MOLECULAR AND MORPHOLOGICAL ANALYSIS OF ISOLATES OF *PRATYLENCHUS COFFEAE* AND CLOSELY RELATED SPECIES[†]

L. W. Duncan,[1] R. N. Inserra,[2] W. K. Thomas,[3] D. Dunn,[1] I. Mustika,[4] L. M. Frisse,[3] M. L. Mendes,[5] K. Morris,[3] and D. T. Kaplan,[6]

University of Florida, Citrus Research and Education Center, 700 Experiment Station Rd., Lake Alfred, FL 33850, U.S.A.;[1] Florida Department of Agriculture and Consumer Services, Division of Plant Industry, P.O. Box 147100, Gainesville, FL 32614-7100, U.S.A.;[2] Division of Molecular Biology and Biochemistry, 5007 Rockhill Rd., University of Missouri, Kansas City, MO 64110, U.S.A.;[3] Balai Penelitian Tanaman Rempah dan Obat, Jalan Tentara Pelajar No. 3, Bogor, 16111, Indonesia;[4] Universidade Estadual de Londrina, Departamento de Agronomia, 86051-990 Londrina, PR, Brazil;[5] and USDA-ARS, Horticultural Research Laboratory, 2120 Camden Rd., Orlando, FL 32803, U.S.A.[6]

ABSTRACT

Duncan, L. W., R. N. Inserra, W. K. Thomas, D. Dunn, I. Mustika, L. M. Frisse, M. L. Mendes, K. Morris, and D. T. Kaplan. 1999. Molecular and morphological analysis of isolates of *Pratylenchus coffeae* and closely related species. Nematropica 29:61-80.

Morphological and genome variation between 32 nematode isolates identified originally as *Pratylenchus coffeae, P. gutierrezi, P. loosi,* and *P. pseudocoffeae* were characterized to estimate phylogenetic relationships among them. All isolates have numerous males, and two lip annuli. Viewed *en face* with scanning electron microscopy, the first lip annulus is divided into lateral and median sectors in several isolates from coffee (Central America and Indonesia), and in one from aster (Florida). The first lip annulus is smooth in all other isolates, including six from coffee (Brazil and Indonesia). Principal component analysis (PCA) of one morphological (smooth face *vs* divided face) and three weakly-allometric morphometric variables (V, a, length of stylet) revealed seven assemblages of isolates. The PCA-derived assemblages conform closely to phylogenetic relationships inferred from analysis of 28S rDNA sequences. Nevertheless, identity within the D2/D3 expansion segment was not absolute for all isolates within the morphological assemblages, indicating the possibility that several assemblages are species complexes. Based on face morphology, five isolates (smooth faces) from coffee near the type locality for *P. coffeae* in eastern Java, Indonesia are different species than preserved museum specimens (divided faces) collected in the same area. Moreover, the D2/D3 sequence of a Java isolate suggests that it may be conspecific with isolates of *P. coffeae sensu lato* (all with identical sequences and smooth faces) from citrus, banana, yam, aglaonema and cocoyam, but not with isolates from citrus in Oman, nor banana in Ghana. Morphology and D2/D3 sequence of a *P. gutierrezi* topotype isolate revealed the likelihood that it is not conspecific with two other isolates with divided faces from coffee in Central America. Morphology and genetic sequence data for isolates from coffee and citrus in Sao Paulo State in Brazil, indicate that they are one or more undescribed species. When compared to the morphology and D2/D3 sequence of *P. loosi* from tea in Sri Lanka, isolates recently described as *P. loosi* from *Paspalum notatum* and *Panicum hemitomon* in Florida are apparently two undescribed species.

Key words: molecular evolution, morphology, morphometrics, nematode phylogeny, *Pratylenchus coffeae, P. gutierrezi, P. loosi, P. pseudocoffeae,* ribosomal DNA, systematics, taxonomy.

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RESUMEN

Duncan, L. W., R. N. Inserra, W. K. Thomas, D. Dunn, I. Mustika, L. M. Frisse, M. L. Mendes, K. Morris y D. T. Kaplan. 1999. Análisis molecular y morfológico de aislamientos de *Pratylenchus coffeae* y especies estrechamente relacionadas. Nematrópica 29:61-80.

Las variaciones morfológicas y genómicas entre 32 nematodos identificados originalmente como *Pratylenchus coffeae, P. gutierrezi, P. loosi, y P. pseudocoffeae* fueron caracterizadas, para estimar las relaciones filogeneticas entre los mismos. Todos los aislamientos presentan numerosos machos y dos anillos labiales. Visto *en face*, en el microscopio electrónico de barrido, el primer anillo labial está dividido en sectores laterales y medios, en varios aislamientos de café (América Central e Indonesia), y en uno de aster (Florida). El primer sector del labio es suave en todos los otros aislamientos, incluyendo seis de café (Brazil e Indonesia). El análisis de componente principal (PCA) de una variable morfológica (*face* suave *vs. face* dividida) y de tres variables morfometricas debilmente alometricas (V, a, largo del estilete) reveló siete ensamblajes de aislamientos. La relación evolutiva estimada entre los 32 isolamientos, se evidencia en la morfología. Los ensamblajes derivados del PCA, se corresponden estrechamente con la relación filogenetica estimada a partir de la extensa homología de secuencia dentro del segmento de extensión D_2/D_3 del gen 28S del rDNA y del análisis RAPD. La homología dentro del segmento de extensión D_2/D_3 no fue absoluta para todos los aislamientos dentro de los ensamblajes morfológicos, indicando la posibilidad de que varios ensamblajes sean especies complejas. Basado en la morfología de *face*, cinco aislamientos (*face* suave) de café, cercanos a la localidad tipo para *P. coffeae* en Java oriental Indonesia, son especies diferentes a las conservadas (*faces* divididas) y coleccionadas en la misma área. Incluso la secuencia D_2/D_3 de un aislamiento de Java, sugiere que pudiera ser coespecífica a aislamientos de *P. coffeae sensu lato* (todos con secuencias homologas y *faces* suave) de cítricos, banana, ñame, aglaonema y *Colocasia esculenta*, pero no con aislamientos de Omán, ni de banana de Ghana. La morfología y secuencia D_2/D_3 de una cepa topotipo de *P. gutierrezi* reveló la probabilidad de que no sea coespecífica a otras dos cepas de café de América Central con *faces* divididas. La morfología y datos de secuencia de cepas de café y cítricos del estado de Sao Paulo en Brazil, indica que hay una o más especies no descritas. Cuando se comparó la morfología y secuencia D_2/D_3 de *P. loosi* de te de Sri Lanka, con cepas de *Paspalum notatum* y *Panicum hemitomon* descritas recientemente como *P. loosi* en Florida, estas parecen ser dos especies no descritas.

Palabras claves: genetica molecular, morfología, morfometricos, nematodo filogenia, *Pratylenchus coffeae, P. gutierrezi, P. loosi, P. pseudocoffeae,* secuencia genetica, sistemática, taxonomía.

INTRODUCTION

Pratylenchus coffeae (Zimmermann) Filipjev and Schuurmanns Stekhoven causes a severe decline of citrus trees in Florida, where it infects all commercial rootstock varieties and a number of weed species in citrus orchards (Kaplan and MacGowan, 1982; O'Bannon and Tomerlin, 1973; O'Bannon *et al.*, 1976; Radewald *et al.*, 1971). During more than 30 years following its detection, the incidence of *P. coffeae* in Florida citrus orchards has remained very low, partly because planting stock from commercial nurseries must be certified free of the nematode. However, *P. coffeae* was also identified from native plants in fields not planted with citrus (Inserra *et al.*, 1990). The presence of *P. coffeae* on native plants, and its low incidence in Florida citrus orchards, suggested that these nematodes may be comprised of different races or species. This suspicion was confirmed when *P. loosi* Loof and *P. pseudocoffeae* Mizokubo were proposed as the correct identities of nematodes, previously considered to be *P. coffeae,* from several native plant species in Florida (Inserra *et al.*, 1996, 1998).

The description in 1992 of *P. gutierrezi* Golden *et al.*, from coffee in Costa Rica, raised further concern about the taxonomic designation of *P. coffeae* for lesion nematodes attacking citrus. The morphology of *P. gutierrezi* is similar to that of *P. coffeae* except that scanning electron microscopy (SEM) revealed distinct medial and lateral lip sectors in the *en face* view of the first lip annulus of the former species, in contrast to the smooth face (fused lip sectors) reported for *P. coffeae* from citrus (Corbett and Clark, 1983). The different lip patterns in these two species underscores a need for SEM examination of type material of *P. coffeae*, and of other populations of nematodes currently designated as *P. coffeae* from various crops and locales. Indeed, recent examination of *P. coffeae* from coffee near the type locality in Indonesia, revealed lip patterns similar to those of *P. gutierrezi* (Inserra *et al.*, 1998).

In order to clarify the taxonomic status of coffee lesion nematodes from citrus in Florida, we characterized both the morphology and the D2/D3 expansion segment of the large subunit of nuclear rDNA of *P. coffeae* and closely related species collected worldwide. We also performed a RAPD analysis of 15 lesion nematode isolates. Our objectives were to 1) estimate phylogenetic relationships among the isolates 2) study relationships between the morphological and molecular characteristics among the isolates, and 3) evaluate taxonomic relationships. In this paper, we provide evidence that several of the species studied represent species complexes, and we propose the need to redefine *P. coffeae*.

MATERIALS AND METHODS

Nematode isolates: Isolates from populations of putative *P. coffeae, P. gutierrezi, P. loosi,* and *P. pseudocoffeae* were collected from various host plants in Florida and/or imported into Florida under special permit (Table 1). Five of the isolates were collected from roots of coffee trees in 5 different provinces of eastern Java which is the type locality of *P. coffeae*. A topotype isolate of *P. gutierrezi* was also obtained. Species identifications given in Table 1 are those made from light microscopy and SEM, prior to analysis of the multivariate relationships of morphological characters and DNA sequences among the isolates. Nematodes were imported in the roots of crop host plants, or in carrot disk culture (Huettel, 1985), or in excised corn roots (Huang and Becker, 1997). Thereafter, all nematode populations were maintained on carrot disks at 24°C.

Morphological studies: Specimens for SEM were cold fixed in 3% glutaraldehyde buffered with 0.1 M phosphate buffer (pH 7.2), post-fixed 1 h in 2% osmium tetroxide, dehydrated in a graded series of ethanol, critical point dried with CO_2, and sputter coated with goldpalladium (Eisenback, 1985). Specimens were observed with a Hitachi S530 microscope at 1 520 kV accelerating voltage.

Live nematode specimens were narcotized with low heat, and mounted in water agar (Esser, 1986) for measurement. Morphological features of diagnostic value for the genus *Pratylenchus* (Loof, 1991) were determined for 20 mature females from all populations. When possible, specimens were obtained from roots of the host plant on which they were collected originally. For several populations, cohorts of 20 females were also measured at different times and following recovery from both host roots and carrot disks.

Morphological and morphometric variables from at least 20 females from all populations were analyzed using principal component analysis (PCA) (Minitab Software, State College, Pennsylvania). Thirteen morphometric variables (Table 2)

Table 1. Regional sources, original host plants, and *en face* patterns of isolates of putative *Pratylenchus coffeae, P. loosi, P. pseudocoffeae,* and *P. gutierrezi* used in this study.

Putative Species[y]	Original Host	Region	*En Face* Pattern	Code[z]
P. coffeae	Citrus	Sao Paulo, Brazil	undivided	C1
P. coffeae	Citrus	Sao Paulo, Brazil	undivided	C2
P. coffeae	Citrus	Sao Paulo, Brazil	undivided	C7
P. coffeae	Citrus	Florida, USA	undivided	C3
P. coffeae	Citrus	Florida, USA	undivided	C4
P. coffeae	Citrus	Florida, USA	undivided	C5
P. coffeae	Citrus	Dhofar, Oman	undivided	C6
P. coffeae	Yam	Martinique	undivided	Y1
P. coffeae	Yam	Pernambuco, Brazil	undivided	Y2
P. coffeae	Yam	Puerto Rico, USA	undivided	Y3
P. coffeae	Banana	Ghana	undivided	B1
P. coffeae	Banana	Honduras	undivided	B2
P. coffeae	Banana	Costa Rica	undivided	B3
P. coffeae	Banana	Malaysia	undivided	B4
P. coffeae	Cocoyam	Sao Paulo, Brazil	undivided	M1
P. coffeae	Diffenbachia	Sao Paulo, Brazil	undivided	M2
P. coffeae	Aglaonema	Florida, USA	undivided	M3
P. coffeae	Ficus	China	undivided	M4
P. loosi	Bahia grass	Florida, USA	undivided	N1
P. loosi	Maidencane	Florida, USA	undivided	N2
P. loosi	Popash	Florida, USA	undivided	N3
P. loosi	Tea	Sri Lanka	undivided	T
P. gutierrezi	Coffee	Costa Rica	divided	K1
P. gutierrezi	Coffee	Guatemala	divided	K2
P. gutierrezi	Coffee	San Antonio, Costa Rica (topotype)	divided	K3
P. coffeae	Coffee	Bogor, Indonesia (preserved)	divided	K4
P. coffeae	Coffee	Sao Paulo, Brazil	undivided	K5
P. coffeae	Coffee	Kaliwining, Indonesia	undivided	K6
P. coffeae	Coffee	Dampit, Indonesia	undivided	K7
P. coffeae	Coffee	Sumberasin, Indonesia	undivided	K8
P. coffeae	Coffee	Wlingi, Indonesia	undivided	K9
P. coffeae	Coffee	Kalibaru, Indonesia	undivided	K10

Table 1. (Continued) Regional sources, original host plants, and *en face* patterns of isolates of putative *Pratylenchus coffeae, P. loosi, P. pseudocoffeae,* and *P. gutierrezi* used in this study.

Putative Species[y]	Original Host	Region	*En Face* Pattern	Code[z]
P. pseudocoffeae	Aster	Florida, USA	divided	A1

[y]Identification of isolates prior to multivariate analysis of morphological/morphometric statistics, and DNA sequence analysis. Isolates K4 and K6-10 are *P. coffeae sensu* Sher and Allen, 1953 (face patterns are not considered).

[z]Codes used in Tables 2-3, and Figs. 2, 4, 5, and 6.

and *en face* lip characteristics were used in one analysis. Lip characteristics were coded for fused or separate median and lateral lip sectors. A second analysis used only the most weakly allometric variables (those that were least significantly correlated with body length: V, a, stylet length, and fused or separated lip sectors) to minimize effects of allometry (Duncan *et al.*, 1998; Mizukubo, 1992). For comparison with the isolates in our collection, we included in the analyses measurements and lip morphology reported for fixed specimens of *P. coffeae sensu* Sher and Allen, 1953, collected in 1952 from coffee roots near the type locale in Indonesia.

DNA was extracted from selected lesion nematode isolates following culture on carrot disks (Kaplan *et al.*, 1997). Sixteen sets of decamer primers (OPQ04-07, OPQ09, OPQ13-14, OPQ18, OPQ20, OPF01, OPF04-06, OP07-09; Operon Technologies, Alameda, CA, U.S.A.) were used for RAPD analyses as described previously (Kaplan *et al.*, 1997).

A D2/D3 analysis and a resulting phylogenetic analysis of 19 isolates was performed essentially as described by Thomas *et al.* (1997), except that specimens were not fixed prior to DNA extraction. DNA sequences were aligned by eye using Eyeball Sequence Editor (ESEE) (Cabot and Beckenbach, 1989). Regions of the alignment involving an insertion or deletion were removed from the analysis to a point where a residue was conserved in all taxa. All trees were rooted with the outgroup method using *R. similis* sequences. Known sequences of *P. penetrans* and *P. vulnus* were also included in the analysis for comparison. Aligned sequences were subjected to maximum parsimony (MP) analysis as implemented in PAUP (Swofford, 1991) and neighbor-joining (NJ) analysis as implemented in MEGA (Kumar *et al.* 1993). Sequences were submitted to Genbank (accession numbers AF170426-AF170444).

DNA extracted from individual lesion nematodes from the 19 isolates used for D2/D3 analysis and rDNA was amplified using PCR reaction conditions described previously (Kaplan *et. al.*, 1997). Primers PC1 (5'-ATGCGCACATTGCATTCAGC-3') and PC2 (5'-GAGCGAGAAACACCTCTCAC-3'), or PL1 (5'-CAGTCAGCTAGCTGCTGGAT-3') and PL2 (5'-ATGAGAGCATAGTCGCT-GTG-3') (Uehara *et al.*, 1998) or TW81 (5'-GTTTCCGTAGGTGAACCTGC-3') and AB28 (5'-ATATGCTTAAGTTCAGCGGGT-3') were used to amplify contiguous portions of the ITS1, 5.8s and ITS2 for *P. coffeae, P. loosi,* and the ITS1 with small portions of the 18s and 5.8s subunits, respectively (Uehara *et al,* 1998; Vrain *et al.*, 1992). Ten independent reactions were performed two times for each nematode isolate/primer combination. Electrophoresis was performed using 1.25% agarose gels in 1XTAE (40mM

Tris acetate, 1mM EDTA, pH 8.0) at 60V for 3 hours. Gels were stained with ethidium bromide (5g/100 ml) and viewed on a UV transilluminator. Molecular weight markers were BioMarker EXT (Bio Ventures, Murfreesboro, TN).

RESULTS

Morphological studies: Isolates of *Pratylenchus* spp. generally had smooth first lip annuli when viewed *en face* with SEM, except for an isolate from aster and 3 isolates from coffee, all of which were shown previously (Golden *et al.*, 1992; Inserra *et al.*, 1998) to have medial lip sectors that were divided from lateral lip sectors (Table 1, Fig. 1). Within any assemblage derived from PCA (described below), the morphology of the first lip annulus was consistent for all isolates.

The ranges of nearly all morphometric variables for all isolates overlapped those reported for *P. coffeae* (Table 2; Loof, 1991). Consequently, the PCA analysis of all morphometric variables and the coded variable for divided or fused lip sectors revealed isolate affinities that corresponded poorly to their putative taxonomic status (Fig. 2A).

All morphometric variables and all but one of the derived variables (ratios) were significantly and positively correlated with body length (allometric), whether all isolates or repeated measurements of a single isolate were considered. When the PCA analysis was restricted to the most weakly allometric variables, seven arbitrary but distinct assemblages of isolates were delineated (Fig. 2B). Group I consisted exclusively of isolates from coffee, including two of the putative *P. gutierrezi* isolates and the fixed *P. coffeae* from Indonesia. The *P. gutierrezi* topotype isolate (Group VII), although closely positioned to Group I, was designated as a distinct group due to differences in stylet length. The *P. pseudocoffeae* isolate from Florida, whether obtained from aster or carrot, clustered in Group II. Most *P. coffeae* isolates from citrus, yam, banana and miscellaneous plants were clustered in the largest assemblage designated Group III. The five *P. coffeae* isolates from coffee in Indonesia were also positioned in Group III, in contrast to the fixed specimens from coffee in Indonesia (Group I). Within Group III, isolates from citrus showed a close affinity for one another, as did isolates from coffee. *P. loosi* from tea in Sri Lanka, designated as the only isolate in Group IV, was not greatly different from isolates in Group III in the analysis. However, the isolate from tea was clearly different from those of *P. loosi* from native plants in Florida (Group VI). All isolates from citrus in Brazil and that from coffee in Brazil (Group V) were similar to one another and distinct from all other isolates.

The groups identified by PCA can be characterized based on the variables used in the analysis (Fig. 3). Isolates in Groups V and VI tend to have more anterior vulva position (<79.5) than other isolates, and those in Group VI often appear more slender (a > 25.5) than those in Group V (a < 25.5). Isolates in Groups I, II and VII have segmented first lip annuli. Those in Group I appear more stout (a < 23.7) than all other isolates, and the specimens in Group VII have longer stylets on average than those than all others. Group II isolates also have longer overlap of the esophageal gland (data not shown) than those of Group I (Inserra *et al.*, 1998). The remaining groups (III and IV) differed for the longer stylets (>16.0 µm) in the isolates of Group III. Within Group III, it is noteworthy that the length of the post-uterine branch in the isolates from citrus is significantly greater than for all other populations (Table 2).

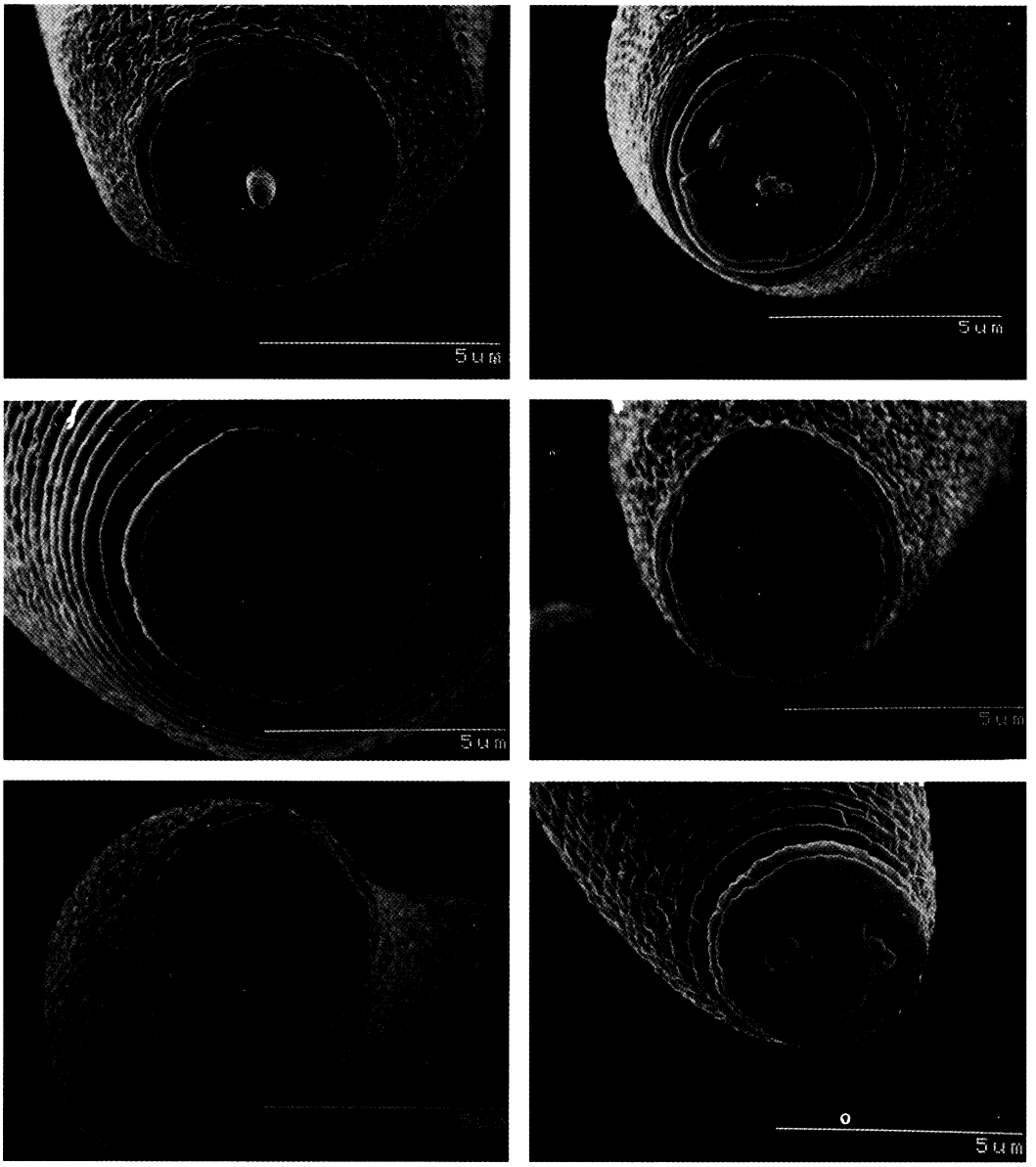

Fig. 1. *En face* scanning electron micrographs of *Pratylenchus* spp. representative of the isolates in the 7 groups derived from principal component analysis. Host plant and locality of the isolates were: top left (Group I), coffee, Guatemala; top right (Group II), aster, Florida; middle left (Group III), yam, Brazil; middle right (Group IV), tea, Sri Lanka; bottom left (Group V), citrus, Brazil; bottom right (Group VI), popash, Florida. Micrographs representative of Group VII, given by Golden *et al.* (1992), appear similar to those of Group I.

The characters used in PCA to derive relationships among isolates were highly variable among individual nematodes (Table 2). Consequently, identification of one or a few specimens requires the use of additional characters not used in our anal-

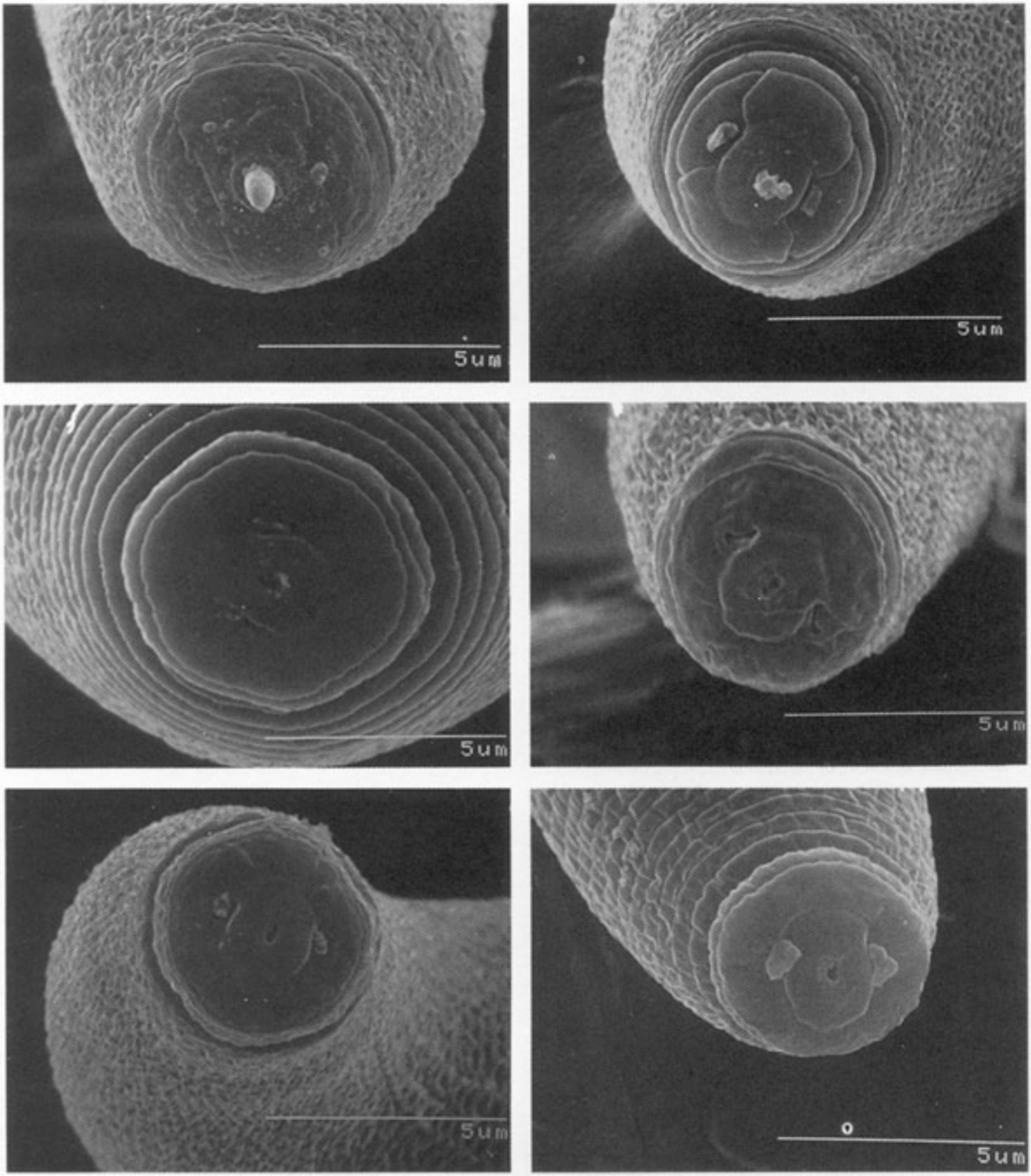

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Table 2. Morphometric measurements and derived variables for females from isolates of *Pratylenchus* spp. described in Table 1. Statistics include mean (n = 20) ± standard deviation with range shown in bold print.

PCA Group	Isolate Code	Body length (L)	Body width	Esophagus length	Excretory pore to head end (E)	Post-uterine branch length (PUB)	Tail length	Stylet length	Vulva (%)	a	b	c	L/PUB	L/E	L/S
I	K1	491.8 ± 7.0	21.6 ± 0.31	86.6 ± 1.0	85.4 ± 1.00	25.7 ± 0.89	23.2 ± 0.37	16.2 ± 0.05	80.0 ± 0.30	22.8 ± 0.38	5.7 ± 0.08	21.3 ± 0.26	19.6 ± 0.81	5.8 ± 0.04	30.4 ± 0.45
		439.0 – 549.5	**18.5 – 24.5**	**78.0 – 96.0**	**78.0 – 96.0**	**17.5 – 33.0**	**20.0 – 24.5**	**16.0 – 16.5**	**78.0 – 82.0**	**19.3 – 26.2**	**4.9 – 6.3**	**18.5 – 23.7**	**14.4 – 31.4**	**5.4 – 6.2**	**26.6 – 34.3**
I	K2	646.5 ± 12.0	28.7 ± 0.70	87.9 ± 1.1	94.3 ± 1.80	36.3 ± 1.70	31.1 ± 0.55	15.6 ± 0.08	80.0 ± 0.38	22.6 ± 0.35	7.4 ± 0.13	20.8 ± 0.40	18.4 ± 0.76	6.9 ± 0.12	41.5 ± 0.75
		553.0 – 728.0	**21.5 – 33.0**	**81.0 – 99.5**	**80.0 – 109.5**	**24.5 – 50.5**	**24.5 – 35.0**	**15.0 – 16.0**	**77.0 – 83.0**	**20.7 – 25.7**	**5.9 – 8.3**	**18.4 – 26.9**	**13.6 – 23.7**	**6.0 – 8.4**	**36.3 – 47.0**
I	K4	514.6 ± 38.1	23.8 ± 2.48	83.3 ± 2.6	84.7 ± 3.6	28.3 ± 3.4	28.6 ± 2.5	16.1 ± 0.24	80.5 ± 1.5	21.8 ± 2.4	6.2 ± 0.37	18.0 ± 1.6	18.4 ± 2.75	6.1 ± 0.52	31.9 ± 2.2
		466.5 – 579.0	**19.5 – 27.0**	**79.5 – 86.0**	**80.0 – 90.0**	**24.5 – 33.0**	**24.5 – 31.5**	**16.0 – 16.5**	**77.9 – 82.7**	**19.1 – 25.1**	**5.6 – 6.9**	**16.1 – 20.4**	**14.9 – 22.3**	**5.5 – 7.0**	**29.2 – 35.1**
II	A1	560.6 ± 8.5	22.1 ± 0.37	91.1 ± 0.6	91.0 ± 1.00	26.3 ± 0.83	29.2 ± 0.41	15.9 ± 0.11	79.7 ± 0.26	25.4 ± 0.34	6.2 ± 0.11	19.2 ± 0.21	21.7 ± 0.81	6.2 ± 0.10	35.3 ± 0.50
		495.8 – 633.0	**19.6 – 24.5**	**85.2 – 96.0**	**85.2 – 99.9**	**19.6 – 32.4**	**26.4 – 33.3**	**15.1 – 16.7**	**77.3 – 81.5**	**22.4 – 27.7**	**5.2 – 7.0**	**16.4 – 20.2**	**15.8 – 30.0**	**5.4 – 6.9**	**31.6 – 39.1**
II	A1	530.8 ± 8.8	22.2 ± 0.33	91.9 ± 1.2	86.7 ± 1.21	27.3 ± 0.72	29.2 ± 0.71	16.1 ± 0.08	80.2 ± 0.23	24.0 ± 0.42	5.8 ± 0.06	18.3 ± 0.33	19.7 ± 0.50	6.1 ± 0.06	32.9 ± 0.55
		466.0 – 608.5	**20.0 – 24.5**	**80.0 – 101.5**	**75.5 – 96.0**	**22.5 – 33.0**	**24.5 – 36.0**	**15.5 – 16.5**	**79.0 – 82.0**	**20.1 – 28.9**	**5.3 – 6.4**	**14.4 – 20.6**	**15.5 – 24.6**	**5.6 – 6.7**	**28.2 – 38.0**
II	A1	551.4 ± 9.4	22.1 ± 0.35	100.1 ± 1.4	88.8 ± 1.50	27.8 ± 1.00	28.6 ± 0.54	16.0 ± 0.06	81.2 ± 0.22	25.1 ± 0.51	5.5 ± 0.05	19.4 ± 0.30	20.3 ± 0.69	6.2 ± 0.08	34.4 ± 0.58
		468.4 – 614.4	**19.6 – 25.4**	**89.1 – 108.7**	**74.4 – 98.0**	**18.6 – 34.3**	**24.5 – 34.3**	**15.6 – 16.4**	**79.4 – 82.8**	**20.3 – 28.0**	**5.1 – 6.0**	**15.1 – 21.4**	**15.1 – 27.1**	**5.6 – 7.0**	**29.1 – 38.4**
II	A1	557.7 ± 7.7	22.9 ± 0.27	92.6 ± 1.0	84.9 ± 1.28	27.4 ± 0.90	28.1 ± 0.54	16.6 ± 0.08	79.9 ± 0.26	24.5 ± 0.38	6.0 ± 0.09	19.9 ± 0.35	20.8 ± 0.73	6.6 ± 0.10	33.7 ± 0.48
		482.0 – 630.0	**20.5 – 25.0**	**82.0 – 104.5**	**70.5 – 93.0**	**19.5 – 33.0**	**21.5 – 33.0**	**16.0 – 17.0**	**78.0 – 81.0**	**20.5 – 26.3**	**5.2 – 6.7**	**17.2 – 24.2**	**16.5 – 27.7**	**5.5 – 7.5**	**28.4 – 37.1**
III	B1	674.4 ± 13.9	28.1 ± 0.54	98.6 ± 1.3	98.1 ± 1.60	41.5 ± 1.40	33.0 ± 0.54	16.8 ± 0.07	80.0 ± 0.39	24.0 ± 0.36	6.9 ± 0.15	20.5 ± 0.43	16.6 ± 0.56	6.9 ± 0.08	40.2 ± 0.76
		562.5 – 807.5	**23.5 – 32.0**	**85.0 – 109.5**	**83.0 – 113.5**	**27.0 – 51.5**	**29.0 – 38.0**	**16.5 – 17.5**	**77.0 – 84.0**	**21.1 – 27.7**	**5.7 – 8.4**	**18.2 – 25.6**	**11.7 – 21.6**	**6.2 – 7.5**	**34.1 – 47.5**
III	B2	659.1 ± 26.4	27.4 ± 1.21	97.4 ± 3.1	90.9 ± 2.50	38.7 ± 1.80	31.5 ± 0.94	16.1 ± 0.19	80.1 ± 0.38	24.1 ± 0.59	6.8 ± 0.18	21.0 ± 0.80	17.2 ± 0.60	7.2 ± 0.18	41.0 ± 1.36
		474.0 – 748.5	**21.5 – 32.0**	**83.0 – 115.0**	**73.0 – 100.5**	**31.0 – 47.0**	**26.5 – 36.0**	**15.0 – 16.5**	**78.0 – 82.0**	**20.6 – 26.2**	**5.6 – 7.6**	**17.9 – 26.7**	**15.3 – 21.4**	**6.5 – 8.1**	**31.6 – 45.4**
III	B3	625.5 ± 6.5	27.2 ± 0.89	92.6 ± 1.3	77.6 ± 2.70	29.6 ± 1.30	32.7 ± 0.35	17.2 ± 0.11	80.1 ± 0.51	23.2 ± 0.62	6.8 ± 0.12	19.2 ± 0.29	21.6 ± 0.85	8.2 ± 0.33	36.5 ± 0.41
		576.0 – 672.0	**21.5 – 33.0**	**81.0 – 100.0**	**59.0 – 92.0**	**22.5 – 40.0**	**30.0 – 35.0**	**16.5 – 18.0**	**74.0 – 82.0**	**19.6 – 27.3**	**6.3 – 7.8**	**16.9 – 20.8**	**16.1 – 27.6**	**6.6 – 10.7**	**32.9 – 38.4**
III	B4	533.4 ± 9.1	20.7 ± 0.48	87.5 ± 1.2	86.2 ± 1.40	28.7 ± 1.40	27.2 ± 0.79	15.8 ± 0.09	80.4 ± 0.22	25.9 ± 0.45	6.1 ± 0.12	20.0 ± 0.83	19.3 ± 0.85	6.2 ± 0.11	33.8 ± 0.53
		438.0 – 620.0	**17.5 – 24.5**	**79.0 – 98.0**	**73.5 – 96.5**	**20.0 – 44.5**	**15.5 – 33.0**	**15.0 – 16.5**	**79.0 – 82.0**	**21.8 – 28.5**	**5.3 – 7.2**	**17.2 – 34.8**	**13.5 – 27.6**	**5.4 – 7.4**	**29.2 – 38.8**
III	C3	700.7 ± 12.2	24.6 ± 0.48	99.4 ± 0.9	103.1 ± 1.50	58.5 ± 5.10	33.0 ± 0.67	16.5 ± 0.07	79.7 ± 0.45	28.6 ± 0.44	7.1 ± 0.12	21.3 ± 0.49	13.9 ± 1.32	6.8 ± 0.11	42.5 ± 0.73
		568.4 – 788.9	**21.5 – 29.4**	**90.1 – 104.8**	**89.1 – 116.6**	**21.5 – 101.9**	**29.4 – 38.2**	**15.6 – 17.0**	**75.7 – 83.4**	**24.2 – 32.1**	**6.1 – 8.1**	**18.4 – 26.1**	**7.5 – 31.9**	**5.8 – 7.6**	**34.4 – 49.9**

Table 2. (Continued) Morphometric measurements and derived variables for females from isolates of *Pratylenchus* spp. described in Table 1. Statistics include mean (n = 20) ± standard deviation with range shown in bold print.

PCA Group	Isolate Code	Body length (L)	Body width	Esophagus length	Excretory pore to head end (E)	Post-uterine branch length (PUB)	Tail length	Stylet length	Vulva (%)	a	b	c	L/PUB	L/E	L/S
III	C3	629.1 ± 13.0	23.5 ± 0.48	96.4 ± 1.3	96.2 ± 1.60	48.6 ± 3.60	30.9 ± 0.73	16.4 ± 0.08	79.2 ± 0.29	26.8 ± 0.58	6.6 ± 0.17	20.4 ± 0.40	14.3 ± 1.10	6.6 ± 0.12	38.3 ± 0.75
		515.9 – 705.5	**19.6 – 29.4**	**82.0 – 105.5**	**81.3 – 108.5**	**19.5 – 97.0**	**24.5 – 36.0**	**15.5 – 17.0**	**76.0 – 81.0**	**22.3 – 31.2**	**5.5 – 8.4**	**18.2 – 25.3**	**7.3 – 27.8**	**5.7 – 7.7**	**31.3 – 42.8**
III	C3	633.8 ± 13.3	22.5 ± 0.40	98.4 ± 1.1	97.4 ± 1.80	53.7 ± 4.60	30.0 ± 0.75	16.5 ± 0.16	79.8 ± 0.20	28.2 ± 0.54	6.4 ± 0.12	21.3 ± 0.54	13.7 ± 1.46	6.5 ± 0.12	38.5 ± 0.69
		525.2 – 725.1	**17.6 – 25.4**	**90.1 – 108.7**	**82.3 – 109.7**	**20.5 – 98.0**	**20.5 – 37.3**	**14.7 – 17.6**	**77.7 – 81.0**	**23.8 – 31.9**	**5.4 – 7.4**	**15.8 – 25.6**	**6.9 – 34.6**	**5.6 – 7.8**	**34.3 – 45.0**
III	C4	748.5 ± 13.1	25.3 ± 0.38	101.3 ± 1.8	103.2 ± 1.30	62.4 ± 5.10	33.0 ± 0.52	16.7 ± 0.15	80.6 ± 0.34	29.6 ± 0.37	7.4 ± 0.14	22.8 ± 0.54	13.8 ± 1.24	7.3 ± 0.11	44.9 ± 0.66
		618.3 – 838.8	**22.5 – 28.4**	**88.2 – 114.6**	**89.1 – 111.7**	**32.3 – 99.9**	**28.4 – 37.2**	**15.7 – 17.7**	**78.3 – 83.5**	**25.2 – 33.0**	**6.3 – 8.4**	**18.6 – 28.4**	**7.0 – 24.8**	**6.5 – 8.7**	**39.4 – 48.8**
III	C5	731.3 ± 19.0	25.8 ± 0.58	103.0 ± 1.8	103.3 ± 2.20	63.4 ± 5.30	34.0 ± 0.61	16.6 ± 0.11	81.1 ± 0.29	28.4 ± 0.48	7.1 ± 0.12	21.5 ± 0.49	12.9 ± 0.96	7.1 ± 0.08	44.0 ± 1.17
		586.0 – 907.4	**21.5 – 31.3**	**91.1 – 119.5**	**87.2 – 127.4**	**31.3 – 110.7**	**29.4 – 39.2**	**15.6 – 17.7**	**77.4 – 82.9**	**25.4 – 33.1**	**6.0 – 8.0**	**17.6 – 27.2**	**6.7 – 20.6**	**6.4 – 7.7**	**33.2 – 54.3**
III	C6	720.9 ± 14.0	25.1 ± 0.68	97.3 ± 0.9	96.5 ± 1.06	41.1 ± 1.28	34.7 ± 0.82	16.7 ± 0.84	79.3 ± 0.28	29.0 ± 0.64	7.4 ± 0.11	20.9 ± 0.41	17.9 ± 0.76	7.5 ± 0.12	43.2 ± 0.75
		588.5 – 885.5	**19.5 – 32.0**	**89.0 – 107.5**	**89.0 – 109.5**	**31.0 – 51.5**	**29.0 – 42.0**	**16.0 – 17.5**	**77.0 – 81.0**	**23.3 – 33.7**	**6.2 – 8.2**	**17.4 – 23.8**	**13.1 – 27.7**	**6.4 – 8.2**	**35.7 – 50.6**
III	K6	621.7 ± 36.0	23.3 ± 2.1	90.1 ± 3.1	91.1 ± 4.3	28.8 ± 4.2	32.0 ± 2.3	16.4 ± 0.22	80.2 ± 0.93	26.8 ± 1.8	6.9 ± 0.40	19.5 ± 1.1	22.0 ± 3.6	6.8 ± 0.35	37.9 ± 2.2
		533.0 – 670.0	**17.5 – 26.0**	**86.0 – 96.0**	**85.0 – 97.0**	**21.5 – 35.0**	**28.0 – 36.0**	**16.0 – 16.5**	**79.0 – 82.0**	**23.9 – 30.5**	**6.0 – 7.5**	**17.4 – 21.4**	**17.6 – 31.2**	**6.3 – 7.5**	**32.3 – 40.6**
III	M1	550.7 ± 5.4	21.1 ± 0.22	88.9 ± 0.7	86.2 ± 1.00	28.7 ± 1.00	26.5 ± 0.48	16.1 ± 0.06	81.6 ± 0.46	26.2 ± 0.33	6.2 ± 0.09	20.9 ± 0.35	19.7 ± 0.68	6.4 ± 0.07	34.2 ± 0.37
		500.5 – 597.5	**19.5 – 23.5**	**84.0 – 96.0**	**80.0 – 96.0**	**20.5 – 41.0**	**22.5 – 31.5**	**15.5 – 16.5**	**78.0 – 86.0**	**23.8 – 29.1**	**5.4 – 6.9**	**18.3 – 24.2**	**14.2 – 25.9**	**5.9 – 7.2**	**31.2 – 37.3**
III	M2	661.8 ± 13.7	28.5 ± 0.79	98.8 ± 1.4	92.8 ± 0.94	34.3 ± 1.60	36.0 ± 0.75	16.1 ± 0.05	79.8 ± 0.28	23.3 ± 0.43	6.7 ± 0.10	18.5 ± 0.44	20.1 ± 0.97	7.1 ± 0.11	41.0 ± 0.77
		572.0 – 810.0	**22.0 – 35.0**	**86.0 – 113.5**	**85.0 – 102.5**	**22.5 – 46.0**	**31.0 – 44.0**	**16.0 – 16.5**	**77.0 – 82.0**	**19.3 – 26.8**	**5.8 – 7.6**	**15.9 – 24.5**	**14.2 – 32.2**	**6.3 – 8.6**	**35.8 – 49.1**
III	M3	646.4 ± 14.4	27.7 ± 0.64	95.7 ± 1.5	89.3 ± 1.80	37.7 ± 2.20	31.7 ± 1.10	16.2 ± 0.08	80.2 ± 0.30	23.4 ± 0.46	6.8 ± 0.08	20.6 ± 0.40	18.3 ± 1.04	7.3 ± 0.13	39.9 ± 0.90
		526.5 – 763.0	**23.5 – 33.0**	**83.0 – 107.5**	**69.0 – 103.5**	**21.5 – 58.8**	**21.5 – 40.0**	**15.5 – 17.0**	**77.8 – 83.4**	**19.7 – 27.3**	**5.9 – 7.4**	**17.9 – 24.5**	**9.9 – 26.9**	**6.4 – 9.0**	**31.9 – 47.7**
III	Y1	558.4 ± 5.7	20.9 ± 0.36	94.6 ± 0.7	88.5 ± 0.74	25.2 ± 1.00	27.2 ± 0.39	16.1 ± 0.06	80.7 ± 0.30	26.9 ± 0.43	5.9 ± 0.07	20.6 ± 0.22	22.9 ± 1.00	6.3 ± 0.05	34.6 ± 0.33
		518.0 – 608.0	**17.5 – 24.5**	**89.0 – 101.5**	**82.0 – 94.0**	**16.5 – 35.0**	**25.0 – 32.5**	**15.5 – 16.5**	**78.0 – 83.0**	**23.1 – 30.2**	**5.5 – 6.4**	**17.9 – 21.8**	**16.8 – 35.9**	**5.9 – 6.7**	**32.4 – 37.0**
III	Y2	725.0 ± 13.8	30.1 ± 0.49	97.9 ± 1.7	103.2 ± 1.30	33.4 ± 1.50	35.4 ± 0.60	16.5 ± 0.09	81.3 ± 0.29	24.2 ± 0.36	7.4 ± 0.16	20.6 ± 0.50	22.5 ± 1.10	7.0 ± 0.11	44.1 ± 0.80
		588.5 – 821.0	**27.0 – 35.0**	**87.0 – 116.5**	**89.0 – 116.5**	**20.5 – 49.0**	**30.0 – 39.0**	**15.5 – 17.0**	**79.0 – 83.0**	**19.6 – 27.0**	**6.1 – 8.9**	**17.3 – 24.8**	**15.9 – 36.4**	**5.5 – 7.9**	**35.7 – 51.3**
III	Y3	659.2 ± 14.0	24.5 ± 0.81	97.6 ± 1.2	98.5 ± 1.50	37.9 ± 2.50	33.1 ± 0.75	16.8 ± 0.08	80.7 ± 0.26	27.2 ± 0.53	6.8 ± 0.11	20.1 ± 0.54	18.3 ± 0.73	6.7 ± 0.12	39 3 ± 0.81
		565.0 – 821.0	**19.5 – 31.5**	**86.0 – 109.5**	**81.0 – 109.5**	**26.0 – 77.0**	**28.0 – 38.0**	**16.5 – 17.5**	**78.0 – 83.0**	**23.9 – 31.2**	**6.05 – 7.7**	**16.3 – 25.9**	**8.9 – 22.9**	**6.0 – 7.7**	**34.2 – 46.9**

Table 2. (Continued) Morphometric measurements and derived variables for females from isolates of *Pratylenchus* spp. described in Table 1. Statistics include mean (n = 20) ± standard deviation with range shown in bold print.

PCA Group	Isolate Code[z]	Body length (L)	Body width	Esophagus length	Excretory pore to head end (E)	Post-uterine branch length (PUB)	Tail length	Stylet length	Vulva (%)	a	b	c	L/PUB	L/E	L/S
IV	T	521.7 ± 13.1	21.6 ± 1.05	91.7 ± 2.8	75.4 ± 1.59	29.1 ± 0.89	28.5 ± 0.53	15.5 ± 0.10	80.2 ± 0.34	24.7 ± 0.97	5.8 ± 0.25	18.3 ± 0.36	18.3 ± 0.75	6.9 ± 0.17	33.7 ± 0.74
		430.0 – 615.0	**16.0 – 31.0**	**80.0 – 108.5**	**63.5 – 87.0**	**25.0 – 36.0**	**24.5 – 31.0**	**15.0 – 16.0**	**78.5 – 82.5**	**19.5 – 29.9**	**4.7 – 7.1**	**16.2 – 20.5**	**14.0 – 22.0**	**5.9 – 8.1**	**28.7 – 38.4**
V	C1	517.1 ± 5.6	21.9 ± 0.36	87.3 ± 0.9	88.2 ± 1.00	23.8 ± 1.00	25.6 ± 0.60	15.2 ± 0.10	79.4 ± 0.18	23.7 ± 0.35	5.9 ± 0.07	20.4 ± 0.46	22.4 ± 0.90	5.9 ± 0.07	34.0 ± 0.35
		461.5 – 552.5	**19.5 – 24.5**	**82.0 – 95.0**	**81.0 – 98.0**	**16.5 – 31.0**	**20.0 – 30.0**	**14.5 – 16.0**	**78.0 – 81.0**	**20.1 – 26.3**	**5.3 – 6.7**	**17.8 – 25.0**	**16.6 – 29.6**	**5.2 – 6.5**	**31.5 – 36.8**
V	C2	527.0 ± 5.8	21.2 ± 0.23	89.5 ± 1.1	87.0 ± 1.30	27.3 ± 0.84	27.3 ± 0.74	15.1 ± 0.06	78.6 ± 0.25	25.0 ± 0.40	5.9 ± 0.09	19.5 ± 0.50	19.6 ± 0.57	6.1 ± 0.09	34.9 ± 0.39
		475.0 – 576.0	**19.0 – 23.0**	**81.0 – 98.0**	**71.5 – 96.0**	**20.5 – 36.0**	**22.0 – 34.0**	**14.5 – 15.5**	**77.0 – 81.0**	**22.4 – 28.2**	**5.3 – 6.8**	**15.9 – 24.6**	**15.7 – 26.3**	**5.4 – 6.8**	**31.7 – 37.7**
V	C7	504.4 ± 26.7	21.0 ± 1.9	87.6 ± 3.8	87.6 ± 4.0	20.8 ± 2.3	26.6 ± 1.6	15.3 ± 0.3	77.9 ± 0.9	24.2 ± 2.6	5.8 ± 0.32	19.2 ± 1.2	24.9 ± 2.6	5.8 ± 0.34	33.1 ± 1.7
		472.0 – 543.0	**19.0 – 24.5**	**78.0 – 91.0**	**82.0 – 93.0**	**17.5 – 25.0**	**23.5 – 29.0**	**15.0 – 15.5**	**76.8 – 79.7**	**19.3 – 28.6**	**5.3 – 6.3**	**16.3 – 20.9**	**20.3 – 27.8**	**5.4 – 6.2**	**31.4 – 35.3**
V	K5	482.5 ± 41.0	18.9 ± 1.8	90.1 ± 3.7	83.8 ± 5.3	20.5 ± 2.4	24.6 ± 2.2	15.3 ± 0.44	78.9 ± 1.5	25.6 ± 1.5	5.4 ± 0.46	19.7 ± 1.5	23.8 ± 2.7	5.8 ± 0.39	31.6 ± 2.5
		393.5 – 560.5	**15.5 – 22.0**	**82.0 – 99.0**	**74.5 – 92.0**	**16.5 – 26.0**	**19.5 – 30.0**	**15.0 – 15.0**	**77.0 – 2.0**	**21.9 – 28.3**	**4.3 – 6.2**	**17.2 – 23.1**	**18.3 – 27.2**	**4.7 – 6.4**	**26.2 – 35.7**
VI	N1	499.9 ± 12.8	17.7 ± 0.25	91.9 ± 0.8	83.6 ± 1.10	30.0 ± 0.82	31.9 ± 0.60	15.3 ± 0.10	77.1 ± 0.22	28.2 ± 0.56	5.4 ± 0.13	15.7 ± 0.44	16.8 ± 0.58	6.0 ± 0.12	32.7 ± 0.67
		421.3 – 601.7	**16.5 – 20.0**	**86.2 – 96.0**	**78.3 – 95.0**	**23.5 – 35.2**	**27.4 – 36.2**	**14.7 – 15.8**	**76.0 – 78.6**	**23.9 – 32.8**	**4.8 – 6.5**	**12.0 – 18.6**	**12.8 – 22.3**	**5.1 – 7.2**	**27.9 – 38.3**
VI	N1	506.5 ± 6.1	20.1 ± 0.31	92.4 ± 1.1	83.9 ± 1.20	28.4 ± 1.00	29.8 ± 0.57	15.0 ± 0.10	77.5 ± 0.22	25.3 ± 0.34	5.5 ± 0.06	17.1 ± 0.28	18.2 ± 0.52	6.0 ± 0.07	33.9 ± 0.33
		458.0 – 548.5	**18.0 – 23.0**	**83.0 – 100.0**	**74.5 – 92.0**	**22.5 – 40.0**	**24.0 – 36.0**	**14.0 – 15.5**	**76.0 – 79.0**	**21.8 – 27.7**	**4.8 – 5.9**	**15.1 – 19.7**	**12.8 – 22.2**	**5.5 – 6.6**	**30.5 – 36.6**
VI	N2	538.2 ± 8.6	19.0 ± 0.34	96.9 ± 0.7	86.8 ± 1.30	29.7 ± 1.30	32.2 ± 0.50	15.0 ± 0.09	77.0 ± 0.23	28.4 ± 0.44	5.6 ± 0.09	16.7 ± 0.27	18.7 ± 0.76	6.2 ± 0.09	36.0 ± 0.56
		452.0 – 602.0	**17.0 – 22.5**	**90.0 – 103.0**	**68.0 – 95.0**	**21.5 – 42.0**	**28.5 – 37.0**	**14.0 – 15.5**	**75.0 – 79.0**	**24.4 – 31.4**	**4.9 – 6.3**	**15.2 – 19.4**	**13.1 – 25.6**	**5.6 – 7.3**	**30.1 – 40.1**
VI	N2	631.4 ± 14.1	23.6 ± 0.49	104.1 ± 1.4	97.4 ± 1.60	34.8 ± 2.10	37.4 ± 0.67	15.1 ± 0.08	77.4 ± 0.18	26.8 ± 0.51	6.1 ± 0.12	16.9 ± 0.32	19.2 ± 0.96	6.5 ± 0.11	41.7 ± 0.83
		510.5 – 744.5	**19.5 – 27.0**	**93.0 – 118.5**	**84.0 – 112.5**	**21.5 – 53.5**	**31.0 – 41.1**	**14.0 – 15.5**	**76.2 – 79.1**	**22.3 – 32.6**	**5.3 – 7.0**	**14.5 – 19.5**	**12.8 – 27.9**	**5.3 – 7.4**	**34.9 – 48.0**
VI	N3	524.7 ± 8.0	19.8 ± 0.47	95.7 ± 1.5	88.1 ± 0.88	26.9 ± 0.80	27.9 ± 0.56	14.8 ± 0.08	78.7 ± 0.32	26.7 ± 0.56	5.5 ± 0.06	18.9 ± 0.36	19.8 ± 0.67	6.0 ± 0.09	35.4 ± 0.50
		452.7 – 592.9	**17.6 – 24.5**	**78.4 – 106.0**	**79.3 – 97.0**	**21.5 – 34.3**	**25.0 – 33.3**	**14.4 – 15.8**	**77.0 – 83.0**	**21.5 – 30.4**	**5.1 – 5.9**	**15.5 – 21.4**	**14.5 – 27.6**	**5.2 – 6.7**	**31.4 – 39.3**
VII	K3	542.2 ± 44.8	23.1 ± 1.51	93.0 ± 6.7	91.7 ± 5.7	26.1 ± 3.4	24.1 ± 2.8	16.9 ± 0.42	80.5 ± 1.3	22.2 ± 0.56	5.8 ± 0.36	22.7 ± 2.1	21.0 ± 2.7	5.9 ± 0.35	32.1 ± 2.3
		447.0 – 641.5	**20.0 – 26.5**	**81.0 – 110.5**	**80.0 – 101.5**	**19.5 – 34.0**	**19.5 – 29.0**	**16.0 – 17.5**	**77.3 – 82.3**	**20.8 – 23.3**	**5.2 – 6.5**	**18.4 – 27.0**	**17.0 – 29.3**	**5.4 – 6.9**	**27.9 – 36.7**

[z]Several isolates measured on more than one occassion to reveal temporal variability caused by host (crop root vs carrot disks), physical environment, or age structure of the population.

ysis. The following key was developed as a first approach to the practical (light and electron microscopy) identification of specimens from these isolates.

KEY TO GROUPS
DERIVED FROM PCA ANALYSIS

1. Segmented face 2
   Smooth face 4
2. Pharyngeal overlap > 61µm.....Group II
   Pharyngeal overlap < 61µm 3
3. Stylet > 16.5µm
   ..... Group VII (*P. gutierrezi*)
   Stylet < 16.5µm ..... Group I
4. Tail hemispherical, subhemispherical, truncate, subdigitate (*sensu* Frederick and Tarjan, 1989) with smooth terminus, bluntly rounded, truncate, or indented 5
   Tail bluntly pointed (*sensu* Frederick and Tarjan, 1989) sometimes indented, with smooth terminus 6
5. Stylet > 15.5 µm;
   √(stylet × V) > 35 ..... Group III
   Stylet < 15.5 µm;
   √(stylet × V) < 35 ..... Group V
6. V < 79% ..... Group VI
   V > 79% ..... Group IV (*P. loosi*)

Variables in the key in addition to those used in PCA include tail shape, length of the pharyngeal overlap, and the statistic √(stylet length × V). Tail shape is a primary character used to differentiate *P. loosi* from *P. coffeae* (Inserra *et al.*, 1996). Length of the pharyngeal overlap is the only character that differs reliably between the isolate in Group II (*P. pseudocoffeae sensu* Mizukubo) and isolates in Groups I (*P. gutierrezi sensu* Golden *et al.*) and VII (*P. gutierrezi* topotype) (Inserra *et al.*, 1998). The longer stylet for nematodes in Group VII compared to those in Group I, is in agreement with measurements given by

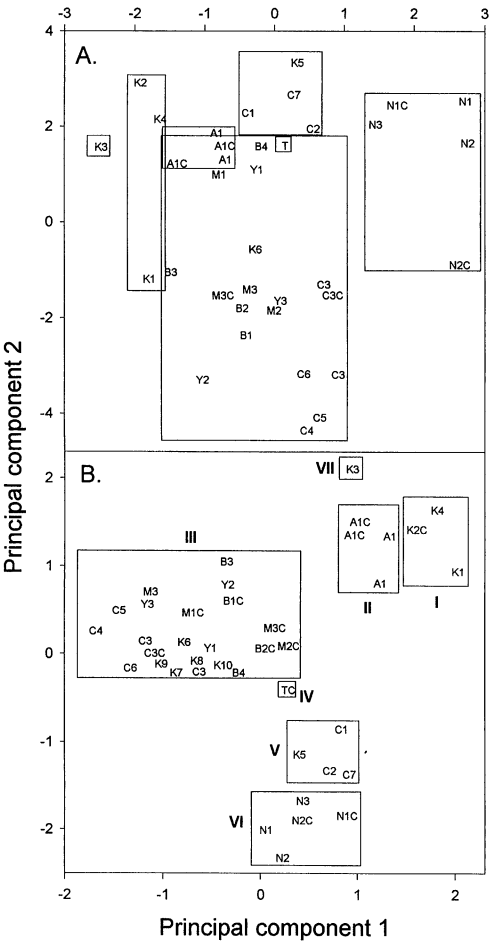

Fig. 2. Principal component analyses of lesion nematode isolates from crops and locations worldwide. Morphology of the first lip annulus and all morphometric characters given in Table 2 were analyzed in (A). Morphology of the first lip annulus, and the weakly-allometric variables V, a, and length of stylet were analyzed in (B). Isolate symbols are from Table 1. Symbols follow by C represent measurements of specimens from carrot disk culture, rather than from original host roots. Cohorts of specimens from some isolates (C3, A1, N1, N2) were measured at different times to reveal intra-population variability.

Golden *et al.* (1992). Although there is overlap in the length of the stylet among specimens in Group VII and those in Group I, the character appears to differ reliably on average, if several specimens

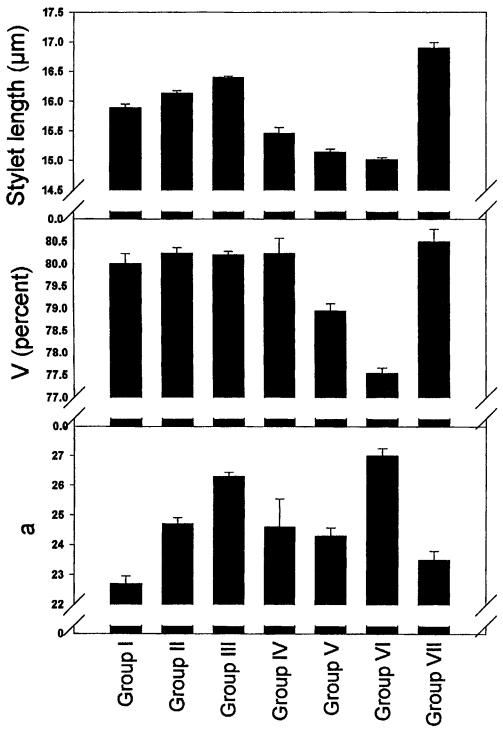

Fig. 3. Average values and standard errors of the three morphometric variables used in principal component analysis to discriminate lesion nematode isolates with-

are examined. From the means and standard deviations of stylet length for the two groups, we generated 5 000 random values (normally distributed) for each group, and then calculated 1 000 means of 5 values each. None of the mean values for the Group I isolates exceeded 16.5 µm, and fewer than 1% of the values for the Group VII isolate were smaller than 16.5 µm. We also found that stylet length and vulva position were not correlated. Consequently if one of those characters was atypical for a specimen from a given isolate, the other often was within range. Thus, the product of the 2 variables provided significantly better separation of nematodes in Groups III and V. More than one third of the 61 nematodes in Group V had stylets longer than 15.5 µm, and 6% of the 321 nematodes in Group III had stylets <15.5µm. However, only 3% of the specimens in Group III had $\sqrt{}$(stylet length × V) < 35. Although 15% of specimens in Group V had $\sqrt{}$(stylet length × V) > 35, only 3% of the means of 5 specimens exceeded that value, when 100 means were calculated from 5 specimens selected randomly.

DNA sequences of the D2/D3 expansion segment for 10 of the lesion nematode isolates, all from within PCA Group III, were closely related (Fig. 4). This clade included citrus parasitic isolates from Florida (C4) and Oman (C6), and several isolates collected from different crops in several other countries (K6, M1, M3, B1-2, and Y1-3). However, sequence identity was not absolute among these isolates in Group III. The sequences of isolates K6, C6, and B1 each varied by 1-5 nucleotides from the remaining isolates in Group III. Of the remaining lesion nematode isolates, pairs of isolates were closely related to each other and were positioned in three separate clades: K1 and K2 (PCA Group I), N1 and N2 (PCA Group VI), and C1 and C2 (PCA Group V). Isolates A1 (PCA Group II), T (PCA Group IV), and K3 (PCA Group VII) were not closely associated with any other isolates. Based on a phylogenetic species concept (Adams, 1998), where species are defined as lineages with an autapomorphy, there are 11 operational species (excluding the outgroup species) among our 19 *Pratylenchus* isolates. In 2 cases, (K1/K2; and K6/C4, B2, M1, M3, Y1, Y2, Y3) isolates with different D2/D3 sequences are considered conspecific because they are not defined by a unique shared derived character in the phylogenetic analysis.

A phenogram to explore the extent of variation among 15 of the lesion nematode isolates was generated from comparison of 227 RAPD bands from 18 decameric prim-

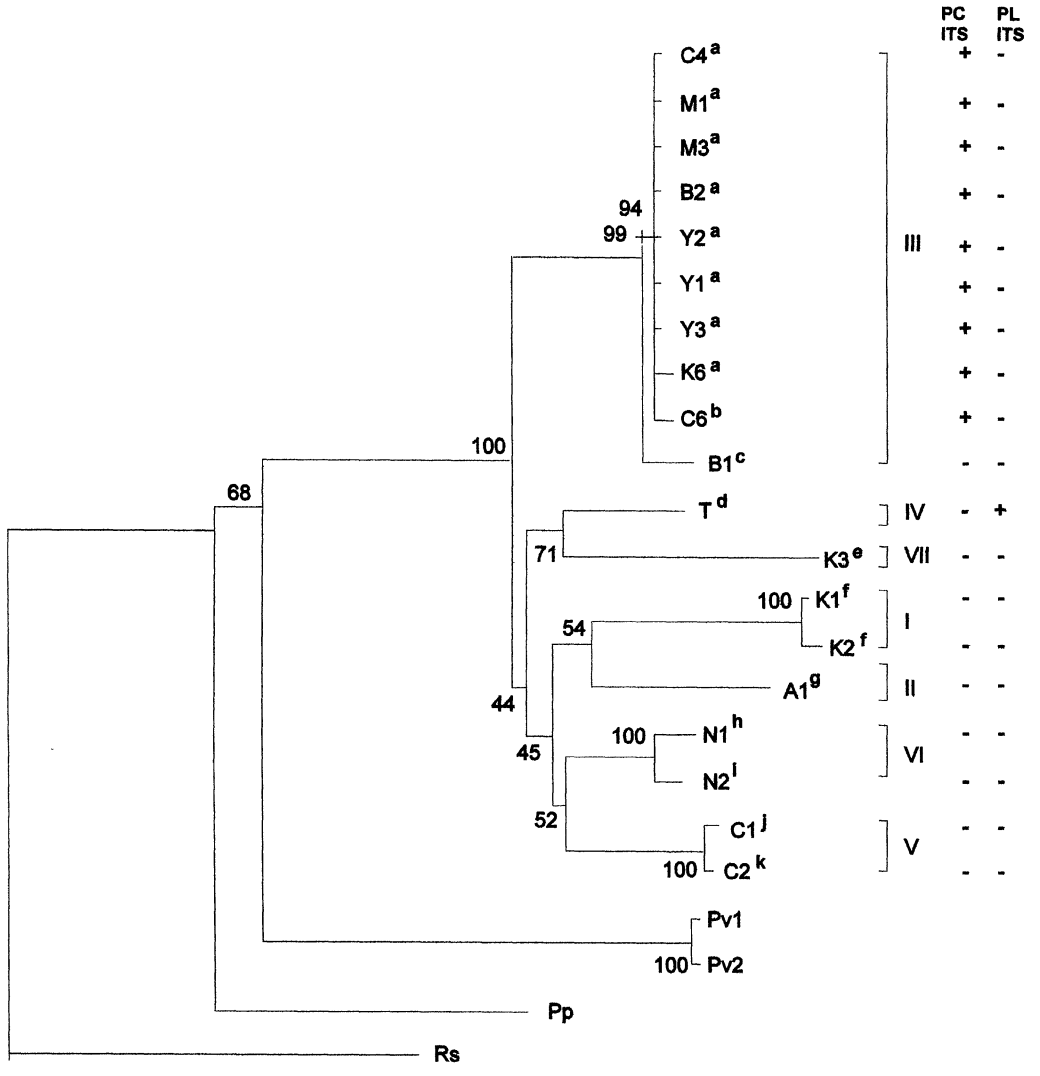

Fig. 4. Estimated phylogenetic relationships among isolates of lesion nematodes from analyses of sequence homology within the D2/D3 expansion segment of the 28S rDNA gene. For comparison, PCA grouping of the isolates is shown by brackets, and reaction to primers designed by Uehara et. al. (1998) are shown as +/−. Isolate symbols are from Table 1. Isolates with the same superscript letter are designated as phylogenetic species. Numbers (0-100) at clade brachpoints are the estimated likelihood that isolates within a clade are correctly affiliated with regard to all of the isolates available in the analysis. *Radopholus similis* (outgroup), *Pratylenchus penetrans* and *P. vulnus* are designated Rs, Pp, and Pv, respectively.

ers (Fig. 5). The relatively low level of similarity between isolates suggests that the lesion nematode genome is highly variable and therefore phylogenetic relationships cannot be based solely upon RAPD data. Some of the nematode isolates included in the RAPD analysis were grouped in reasonable accordance with the PCA and D2/D3 analyses; however, the isolates in Group III presented very different RAPD profiles.

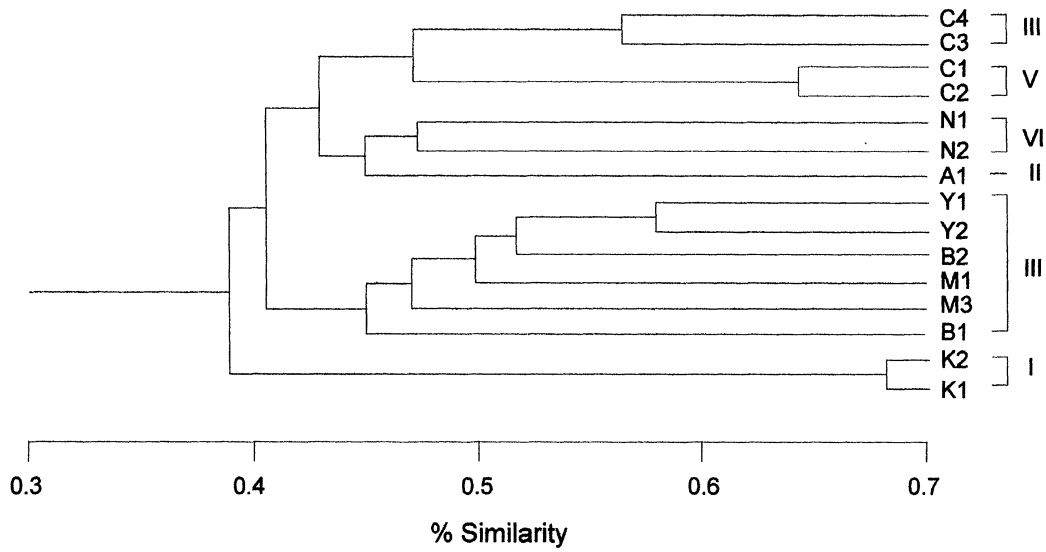

Fig. 5. Estimated phylogenetic relationships among isolates of lesion nematodes from analyses of 227 RAPD bands from 18 decameric primers. Isolate symbols are from Table 1. Scale represents proportional similarity of band patterns among isolates connected at specific branch points. PCA grouping of the isolates is shown by brackets.

Several polymorphic DNA fragments appeared to be candidates for use as genetic markers of traits of interest. Primers OP-P07 and OP-F05 amplified bands that were not present in lesion nematode isolates that did not parasitize citrus. Primers OP-P07, OP-Q13 and OP-Q14 amplified DNA bands that distinguished the citrus parasitic lesion nematodes from Florida and Brazil. Primers OP-F05 and OP-Q18 discriminated between the two citrus parasitic lesion nematodes from Florida.

Primers PC1 and PC2, designed by Uehara *et al.* (1998) to selectively amplify a 632bp portion of the rDNA of *P. coffeae*, amplified a product of appropriate size from all lesion nematodes in Group III except from isolate B1 (Figure 6-A). Primers PL1 and PL2 designed by Uehara *et al.* (1998) amplified a DNA fragment (ca. 668bp) from lesion nematode isolate T (Fig. 6-B). Primers TW81 and AB28, that amplify the ITS1 and limited portions of the 18s and 5.8s genes, demonstrated that reaction conditions were conducive to amplification (Fig. 6-C). They also demonstrated that the size of the ITS1 varied between isolates. The DNA fragments amplified from isolate T (*P. loosi*) and K3 (*P. guttierezi*) were quite distinct, whereas the size of the ITS1 were more similar among the remaining isolates. Within isolates, the size of the ITS1 DNA fragment was consistent.

The range of the mean estimates of the weakly-allometric variables for isolates from citrus in Florida was approximately half that for all of the isolates with identical sequences to those from Florida citrus (Table 3).

DISCUSSION

The results of PCA suggest the existence of at least 7 species of *Pratylenchus* among these isolates. DNA comparisons among the isolates were consistent with the PCA-derived groups and further reveal the possibility that Groups I, III, V and VI represent species complexes. Thus, while

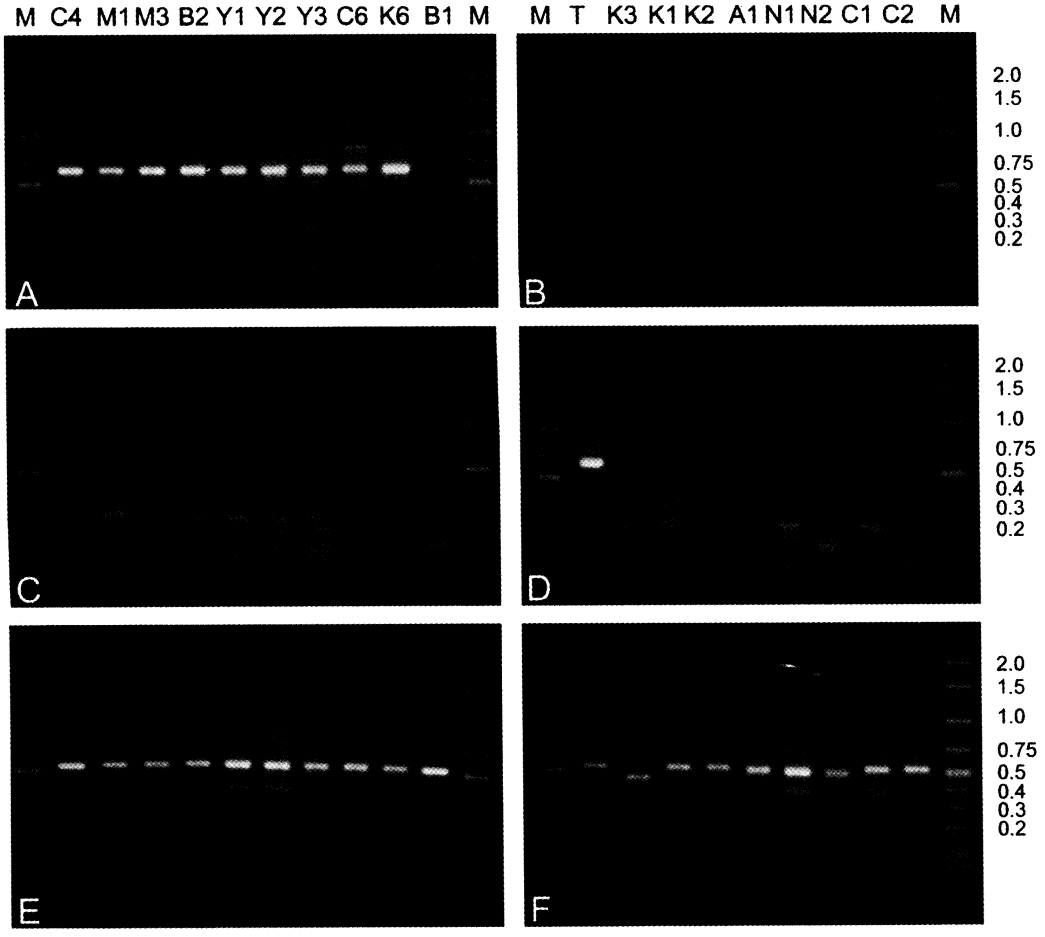

Fig. 6. Amplification of rDNA spacer regions using three sets of primers: A-B) Primers PC1 and PC2 amplified ITS rDNA from 9 lesion nematode isolates (C4, M1, M3, B2, Y1, Y2, Y3, C6, K6) previously identified as *P. coffeae*; C-D) Primers PL1 and PL2 amplified ITS rDNA from one lesion nematode isolate (T), the only *P. loosi* in the study; E-F) ITS1 amplified from all lesion nematode isolates (note differences in size of product). Molecular weights (M) are indicated in base pairs. Isolate symbols are from Table 1.

the DNA sequence of the D2/D3 expansion segment indicates a close relationship among all isolates examined in Group III, the isolates from citrus in Oman and banana in Ghana are nonetheless distinct from the others in the group. A similar degree of sequence divergence was revealed within all groups consisting of more than a single isolate. In all but 2 cases, the sequence differences indicated the existence of more than a single phylogenetic species within each group (Adams, 1998; Al-Banna *et al.*, 1997). Additional study of the genetic isolation/compatibility among the isolates within the PCA groups is needed to determine the level of congruence between the biological species and the phylogenetic species in this study.

Duncan *et al.* (1998) found body length of *P. coffeae sensu* Sher and Allen on Florida citrus to be seasonal and correlated with concentration of starch in fibrous roots of

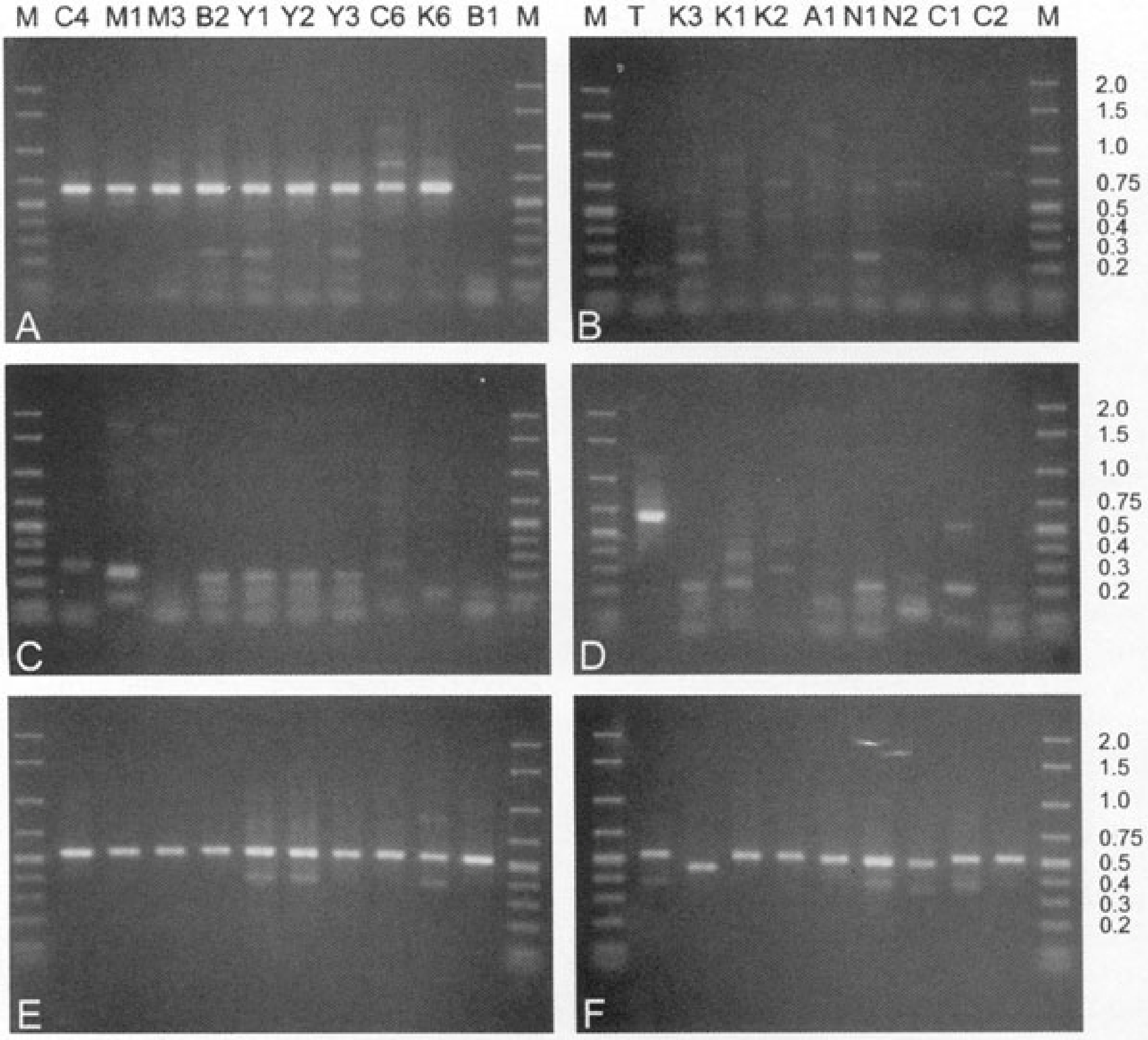

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Table 3. Percentage difference between the highest and lowest average value (n = 20) of several morphometric variables, for 3 lesion nematode islolates from Florida citrus (C3-C5), and of all isolates (Group III) with homologous D2/D3 sequence to the isolate C4 from Florida citrus.

Isolate assemblage	Body length (L)	Body width (W)	Post-uterine branch length (PUB)	Stylet length	Vulva (%)	a (L/W)	L/PUB
C3-C5	18.9	14.2	30.5	1.6	2.3	10.2	10.9
Group III	36.0	31.4	51.6	4.2	3.0	26.2	77.8

the host. In that study, the range of average body length measured monthly during 33 months was 20%. In the present study, a similar range in body length (19%) was noted among 3 isolates of *P. coffeae sensu* Sher and Allen from Florida citrus. The comparison of the range of morphometric variables between the Florida isolates from citrus and all other isolates with homologous D2/D3 sequence, provides an estimate of the intra- and inter-population variability that exists among this group. For most variables the difference was ca. two-fold. The major exception to this difference was for the derived variable L/post-uterine branch length, which is explained by the uniquely long post-uterine branch in the citrus isolates.

The isolates in Group VI appear to be undescribed species. Neither the PCA analysis nor the genetic analyses involving these nematodes from native plants in Florida support the recent proposal by Inserra *et al.* (1996) that they are *P. loosi*. The three isolates in Group VI, each from different plant species, have a more anterior vulva than the single *P. loosi* isolate in this and other studies (Loof, 1960). This difference was noted previously for two of these isolates compared to the published description of *P. loosi* (Inserra *et al.*, 1996). Based on the different rDNA sequences of the isolates from *Paspalum notatum* and *Panicum hemitomon,* mating studies are necessary to determine whether or not each of the isolates is a distinct biological species. Polymorphic DNA fragments that were identified by RAPD analysis may be useful to identify hybrid progeny of these isolates in controlled mating studies (Kaplan *et al.*, 1997).

All of the isolates in Group V were recovered from roots of either citrus or coffee collected in the Brazilian state of Sao Paulo. Although the morphology of these nematodes is consistent with that of *P. coffeae sensu* Sher and Allen, they differ from the 17 other isolates identified as *P. coffeae sensu* Sher and Allen (one isolate in Group I, the remainder in Group III) for their shorter stylets (<15.5 μm). The D2/D3 sequence of the nematodes from Sao Paulo also differs from that of the 10 isolates studied in Group III. The fact that they are genetically and morphologically unlike the other isolates of putative *P. coffeae* from worldwide sources suggests that these nematodes from Sao Paulo are likely to be an undescribed biological species or species complex.

It is noteworthy that the D2/D3 region of the single isolate of *P. loosi* (Group IV) is distinct from all isolates considered to be *P. coffeae.* Practical discrimination between *P. loosi* and *P. coffeae* is difficult, as emphasized by the PCA analysis of these nematodes, depending mainly on the shape of the tail. Given its similarity to the isolates in Group III, morphological study of additional

genetically confirmed isolates of *P. loosi* is needed to better reveal its variability compared to that of other closely related species. Nevertheless, these findings complement those of Uehara *et al.* (1998) who recently identified polymorphisms in the rDNA internal transcribed spacer region (ITS) and designed primers which differentiated populations of *P. coffeae* from those of *P. loosi* in Japan. The ITS1 region has been demonstrated to be a useful genetic marker for several nematode species including animal, plant and insect parasites (Campbell *et al.*, 1995; Ferris *et al.*, 1995; Nasmith *et al.*, 1996; Szalanski, *et al.*, 1997; Thiery and Mugniery, 1996; Vrain *et al.*, 1992; Zijlstra *et al.*, 1997). However, micro-heterogeneity (variable ITS1 seq-uence) within individual nematodes has been reported for *Belonolaimus* (Cherry *et al.*, 1997) and *Meloidogyne* (Zijlstra *et al.*, 1995, 1997). Szalanski *et al.* (1997) also identified variant ITS1 regions between *Heterodera zeae* from the United States and India and within isolates collected in India. In contrast, ITS1 sequence was consistent within and between burrowing nematode isolates collected worldwide that were morphologically similar to *R. similis* (D. Kaplan, unpublished). Micro-heterogeneity was not apparent in lesion nematodes in this study, because the size and number of amplified products was consistent within isolates. Variation in the size of the amplified ITS1 fragment suggests that differences in the genetic sequence of these DNA fragments are present and may be useful as diagnostics. Primers designed by Uehara *et al.* (1998) to amplify the ITS1 and ITS2 of rDNA from *P. loosi* did amplify a fragment, estimated to be of appropriate size, from lesion nematode isolate T, the only *P. loosi* in our collection. The primers PC1 and PC2, designed to identify *P. coffeae,* amplified a fragment estimated to be of appropriate size from all nematodes tested in Group III, except isolate B1 which was separated from the other isolates on the basis of the D2/D3 analysis. However the PC1 and PC2 primers also amplified a fragment from isolate C6, designated by D2/D3 analysis as a different species than the other isolates in Group III. The primers developed by Uehara *et al.* (1998) were based on nematode populations in Japan. The results of this study indicate that a larger sample of populations of these nematodes should be tested to determine whether these primers will serve as an effective means of identifying *P. coffeae* and *P. loosi.*

The isolates K1 and K2 in Group I from coffee in Central America and A1 in Group II from *Aster ellioti* in Florida, were recently identified as *P. gutierrezi* and *P. pseudocoffeae,* respectively (Inserra *et al.*, 1998). Lack of significant homology of the D2/D3 sequence between the isolates in Groups I and II is noteworthy, because the morphological differences among them are few. Although the isolates in the two groups do not overlap in the PCA, their separation from one another was less than that among Group III isolates with identical D2/D3 sequence. Separation of Groups I and II by PCA occurred due to differences in the variable 'a'. However, a more reliable character to separate isolates from Groups I and II is length of pharyngeal overlap, which is longer in Group II than in Group I (Inserra *et al.*, 1998). Although length of the pharyngeal overlap is considered by some authorities to be too variable for use in diagnosis, the considerable difference in average pharyngeal overlap may make it a reliable diagnostic character. A genetic comparison of type specimens of *P. pseudocoffeae* and the isolate from aster in Florida remains an important future consideration.

The likely existence of several undescribed *Pratylenchus* spp. from coffee in Central America was demonstrated

recently when three non-interbreeding isolates of *P. coffeae sensu* Sher and Allen, one with segmented and two with smooth faces, were recovered from the roots of coffee in Guatemala (Villain *et al.*, 1998). Group I isolates from coffee in Costa Rica (K1) and Guatemala (K2), initially identified as *P. gutierrezi*, differ for D2/D3. However, each is not defined by a unique derived character and the isolates are therefore a metaspecies in this analysis (Adams, 1998). Moreover, both isolates are distinct from *P. gutierrezi* (Group VII) on the basis of PCA and D2/D3 sequence. Further study of the reproductive compatibilities, and morphological variation of isolates K1-K3 is warranted.

There is currently ambiguity regarding type material of *P. coffeae*. Zimmermann (1898) did not designate a type location when he described *P. coffeae*, specifying only that it caused a decline of coffeae in eastern Java. Sher and Allen (1953) designated a neotype specimen from Bangor Java (deposited in the University of California Davis nematode collection). However, Bangor is an unknown locality and the label of the neotype was changed to Bogor (western Java) under unknown circumstances. In our study, isolates in Groups I and III are of particular interest with regard to the identity of *P. coffeae*. Group III isolates are all *P. coffeae sensu* Sher and Allen (1953) and Corbett and Clark (1983), and both groups contain isolates (K4 and K6-K10) recovered from coffee roots near the type locality. However, the divided face of the K4 isolate differs from all isolates of putative *P. coffeae* in Group III. Inserra *et al.* (1998) noted that the segmented face of the *P. coffeae* specimen (K4) from coffee in Djember, eastern Java (apparently from the collection used by Sher and Allen in their revision of the genus), casts doubt about the identities of both *P. gutierrezi* and *P. coffeae* from Florida citrus, which are separated primarily based on segmented and smooth faces, respectively (Golden *et al.*, 1992; Corbett and Clark, 1983). Nevertheless, no specimens having divided faces were detected among the isolates collected from 5 provinces of eastern Java. It is therefore likely that isolates K6-K10 represent *P. coffeae*. An ongoing survey of nematodes infecting coffee in Java (P. Baujard, pers. comm.) can test this hypothesis.

In their revision of the genus, Sher and Allen (1953) synonomized *P. musicola* (Cobb) Filipjev and *P. mahogani* (Cobb) Filipjev with *P. coffeae*, ". . . until further evidence is available." The wide morphometric variation among isolates with identical D2/D3 sequence supports a conservative approach to the classification of this group. Nevertheless, the distinct D2/D3 sequence of the isolate K6 (Java), compared to those of other isolates (C6 and B1) in Group III, indicates that the group is not phylogenetically conspecific. Study of the reproductive isolation/compatibility of these isolates can help resolve the uncertainty of their taxonomic status, and could provide an ideal system to investigate the relationship between level of D2/D3 sequence homology and biological speciation in this group of nematodes.

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