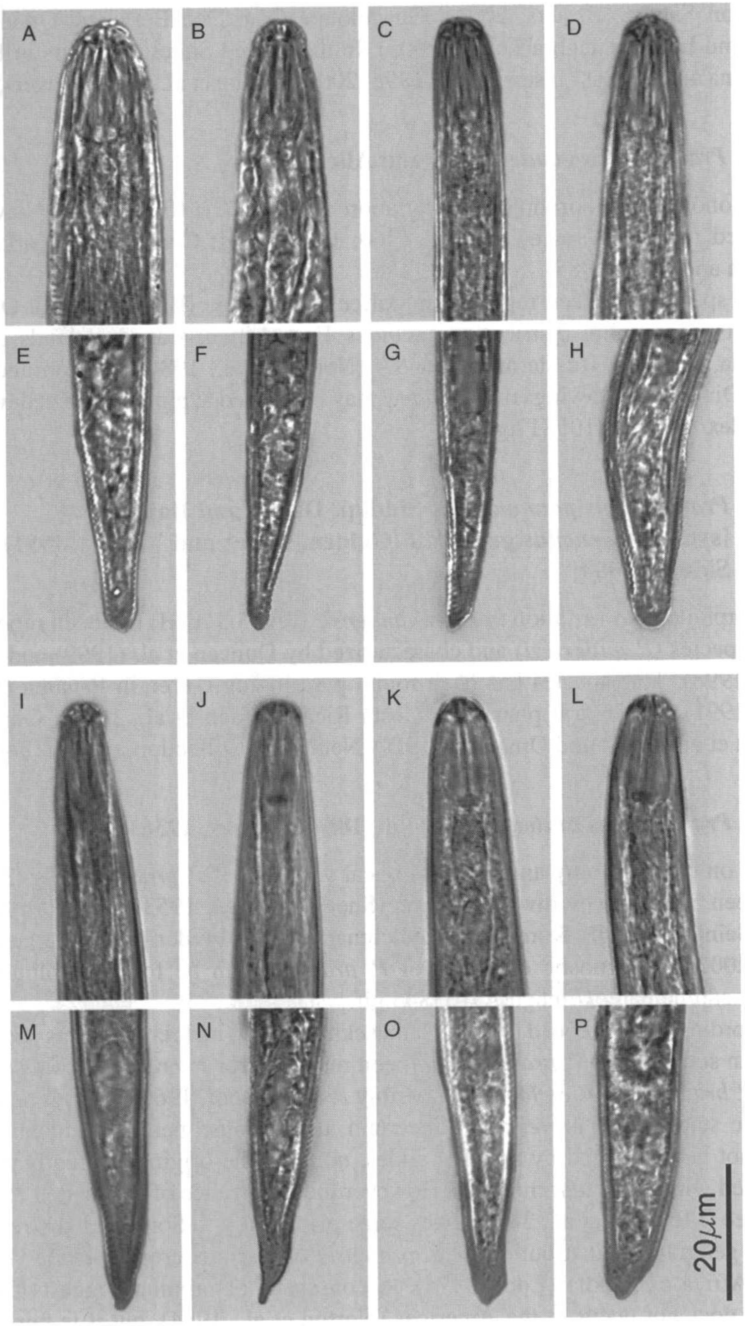

Fig. 3.2 Photomicrographs of female heads and tails, showing variations in tail shape. **(A–H)** *P. loosi*, **(I–P)** *P. goodeyi*. (Photos by Z. A. Handoo)

American Samoa (Brooks, 2004), Guadeloupe (Van Den Berg and Quénéhervé, 2000) and Iran (Hajieghrari et al., 2005). In the United States, it occurs in Florida, Louisiana and Kansas (Inserra et al., 1996; 2001; Norton et al., 1984; Powers, 2008).

3.2.2.4 *Pratylenchus goodeyi* Sher and Allen, 1953

The taxonomy and morphological variation of *P. goodeyi* (Fig. 3.2 I–P) have been described in various reviews (Loof, 1960; 1978; 1991; Corbett and Clark, 1983; Machon and Hunt, 1985).

This species has been reported on coffee in Tanzania (Bridge, 1984). On other hosts, its geographic distribution includes East Africa, Canary Islands, Kenya, Tanzania, England, Russia and the USA (Norton et al., 1984; Machon and Hunt, 1985). Diagnostic drawings of *P. goodeyi* may be viewed at plpnemweb.ucdavis.edu/Nemaplex/images/G105S12.gif.

3.2.2.5 *Pratylenchus panamaensis* Siddiqi, Dabur and Bajaj, 1991 [syn. *Pratylenchus gutierrezi* (Golden, López and Vilchez, 1991) Siddiqi, 2000]

The morphological variation in *P. panamaensis* (Fig. 3.3 A–H) has been reported as a new species (*P. gutierrezi*) and characterized by Duncan et al. (1999) and Inserra et al. (1998). This species has been found parasitizing coffee in Panama (Siddiqi et al., 1991), the central plateau of Costa Rica (Golden et al., 1992), Guatemala (Inserra et al., 1998) and Oman (in USDA Nematode Collection, entry #1546).

3.2.2.6 *Pratylenchus pratensis* (de Man, 1880) Filipjev, 1936

Studies on the taxonomy and morphological variation of *P. pratensis* (Fig. 3.3 I–P) have been advanced by diverse authors (Sher and Allen, 1953; Loof, 1960; 1974; 1978; Seinhorst, 1968; Roman and Hirschmann, 1969; Frederick and Tarjan, 1989; Ryss, 2002a). Diagnostic drawings of *P. pratensis* can be found at plpnemweb.ucdavis.edu/nemaplex/images/G105S45.gif.

According to Whitehead (1968), Somasekhar (1959) had reported this species on coffee in south India. *P. pratensis* has been mistaken for *P. crenatus* Loof, *P. penetrans, P. brachyurus, P. coffeae* and possibly *P. loosi* (Loof, 1960; 1974). The coffee-parasitic status of *P. pratensis* is uncertain also because nematode identification could not be confirmed by voucher slides, nor was the original coffee population examined with molecular methods. However the occurrence of the related *P. vulnus* on coffee (Monteiro et al., 2001) gives some plausibility to Somasekhar's report.

The geographic distribution of *P. pratensis* on various crops includes Europe, South Africa and India (Loof, 1974). A consensus of opinion suggests that this species does not occur in the Americas (Norton et al., 1984), but it is fairly common in Europe (Peña et al., 2007). However, the morphologically similar species *P. pratensisobrinus* Bernard occurs in Alaska (Bernard, 1984) and *P. pseudopratensis* Seinhorst in Konza Prairie, eastern Kansas (USA) (Powers, 2008). This

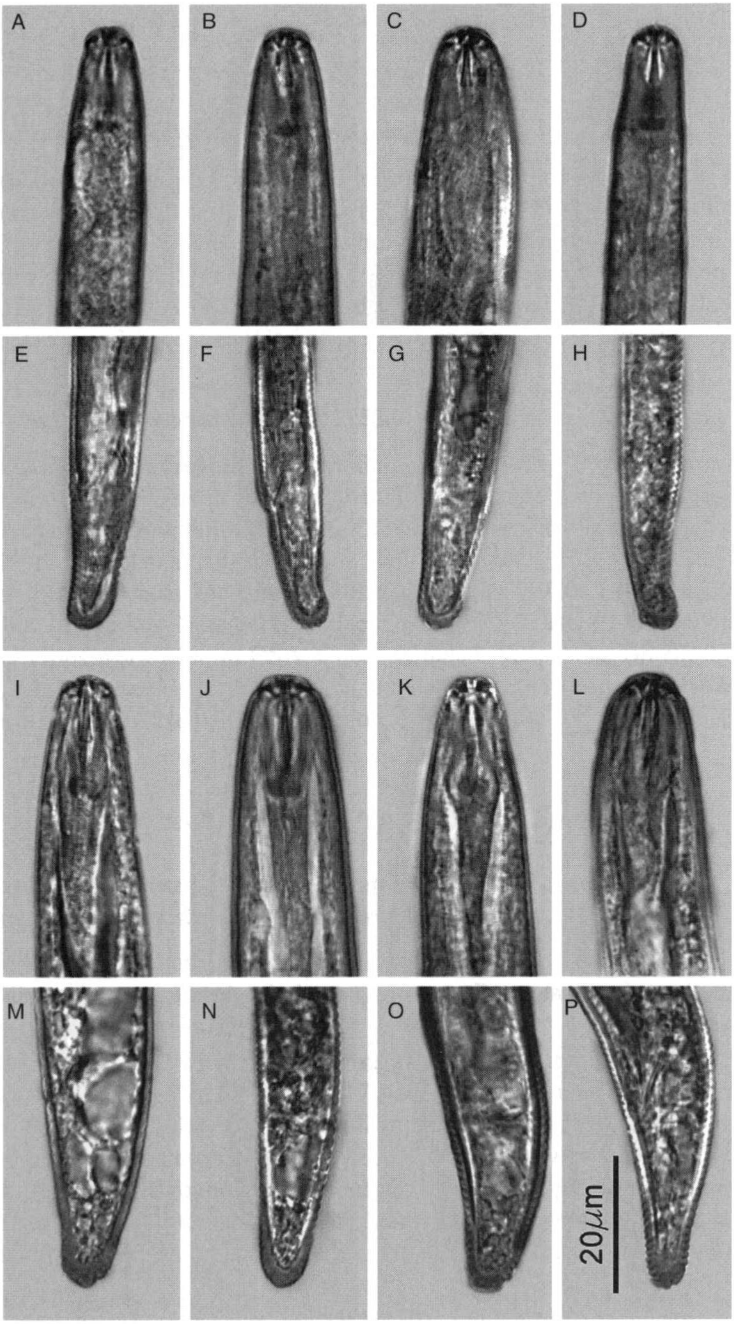

Fig. 3.3 Photomicrographs of female heads and tails, showing variations in tail shape. (**A–H**) *P. panamaensis*, (**I–P**) *P. pratensis*. (Photos by Z. A. Handoo)

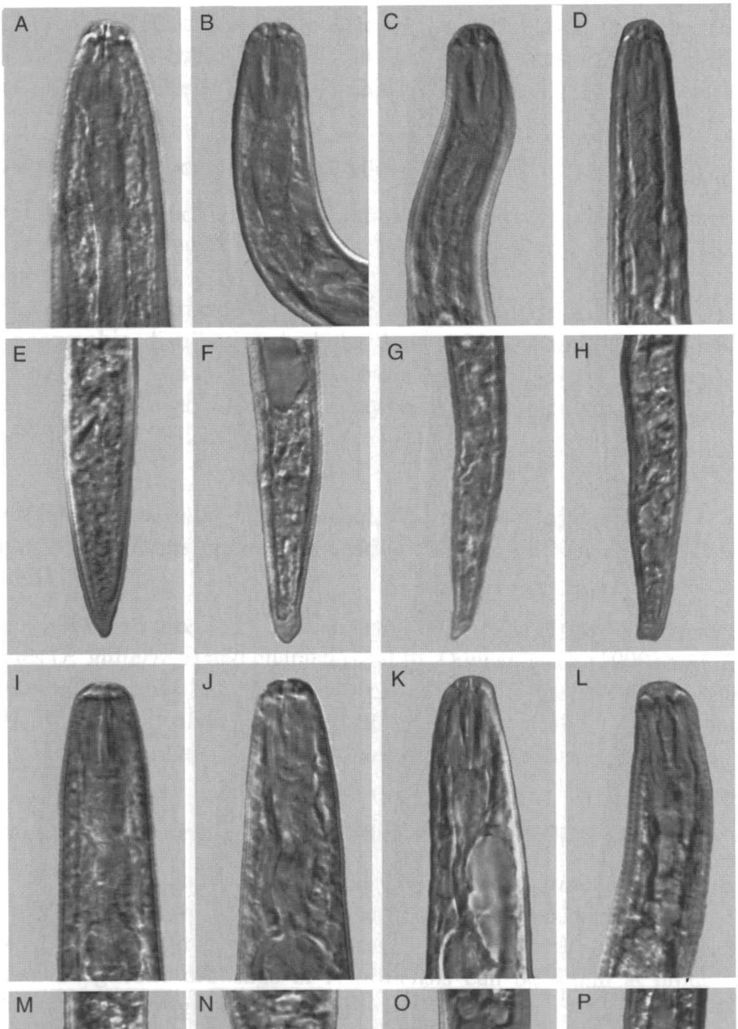

Fig. 3.4 Photomicrographs of female heads and tails, showing variations in tail shape. (**A–H**) *P. vulnus*, (**I–P**): *P. zeae*. (Photos by Z. A. Handoo)

issue will not be satisfactorily resolved until molecular sequences are available for *P. pratensis*-like taxa.

3.2.2.7 *Pratylenchus vulnus* Allen and Jensen, 1951

The taxonomy and morphological variations in *P. vulnus* populations (Fig. 3.4 A–H) have been described in several reviews (Corbett, 1974; Doucet et al., 1996; 1998; 2001; Gao et al., 1999). This species has been recently discovered on coffee in Brazil (Monteiro et al., 2001). It has also been found on other crops in Southern Europe, Russia, Egypt, South Africa, India, Japan, China, Philippines, New Zealand, USA, Mexico, Cuba and Argentina (Corbett, 1974; Gao et al., 1999; Lax et al., 2004).

3.2.2.8 *Pratylenchus zeae* Graham, 1951

The taxonomy and morphological variation of *P. zeae* (Fig. 3.4 I–P) have been described in various papers in the more than 50 years since it was described (Roman and Hirschmann, 1969; Fortuner, 1976; Olowe and Corbett, 1983, 1984a,b; Troccoli et al., 1996; Inserra et al., 2005a).

This species occurs on coffee in Brazil (Ferraz, 1980; Campos, 2002) and Colombia (in USDA Nematode Collection, entry #4686). It is also distributed on other crops worldwide in USA, Cuba, Trinidad, Venezuela, Brazil, throughout Africa, Madagascar, Egypt, Iraq, India, Japan, Australia (Fortuner, 1976) and in Indonesia (in USDA Nematode Collection, entry #2356).

3.2.3 *Key to Coffee-Associated* Pratylenchus *Species*

1 Lip region composed of 2 annules (rarely three) 2
1a Lip region composed of 3–4 annules 5

2(1) Tail terminus smooth 3
2a Tail terminus annulated or indented .... ***P. panamaensis*** (= ***P. gutierrezi***)

3(2) Lip region low, with outer margins angular; stylet typically 19–22 μm long (in Loof (1960) range is 17–22, but 17 very rare) with massive rounded knobs; V% = 82–89; tail subcylindrical, with truncate to subhemispherical or broadly rounded terminus; males rare................ ***P. brachyurus***
3a Lip region high, roundly convex; stylet less than 18 μm long, V% = 76–85; tail narrowly rounded to subacute with hemispherical to bluntly pointed, truncate smooth terminus 4

4(3a) V% = 78(76–82); a = 25(21–30); tail terminus truncate or broadly rounded, occasionally indented .... ***P. coffeae***
4a V% = 82(79–85); a = 32(28–36); tail terminus narrowly rounded to subacute .... ***P. loosi***

5(1a) Tail terminus smooth 6

5a Tail terminus annulated; stylet 12–16 μm long; lip region with three annules; V% = 76–80; tail subcylindrical with obtuse to rounded asymmetrical annulated terminus . . . . ***P. pratensis***

6(5) V% = 66–76. 7

6a V% = 78 – 84; body slender, a = 25–40; spermatheca oval, oblong filled with sperm, posterior uterine sac long, tail terminus bluntly pointed to narrowly rounded; males common . . . . ***P. vulnus***

7(6) Lip region low with 3 annules; stylet 15–17 μm long with broad, anteriorly flattened knobs, tail terminus narrowly rounded to subacute; males extremely rare . . . . ***P. zeae***

7a Lip region high with 4 annules; stylet 16–17 μm long with rounded flattened knobs; tail sharply conical with dorsal tail contour characteristically sinuate anterior to terminus; tail terminus with a small terminal peg; males common . . . . ***P. goodeyi***

3.3 Phylogenetic Trees and Molecular Characterization

Based on morphological data, Ryss (2002a,b) have presented multi-entry and mono-entry keys and diagnostic relationships within *Pratylenchus* sp., along with proposals for phylogeny and evolution of this genus. Also, a morphological tree for a somewhat different set of taxa has been constructed using cladistic methods (Carta et al., 2002). Not surprisingly, these morphological frameworks are often inconsistent with some molecular phylogenetic trees inferred from 28S rDNA sequences (Al-Banna et al., 1997; Duncan et al., 1999; Carta et al., 2001; De Luca et al., 2004). This is partly due to a more limited and different set of species used in the molecular studies, and also due to selection of outgroups, which can have a major impact on branching order (Carta et al., 2001).

The first phylogenetic study of some *Pratylenchus* species with *Radopholus* sp., *Hirschmanniella* sp. and *Nacobbus* sp. demonstrated a polyphyletic tree using the D3 segment of rDNA (Al-Banna et al., 1997). A second study on the *P. coffeae* species complex subdivided and defined many populations into genetic units using both D2 and D3 rDNA regions (Duncan et al., 1999). A study conducted using more *Pratylenchus* species and different outgroups has restored *Pratylenchus* monophyly (Carta et al., 2001). An analysis of sequences of multiple individuals of one or more populations of *P. thornei* Sher and Allen, *P. neglectus* (Rensch) Filipjev and Schuurmans Stekhoven, *P. mediterraneus* Corbett, *P. pinguicaudatus* Corbet and *P. vulnus* has demonstrated high variability among individuals of *P. neglectus* (De Luca et al., 2004). A different assemblage of taxa using variously coded morphological characters has been used to construct trees (Ryss, 2002b; Carta et al., 2002) with different topologies from molecular trees; these differences cannot simply be attributed to differences in species composition.

While the number of sequences and taxa used for testing hypotheses of relationships within the genus *Pratylenchus* has grown through the last decade, it is clear that molecular trees will continue to require expansion, clarification and eventual integration with morphological data.

Molecular methods are often essential to confirm species identity, as with the discovery of *P. jaehni* Inserra, Duncan, Troccoli, Dunn, Santos, Kaplan and Vovlas, which has been revealed from a 28S rDNA phylogeny (Duncan et al., 1999; Inserra et al., 2001). While straightforward PCR-RFLP diagnostics are available for some coffee-parasitic *Pratylenchus* species (Pourjame et al., 1999), many such tests have not been validated with multiple populations or related species. Obtaining the DNA controls necessary for this standardization may also present a challenge, as some species may be difficult to obtain or require labor-intensive culture methods to maintain. Nevertheless, when characterizing potentially new or economically important populations, the generation of gene sequences for comparison with those in GenBank® is highly recommended.

To construct a phylogenetic tree of the coffee-associated *Pratylenchus* spp., 28S and 18S rDNA sequences have been obtained either from GenBank® or our own unpublished data. For the 28S rDNA D2-D3 region these include the following species and GenBank® accession numbers: *P. panamaensis* (= syn. *P. gutierrezi*) isolate K1, AF170440; *P. loosi* isolate N1, AF170437; *P. coffeae* isolate M1, AF170435; *P. zeae*, AF303950; unpublished sequence for peanut-parasitic *P. brachyurus* NL8 isolate from Florida; *Radopholus similis* (Cobb) Thorne, outgroup D3, U47558. ClustalW alignments (Thompson et al., 1994) have been made for sequences of 28S D2-D3 rDNA from the five *Pratylenchus* species from coffee listed previously plus two species from other hosts (*P. hexincisus* Taylor and Jenkins, AF303949 and *P. pseudocoffeae* Mizukubo, AF170444) plus two outgroups: *Meloidogyne exigua* Göldi, AF435804 and *Hirschmanniella pomponiensis* Abdel-Rahman and Maggenti, DQ077795.

Sequences for 18S rDNA include: *P. brachyurus*, AY279545; *P. goodeyi*, AJ966498; *P. pratensis*, AY284611; *P. vulnus*, AY286311 and *R. similis*, outgroup, AJ966502. A separate ClustalW alignment has been made for 18S rDNA of these five species from coffee, plus seven from other hosts: *P. crenatus*, AY284610; *P.* cf. *flakkensis* Seinhorst, DQ080595 (species unconfirmed); *P. hexincisus*, AY919242; *P. neglectus*, AY279544; *P. penetrans*, AY286308; *P. scribneri* Steiner in Sherbakoff and Stanley, AY286309; *P. thornei*, AJ966499, plus one outgroup (*R. similis*, AJ966502).

Based on the branch order in the two corresponding rDNA trees with overlapping taxa (not shown), a single synthetic composite has been constructed using PAUP* version 4.0b10 (Swofford, 1998), with parenthetical NEXUS tree format as an unresolved ladder-like topology in TreeView ver. 1.6.6 (Page, 1996). The resulting tree has been decorated with face views drawn from SEM images of nematodes obtained from published literature: *R. similis* (Sher and Bell, 1975), *R. neosimilis* Sauer (Sauer, 1985), *P. zeae* (Baujard et al., 1990; López and Salazar, 1990), *P. goodeyi* (Corbett and Clark, 1983; Hernández et al., 2001), *P. vulnus* (Corbett and Clark, 1983; Sauer, 1985; Hernández et al., 2001), *P. pratensis* (Corbett and Clark, 1983), *P. brachyurus* (Corbett and Clark, 1983; Baujard et al., 1990; López and Salazar, 1990), *P. gutierrezi* (Golden et al., 1992; Inserra et al., 1998; Duncan et al., 1999), *P. loosi* (Corbett and Clark, 1983; Baujard et al., 1990; Duncan et al., 1999; Pourjame et al., 1999; Inserra et al., 2001) and *P. coffeae* (Corbett and Clark, 1983; Inserra et al., 1998; Duncan et al., 1999; Inserra et al., 2001).

The schematic phylogenetic tree of coffee-associated nematodes, including drawings based upon SEM face views, is shown in Fig. 3.5. Compared to a previous

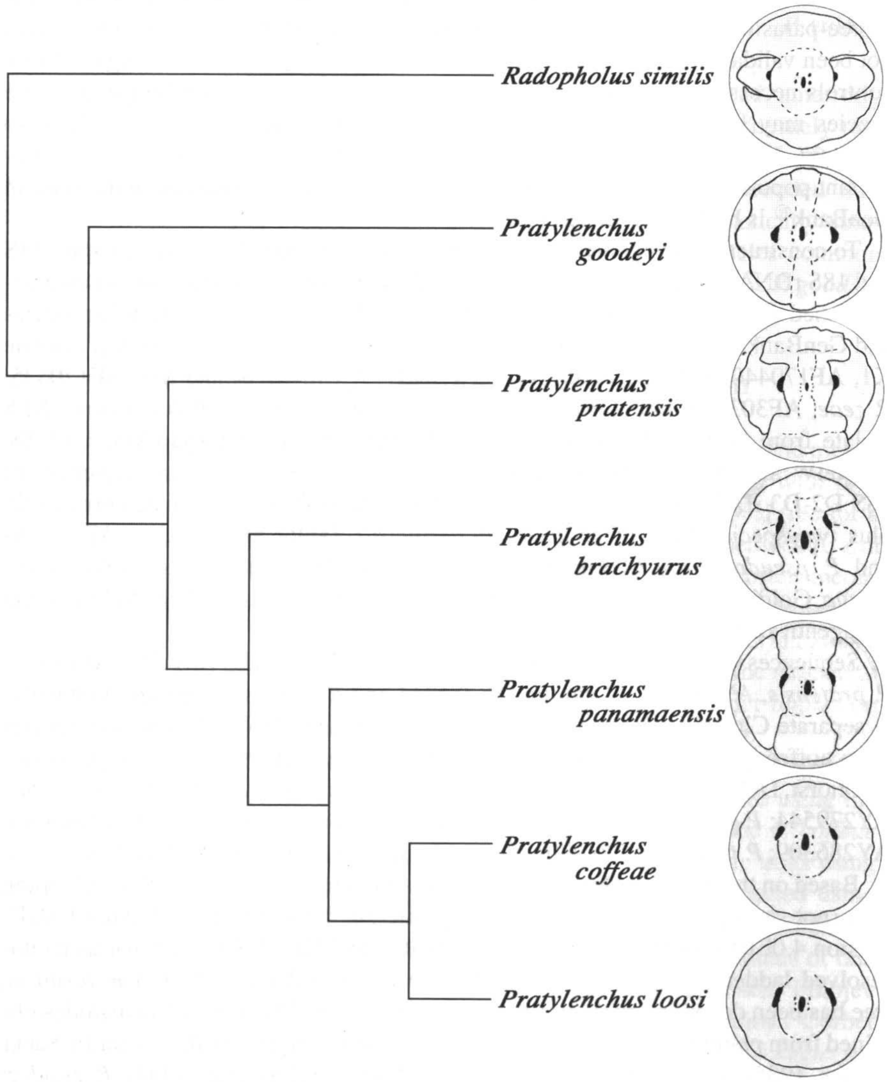

Fig. 3.5 Synthetic composite tree of six root-lesion nematode species derived from 28S rDNA and 18S rDNA trees with overlapping taxa, based on branch order and constructed with PAUP and TreeView. Scanning electron microscopic face views were drawn from the literature

molecular tree based solely on the D3 region of the 28S rDNA (Carta et al., 2001), the phylogenetic position of *P. brachyurus* inferred from the composite tree is more distant from *P. coffeae* and relatives than before. This updated position for *P. brachyurus* is more in line with the phylogenetic tree position based on morphology (Ryss, 2002b). In addition, *P. pratensis* and *P. coffeae* also appear highly divergent in the synthetic tree, unlike their position within the same clade in the morphological tree (Ryss, 2002b). The topology of the tree in Fig. 3.5 is congruent with the one shown in the most recent molecular phylogeny of this group (Inserra et al., 2007).

3.4 Concluding Remarks

Integrated studies on the morphological variation of *Pratylenchus* populations combined with molecular sequencing should result in improved methods of species delimitation. Of particular concern is the presence in the literature of nematode SEM face images that are sometimes variable and of poor quality, in part due to the use of formalin-fixed and dried specimens. This problem could be solved through more widespread application of low temperature-SEM (LT-SEM), a technique that reveals morphological features undistorted by chemicals and drying under pressure (Carta et al., 2003). Rapid cryo-fixation has revealed distinguishing features in root-lesion nematode faces even to the subspecies level (Carta et al., 2002). Determination of the number of lip annules is another serious problem, especially when few specimens are available for examination. This situation may improve through the use of new microscopic technology, such as the modular, high-resolution CytoViva® condenser (CytoViva Inc., Auburn, USA), with a cardioid annular ring that can achieve more than twice the resolution of standard circular condensers (Vainrub et al., 2006).

Increases in speed and capability and decreases in cost should lead to more frequent use of DNA sequencing by diagnostic labs for routine or selective species verification. Rapid new pyrosequencing technology, which generates short fragments (Shendure et al., 2004), may drive the development of rapid new diagnostics which are based upon short DNA fragments from multiple molecular markers.

Comparative pathogenicity studies have not been conducted for most coffee-parasitic root-lesion nematodes (Campos and Villain, 2005). Such studies would be highly desirable to assist in pest management decisions after a nematode species has been identified in a field. Systematic comparisons among species parasitizing either *C. arabica* L. (a commodity representing about 75% of world coffee exports, mostly in South and Central America) or the easier grown *C. canephora* Pierre ex Froehner (about 25% of exports, mostly grown in Africa and Asia) (Anonymous, 1986; Campos and Villain, 2005), would be especially valuable.

A concerted international research effort to centralize collection, preservation and molecular analysis of specimens, with satellite locations to perform morphology and pathogenicity studies, could greatly advance effective crop management of coffee-parasitic *Pratylenchus* species.

Acknowledgments The authors thank Donna Ellington, Maria Hult and Sharon Ochs for technical assistance. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U. S. Department of Agriculture.

References

Al-Banna L, Williamson V, Gardner SL (1997) Phylogenetic analysis of nematodes of the genus *Pratylenchus* using nuclear 26S rDNA. Mol Phylogenet Evol 7:94–102

Anderson RV, Townshend L (1980) Variation of the first head annule in Canadian populations of *Pratylenchus penetrans* (Nematoda: Pratylenchidae) from three host plants. Can J Zool 58:1336–1340

Anonymous (1986) Cultura do cafe no Brasil. Instituto Brasileiro do Cafe, Rio de Janeiro

Bajaj HK, Bhatti DS (1984) New and known species of *Pratylenchus* Filipjev, 1936 (Nematoda: Pratylenchidae) from Haryana, India, with remarks on intraspecific variations. J Nematol 16:360–367

Baujard P, Mounport D, Martiny B (1990) Etude au microscope electronique a balayage de quatre espèces du genre *Pratylenchus* Filip'ev, 1936 (Nemata: Pratylenchidae). Rev Nematol 13:203–210

Bernard EC (1984) Hoplolaimoidea (Nematoda: Tylenchida) from the Aleutian islands with descriptions of four new species. J Nematol 16:194–203

Bridge J (1984) Coffee nematode survey of Tanzania. Report on the visit to examine plant-parasitic nematodes of coffee in Tanzania. Commonwealth Institute of Parasitology, St. Albans

Brooks FE (2004) Plant-parasitic nematodes of banana in American Samoa. Nematropica 34:65–72

Cafe Filho AC, Huang CS (1989) Description of *Pratylenchus pseudofallax* n. sp. with a key to species of the genus *Pratylenchus* Filipjev, 1936 (Nematoda: Pratylenchidae). Rev Nematol 12:7–15

Campos VP (2002) Coffee nematode survey in Minas Gerais State, Brazil. Research report of the grant by PNP&D/cafe. EMBRAPA, Brasilia

Campos VP, Sivapalan P, Gnanapragasam NC (1990) Nematode parasites of coffee, cocoa and tea. In: Luc M, Sikora RA, Bridge J (eds) Plant-parasitic nematodes in subtropical and tropical agriculture. CAB International, Wallingford

Campos VP, Villain L (2005) Nematode parasites of coffee and cocoa. In: Luc M, Sikora RA, Bridge J (eds) Plant parasitic nematodes in subtropical and tropical agriculture, 2nd edition. CAB International, Wallingford

Carta LK, Handoo ZA, Skantar AM et al (2002) A redescription of *Pratylenchus teres* Khan and Singh, 1974 (Nemata: Pratylenchidae), with the description of a new subspecies from South Africa, and a phylogenetic analysis of related species. Afr Plant Prot 8:13–24

Carta LK, Skantar AM, Handoo ZA (2001) Molecular, morphological and thermal characters of 19 *Pratylenchus* spp. and relatives using the D3 segment of the nuclear LSU rRNA gene. Nematropica 31:195–209

Carta LK, Wergin WP, Erbe EF et al (2003) A comparison of low temperature and ambient temperature SEM for viewing nematode faces. J Nematol 35:78–81

Corbett DCM (1974) *Pratylenchus vulnus*. CIH descriptions of plant-parasitic nematodes, 3, # 37, Commonwealth Institute of Helminthology, St. Albans

Corbett DCM (1976) *Pratylenchus brachyurus*. CIH descriptions of plant-parasitic nematodes, 6, # 89, Commonwealth Institute of Helminthology, St. Albans

Corbett DCM, Clark SA (1983) Surface features in the taxonomy of *Pratylenchus* species. Rev Nematol 6:85–98

De Luca F, Fanelli E, Di Vito M et al (2004) Comparison of the sequences of the D3 expansion of the 26S ribosomal genes reveals different degrees of heterogeneity in different populations and species of *Pratylenchus* from the Mediterranean region. Eur J Plant Pathol 110:949–957

De Man JG (1880) Die einheimischen, frei in der reinen Erde und im süssen Wasser lebenden Nematoden monographisch bearbeitet. Tijdschrift der Nederlandse Dierk. Vereenig. 5:1–104

De Man JG (1884) Nematodes der Niederlandischen Fauna. EJ Brill, Leiden

Doucet M, Pinochet J, Di Rienzo JA (1996) Comparative analysis of morphological and morphometrical characters in six isolates of *Pratylenchus vulnus* Allen and Jensen, 1951 (Nemata: Tylenchida). Fundam Appl Nematol 19:79–84

Doucet ME, Lax P, Pinochet J (1998) Variability of some external characters in *Pratylenchus vulnus* Allen and Jensen, 1951 (Nematoda: Tylenchida). Fundam Appl Nematol 21:205–206

Doucet MP, Lax P, Di Rienzo JA et al (2001) Temperature-induced morphometrical variability in an isolate of *Pratylenchus vulnus* Allen and Jensen, 1951 (Nematoda: Tylenchida) Nematology 3:1–8

Duncan LW, Inserra RN, Thomas WK et al (1999) Molecular and morphological analysis of isolates of *Pratylenchus coffeae* and closely related species. Nematropica 29:61–80

Ebsary BA (1991) Catalog of the order Tylenchida (Nematoda). Research Branch, Agriculture Canada, Ottawa

Ferraz S (1980) Reconhecimento das especies de fitonematóides presente nos solos do estado de Minas Gerais. Experientia 26:255–328

Filipjev IN (1936) On the classification of the Tylenchinae. Proceedings Helminthol Soc Wash 3:80–82

Fortuner R (1976) *Pratylenchus zeae*. CIH descriptions of plant-parasitic nematodes, 6, # 77, Commonwealth Institute of Helminthology, St. Albans

Fortuner R (1984) List and status of the genera and families of plant-parasitic nematodes. Helminth Abstr, ser B 53:87–133

Frederick JJ, Tarjan AC (1989) A compendium of the genus *Pratylenchus* Filipjev, 1936 (Nemata: Pratylenchidae). Rev Nematol 12:243–256

Gao X, Cheng H, Fang C (1999) New diagnostic characters for *Pratylenchus vulnus*. Nematol Mediter 27:9–13

Gnanapragasam NC, Mohotti KM (2005) Nematode parasites of tea. In: Luc M, Sikora RA, Bridge J (eds) Plant parasitic nematodes in subtropical and tropical agriculture, 2nd edition. CAB International, Wallingford

Golden AM, Lopez RC, Vilchez RH (1992) Description of *Pratylenchus gutierrezi* n. sp. (Nematoda:Pratylenchidae) from coffee in Costa Rica. J Nematol 24:298–304

Hajieghrari B, Mohammadi M, Kheiri A et al (2005) A study about geographical distribution of root-lesion nematode (*Pratylenchus loosi*, Loof 1960) in tea gardens at Guilan Province, Iran. Commun Agr Appl Biol Sci 70:889–892

Handoo ZA, Golden AM (1989) A key and compendium to the species of the genus *Pratylenchus* Filipjev, 1936 (lesion nematodes). J Nematol 21:202–218

Hernández M, Jordana R, Goldaracena A et al (2001) SEM observations of nine species of the genus Pratylenchus Filipjev, 1936 (Nematoda: Pratylenchidae). J Nematode Morphol System 3:165–174

Herve G (1997) Caracterisation biologique et moleculaire de populations de nematodes phytoparasites dans les plantations de cafeiers d'Amerique Centrale. Ecole Nationale Superieure Agronomique de Rennes, D.E.A. dissertation, Rennes

Inserra RN, Duncan LW, Dunn D et al (1998) *Pratylenchus pseudocoffeae* from Florida and its relationship with *P. gutierrezi* and *P. coffeae*. Nematologica 44:683–712

Inserra RN, Duncan LW, Dunn D et al (2005a) *Pratylenchus jordanensis* a junior synonym of *P. zeae*. Nematropica 35:161–170

Inserra RN, Duncan LW, Troccoli A et al (2001) *Pratylenchus jaehni* sp. n. from citrus in Brazil and its relationship with *P. coffeae* and *P. loosi* (Nematoda: Pratylenchidae). Nematology 3:653–665

Inserra RN, Duncan LW, Vovlas N et al (1996) *Pratylenchus loosi* from pasture grasses in Central Florida. Nematologica 42:159–172

Inserra RN, Stanley JD, O'Bannon JH et al (2005b) Nematode quarantine and certification programmes implemented in Florida. Nematol Mediterr 33:113–123

Inserra RN, Troccoli A, Gozel U et al (2007) *Pratylenchus hippeastri* n. sp. (Nematoda: Pratylenchidae) from amaryllis in Florida with notes on *P. scribneri* and *P. hexincisus*. Nematology 9:25–42

Karssen G, Waeyenberge L, Moens M (2000) *Pratylenchus* sp. nov. (Nematoda: Pratylenchidae), a root-lesion nematode from European coastal dunes. Ann Zoolog 50:255–261

Kubo RK, Oliveira CMG, Antedomênico SR et al (2004) Occurrence of *Pratylenchus* in coffee plantations of São Paulo State. Nematol Bras 28:159–166

Lax P, Doucet M, Di Rienzo JA et al (2004) Inter-population variability in *Pratylenchus vulnus* Allen and Jensen, 1951 (Nematoda: Tylenchida). Nematology 6:257–260

Loof PAA (1960) Taxonomic studies on the genus *Pratylenchus* (Nematoda). Tijdschr Plantenziekten 66:29–90

Loof PAA (1974) *Pratylenchus pratensis*. CIH Descriptions of plant-parasitic nematodes, 4, # 52, Commonwealth Institute of Helminthology, St. Albans

Loof PAA (1978) The genus *Pratylenchus* Filipjev, 1936 (Nematoda: Pratylenchidae): A review of its anatomy, morphology, distribution, systematics and identification. Swedish Universities Agricultural Science Research Information Centre, Uppsala

Loof PAA (1991) The family Pratylenchidae Thorne, 1949. In: Nickle WR (ed) Manual of Agricultural Nematology. M. Dekker, New York

López R, Salazar L (1990) Morphology of some *Pratylenchus* spp. (Nemata: Pratylenchidae) found in Costa Rica, as seen with the scanning electron microscope. Agron Costarric 14:189–195

Machon JE, Hunt DJ (1985) *Pratylenchus goodeyi*. CIH Descriptions of plant-parasitic nematodes, 8, # 120, Commonwealth Institute of Helminthology, St. Albans

Mai WF, Bloom JR, Chen TA (1977) Biology and ecology of the plant-parasitic nematode *Pratylenchus penetrans*. Pennsylvania State University, Bulletin 815, University Park

Mizukubo T (1992) Morphological and statistical differentiation of *Pratylenchus coffeae* complex in Japan (Nematoda: Pratylenchidae). Appl Entom Zool 27:213–224

Monteiro AR, Antedomênico SR, Ferraz LCCB et al (2001) Primeira ocorrência de *Pratylenchus vulnus* Allen and Jensen, 1951 em cafeeiro. Proceedings XXIII Congr Bras Nematol:88

Norton DC, Donald P, Kimpinski J et al (1984) Distribution of plant parasitic nematode species in North America. Society of Nematologists, Lakeland

Olowe T, Corbett DCM (1983) Morphology and morphometrics of *Pratylenchus brachyurus* and *P. zeae*. I. Effect of fixative and processing. Indian J Nematol 13:141–154

Olowe T, Corbett DCM (1984a) Morphology and morphometrics of *Pratylenchus brachyurus* and *P. zeae*. II. Influence of environmental factors. Indian J Nematol 14:6–17

Olowe T, Corbett DCM (1984b) Morphology and morphometrics of *Pratylenchus brachyurus* and *P. zeae*. III. Influence of geographical location. Indian J Nematol 14:30–35

Page RDM (1996) Treeview: an application to display phylogenetic trees on personal computers. Comput Appl Biosci 12:357–358

Park B-Y, Choi D-R, Lee J-K et al (2002) An unrecorded species of *Pratylenchus loosi* Loof (Tylenchida: Pratylenchidae) from tea in Korea. Korean J Appl Entomol 41:299–303

Peña E, Karssen G, Moens M (2007) Distribution and diversity of root-lesion nematodes (*Pratylenchus* spp.) associated with *Ammophila arenaria* in coastal dunes of Western Europe. Nematology 9:881–901

Pourjame E, Waeyenberge L, Moens M et al (1999) Morphological, morphometrical and molecular study of *Pratylenchus coffeae* and *P. loosi* (Nematoda: Pratylenchidae). Mededelingen – Faculteit Landbouwkundige en Toegepaste Biologische Wetenschappen, Universiteit Gent 64:391–401

Powers T (2008) The *Pratylenchus* project. http://nematode.unl.edu/pratyspp.htm, visited on Feb 22nd 2008

Rashid A, Khan AM (1978) Morphometric studies on *Pratylenchus coffeae* with description of *Pratylenchus typicus* Rashid, 1974. Indian J Nematol 6:63–72

Roman J, Hirschmann H (1969) Morphology and morphometrics of six species of *Pratylenchus*. J Nematol 1:363–386

Ryss AY (1988) Parasitic root nematodes of the family Pratylenchidae (Tylenchida) of the world fauna. (In Russian) Nauka, Leningrad

Ryss AY (2002a) Genus *Pratylenchus* Filipjev: multientry and monoentry keys and diagnostic relationships (Nematoda:Tylenchida:Pratylenchidae). Zoosystemat Ross 10:241–255

Ryss AY (2002b) Phylogeny and evolution of the genus *Pratylenchus* according to morphological data (Nematoda:Tylenchida). Zoosystemat Ross 10:257–273

Sakwe PN, Geraert E (1994) Species of the genus *Pratylenchus* Filipjev, 1936 (Nematoda: Tylenchida) from Cameroon. Fundam Appl Nematol 17:161–173

Sauer MR (1985) A scanning electron microscope study of plant and soil nematodes. CSIRO, Northfield

Schmitt DP, Barker KR (1981) Damage and reproductive potentials of *Pratylenchus brachyurus* and *P. penetrans* on soybean. J Nematol 13:327–332

Seinhorst JW (1968) Three new *Pratylenchus* species with a discussion of the structure of the cephalic framework and of the spermatheca in this genus. Nematologica 14:497–510

Seinhorst JW (1977) *Pratylenchus loosi*. CIH Descriptions of plant-parasitic nematodes, 7, # 100, Commonwealth Institute of Helminthology, St. Albans

Shendure J, Mitra RD, Varma C et al (2004) Advanced sequencing technologies: methods and goals. Nature Rev Genet 35:335–344

Sher SA, Allen MW (1953) Revision of the genus *Pratylenchus* (Nematoda: Tylenchidae). Univ California public Zool 57:441–470

Sher SA, Bell AH (1975) Scanning electron micrographs of the anterior region of some species of Tylenchoidea (Tylenchida: Nematoda). J Nematol 7:69–83

Siciliano-Wilcken SR, Inomoto M, Ferraz LC et al (2002a) Morphometry of *Pratylenchus* populations from coffee, banana, ornamental plant and citrus in Brazil. Proceedings IV Int Cong Nematol:356

Siciliano-Wilcken SR, Inomoto M, Ferraz LC et al (2002b) RAPD of *Pratylenchus* populations from coffee, banana, ornamental plant and citrus in Brazil. Proceedings IV Int Cong Nematol:163

Siddiqi MR (1972) *Pratylenchus coffeae*. CIH Description of plant-parasitic nematodes, 1, # 6, Commonwealth Institute of Helminthology, St. Albans

Siddiqi MR (1997) Techniques and methodologies for nematode disease diagnosis and nematode identification. FAO Plant Production and Protection Paper # 144, Rome

Siddiqi MR (2000) Tylenchida Parasites of Plants and Insects, 2nd edition. CAB International, Wallingford

Siddiqi MR, Dabur KR, Bajaj HK (1991) Descriptions of three new species of *Pratylenchus* Filipjev, 1936 (Nematoda: Pratylenchidae). Nematol Mediterr 19:1–7

Silva RA, Inomoto MM (2002) Host-range characterization of two *Pratylenchus coffeae* isolates from Brasil. J Nematol 34:135–139

Swofford DL (1998) PAUP – Phylogenetic Analysis Using Parsimony (and other methods), version 4.0. Sinauer Associates, Sunderland

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680

Trett MW, Perry RN (1985) Functional and evolutionary implications of the anterior sensory anatomy of species of root-lesion nematode (genus *Pratylenchus*). Rev Nematol 8:341–355

Troccoli A, Costilla MA, Lamberti F (1996) Note Morfo-biometriche su *Pratylenchus zeae* Graham, 1951. Nematol Mediterr 24:43–47

Vainrub A, Pustovyy O, Vodyanoy V (2006) Resolution of 90 nm (λ/5) in an optical transmission microscope with an annular condenser. Optic Lett 31:2855–2857

Van Den Berg E, Quénéhervé P (2000) *Hirschmanniella caribbeana* sp. n. and new records of *Pratylenchus* spp. (Pratylenchidae: Nematoda) from Guadeloupe, French West Indies. Nematology 2:179–190

Van Den Berg E, Queneherve P, Tiedi LR (2005) Six known plant-feeding nematodes from Guadeloupe, Martinige and French Guiana (Nemata: Tylenchina). J Nematode Morphol Systemat 7:109–129

Villain L (2000) Caracterisation et bioecologie du complexe parasitaire du genre Pratylenchus (Nemata: Pratylenchidae) present sur cafeiers (Coffea spp.) au Guatemala. Ecole Nationale Superieure Agronomique de Rennes, PhD thesis, Rennes

Villain L, Baujard P, Molina A et al (1998) Morphological and biological characterization of three *Pratylenchus* spp. populations parasitizing coffee trees in Guatemala. Nematologica 44:600–601

Whitehead AG (1968) Nematodea. In: Le Pelley RH (ed) Pests of Coffee. Longmans, Green and Co. Ltd., London