IDENTIFICATION OF SECOND-STAGE JUVENILES OF *TYLENCHULUS* SPP. ON THE BASIS OF POSTERIOR BODY MORPHOLOGY

R. N. Inserra,[1] N. Vovlas,[2] and M. Di Vito[2]

Florida Department of Agriculture & Consumer Services, Division of Plant Industry, Nematology Section, P.O. Box 147100, Gainesville, FL 32614,[1] and Istituto Nematologia Agraria, CNR, via Amendola 165/A, 70126 Bari, Italy.[2]

ABSTRACT

Inserra, R. N., N. Vovlas, and M. Di Vito. 1994. Identification of second-stage juveniles (J2) of *Tylenchulus* spp. on the basis of posterior body morphology. Nematropica 24:25–33.

Tail lengths of live J2 were measured in two, three, and eight populations of *Tylenchulus graminis*, *T. palustris* and female *T. semipenetrans*, respectively. Except for one population of *T. palustris* from Costa Rica and two populations of female *T. semipenetrans* from Italy, all other populations were from Florida, U.S.A. Ranges in tail length values were 59.5–72.5, 42.0–54.0, and 55.0–70.0 µm for *T. graminis*, *T. palustris*, and female *T. semipenetrans* J2, respectively. The canonical discriminant analysis for this character allows the separation of *T. palustris* J2 from *T. graminis* and female *T. semipenetrans* J2, which did not differ. The nearly hyaline portion of the posterior body without fat globules > 2 µm diam was measured in selected populations of *T. graminis*, *T. palustris*, and female *T. semipenetrans* J2 from Florida. The ranges in values for this class of measurements were 59.0–75.0, 24.5–59.0, and 35.0–60.0 µm for each of the three species, respectively. Another class of measurements of the nearly hyaline portion without fat globules > 3 µm diam was also taken in selected populations of the three species from Florida. The ranges in values for this class of measurements were 64.0–78.0, 42.0–59.0, and 40.0–64.0 for each of the three species, respectively. The canonical analysis for all characters, including tail length, allows the separation of *T. graminis* J2 from *T. palustris* and *T. semipenetrans* J2, which did not differ. The remaining species of the genus, *T. furcus*, has J2 with a characteristic furcate tail tip, unlike the tapered tails of other *Tylenchulus* species.

Key words: citrus nematode, morphology, regulatory nematology, systematics, *Tylenchulus furcus*, *Tylenchulus graminis*, *Tylenchulus palustris*, *Tylenchulus semipenetrans*.

RESUMEN

Inserra, R. N., N. Vovlas y M. Di Vito. 1994. Identificación de juveniles de segundo estadío (J2) de *Tylenchulus* spp. basada en la morfología de la parte posterior del cuerpo. Nematrópica 24:25–33.

Se midió el largo de la cola en juveniles vivos de segundo estadío en dos, tres y ocho poblaciones de *Tylenchulus graminis*, *T. palustris*. y *T. semipenetrans*, respectivamente. Con excepción de la población de *T. palustris* de Costa Rica y las dos poblaciones de *T. semipenetrans* de Italia, todas las otras poblaciones provienen de Florida, U.S.A. La distribución de valores de el largo de la cola obtenidos fueron, 59.5–72.5, 42.0–54.0 y 55.0–70.0 µm para *T. graminis*, *T. palustris* y *T. semipenetrans*, respectivamente. El análisis estadístico canónico discriminante de este carácter, permite, la separación de juveniles de segundo estadío de *T. palustris*, *T. graminis* y *T. semipenetrans*, los cuales no difieren entre si. La porción hialina de la parte posterior del cuerpo sin glóbulos de lípidos > de 2µm diam fue medida en poblaciones seleccionadas de *T. graminis*, *T. palustris* y *T. semipenetrans*, provenientes de Florida. La distribución de valores para esta clase de medidas fueron 59.0–75.0, 24.5–59.0 y 35.0–60.0 µm para cada una de las tres especies respectivamente. Otra serie de medidas de la misma región del cuerpo, pero sin glóbulos de lípidos de > de 3 um diam fue tomada de poblaciones seleccionadas de las tres

especies de Florida. La distribución de valores para esta serie de medidas fue de 64.0–78.0, 42.0–59.0 y 40.0–64.0 para cada una de las tres especies, respectivamente. El análisis estadístico canónico discriminante de todos los caracteres (incluyendo el largo de la cola) permite la separación de los juveniles de segundo estadío de *T. graminis* de *T. palustris* y *T. semipenetrans* los cuales no varían entre ellos. Por otra parte, T. furcus, presenta juveniles de segundo estadío con la punta de la cola bifurcada, en contraste con las colas ahusadas de las otras especies del género *Tylenchulus.*

Palabras clave: nematodo de las cítricas, morfología, legislación, sistemática, *Tylenchulus furcus, Tylenchulus graminis, Tylenchulus palustris, Tylenchulus semipenetrans.*

INTRODUCTION

The genus *Tylenchulus* Cobb, 1913 presently contains four species: *T. furcus* Van Den Berg & Spaull, 1982; *T. graminis* Inserra *et al.*, 1988; *T. palustris* Inserra *et al.*, 1988; and *T. semipenetrans* Cobb, 1913. These species are separated mainly on the basis of morphological characters of males and swollen females (3). Second-stage juveniles (J2) of *Tylenchulus* species have similar morphometric parameters and morphology (3). Their close similarity makes differentiation difficult except for *T. furcus* J2, which have a furcate tail distinctly different from the tapered tails of the other *Tylenchulus* species so far described (Fig. 1) (3,8). In Florida, the citrus nematode, *T. semipenetrans,* is a regulated species, and it can occur in association with *T. graminis* and *T. palustris.* The separation of J2 of these three *Tylenchulus* species is very important for regulatory and diagnostic purposes because J2 are found in soil samples more frequently than swollen stages and males.

A study was conducted in 1990–1992 to separate *T. graminis, T. palustris,* and *T. semipenetrans* J2 by comparing the morphological features of their posterior body, which according to their original description (3) shows more differential characters than the remaining body. Particular attention was focused on the position of rectum and anus, which are visible in 45–80% of *T. graminis* and *T. palustris* J2 (2,3) but have not been previously reported in J2 females of *T. semipenetrans* (5,9). The objectives of this study were to detect rectum and anus in female *T. semipenetrans* J2 and to separate *T. graminis, T. palustris,* and female *T. semipenetrans* J2 on the basis of the morphological features of the posterior body.

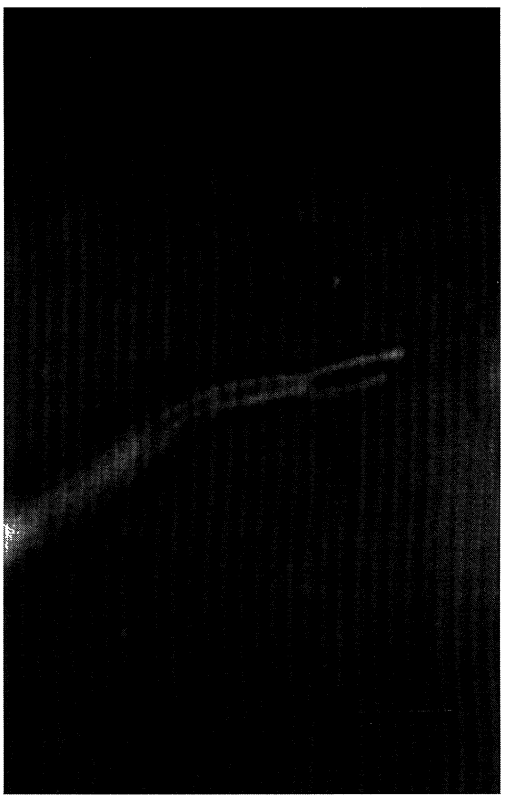

Fig. 1. Photomicrograph of the posterior body portion of *Tylenchulus furcus* J2. Note the furcate shape of tail tip. Scale bar = 5 µm. (Paratype kindly provided by Dr. E. Van Den Berg).

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Table 1. Host and location of *Tylenchulus graminis, T. palustris,* and *T. semipenetrans* populations used in this study.

Species	Population	Host	Location
T. graminis	TG1	*Andropogon virginicus* L. (broomsedge)	Alachua County, Florida
T. graminis	TG2	*Andropogon virginicus* L. (broomsedge)	Lake County, Florida
T. palustris	TP1	*Aster elliottii* T. & G. (aster)	Columbia County, Florida
T. palustris	TP2	*Borrichia arborescens* (L.) DC. (sea oxeye)	Dade County, Florida
T. palustris	TP3	*Borrichia arborescens* (L.) DC. (sea oxeye)	Dade County, Florida
T. palustris	TP4	Unknown	Jaco area, Costa Rica
T. semipenetrans	TS1	*Citrus limon* (L.) Burm. f. (rough lemon)	Polk County, Florida
T. semipenetrans	TS2	*Citrus limon* (L.) Burm. F. (rough lemon)	Polk County, Florida
T. semipenetrans	TS3	*Citrus limon* (L.) Burm. F. (rough lemon)	Highlands County, FLorida
T. semipenetrans	TS4	*Citrus limon* (L.) Burm. F. (rough lemon)	Broward County, Florida
T. semipenetrans	TS5	*Citrus limon* (L.) Burm. F. (rough lemon)	Pasco County, Florida
T. semipenetrans	TS6	*Citrus limon* (L.) Burm. F. (rough lemon)	Pasco County, Florida
T. semipenetrans	TS7	*Citrus aurantium* L. (sour orange)	Apulia region, Italy
T. semipenetrans	TS8	*Olea europaea* L. (olive tree)	Liguria region, Italy

MATERIALS AND METHODS

Populations of *T. graminis, T. palustris,* and *T. semipenetrans* were collected from different geographical areas (Table 1). Juveniles were extracted from soil by the Baermann funnel technique and the centrifugal flotation method (4), and from roots by root incubation or by high pressure water spray. Nematodes in water suspension were recovered on a 35-µm-pore sieve. *Tylenchulus semipenetrans* J2 females were separated from J2 males and used for morphological comparisons with *T. graminis* and *T. palustris* J2 (9). *Tylenchulus semipenetrans* J2 males were not included in these comparative examinations because they can be separated from *T. semipenetrans* J2 females and J2 of *T. graminis* and *T. palustris* by the presence in the posterior body of a clear, square-like area which is absent in *T. semipenetrans* J2 females and J2 of *T. graminis* and *T. palustris* (3,9). However, tail length values of *T. semipenetrans* J2 males were obtained from the TS1 population from Florida (Table 2) and compared with tail length values of J2 males reported by Van Gundy for a population from California (9). The separation of J2 females from J2 males was not possible in the populations of *T. graminis* and *T. palustris.*

Live and healthy J2 were narcotized with light heat, placed on the surface of water agar under a cover slip (1) and observed with the aid of a compound microscope under oil immersion. Specimens of the populations were examined for the presence of the rectum and anus in order to determine their tail length. To facilitate the detection of the rectum and

Table 2. Range, mean, and standard deviation (SD) of tail length of *Tylenchulus graminis* (TG) *T. palustris* (TP), and *T. semipenetrans* (TS) populations used in this study.

	Tail length values (µm)			
Nematode Population	Range	Mean	SD	n[z]
TG1	59.5–68.5	64.0	2.5	20
TG2	60.5–72.5	67.0	3.1	20
TP1	44.0–51.0	47.0	1.9	20
TP2	45.0–54.5	50.0	2.3	20
TP3	44.0–54.0	49.5	3.1	13
TP4	42.0–47.0	44.0	2.0	6
TS1	58.0–70.0	64.5	3.5	16
TS2	55.0–61.0	57.0	2.0	16
TS3	59.0–66.5	62.5	2.1	20
TS4	59.0–64.5	61.0	2.4	5
TS5	58.0–67.5	63.1	2.6	20
TS6	56.0–63.5	60.0	1.9	20
TS7	55.0–66.0	59.5	2.9	20
TS8	57.0–64.0	60.0	2.0	20

[z]n = number of specimens measured.

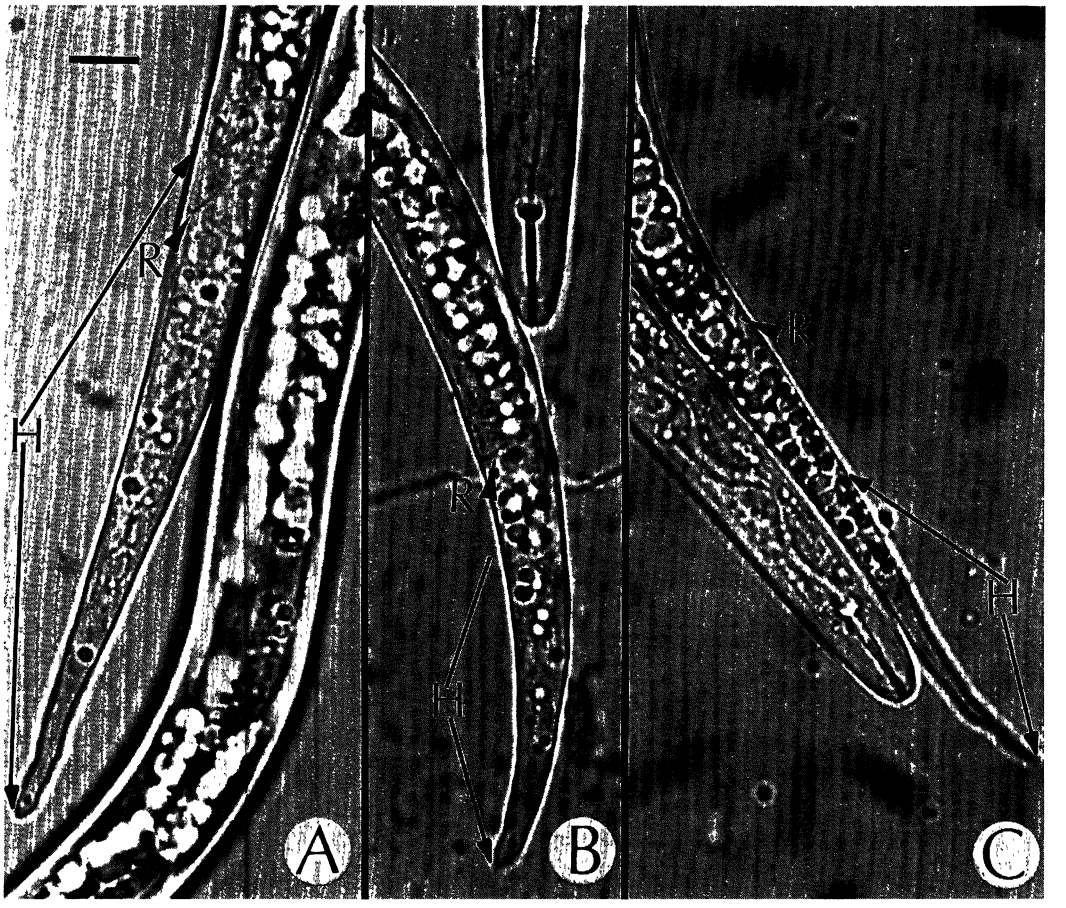
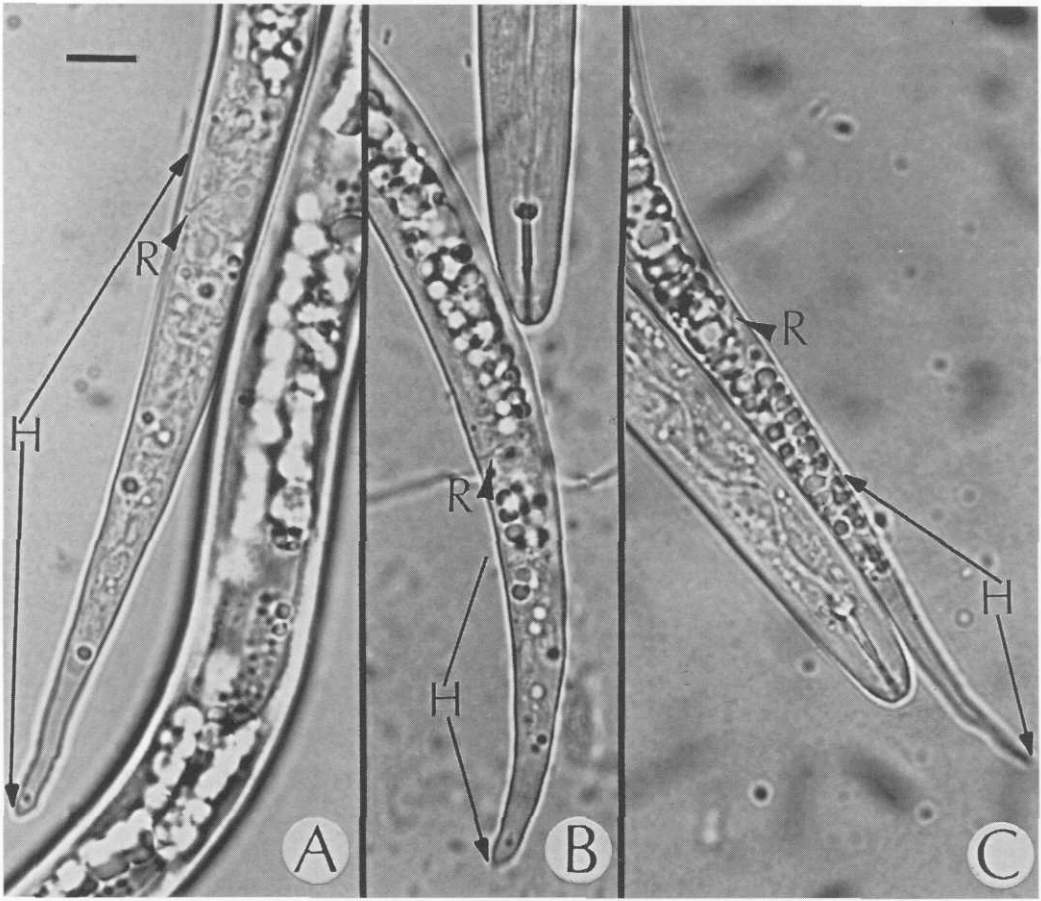


Fig. 2. Photomicrographs of the posterior body portion of *Tylenchulus* species J2 from Florida. A) Posterior and middle body of *T. graminis*. Note in the posterior body the rectum (R) and the absence in the tail of fat globules > 2 µm diam. A few large fat globules are visible in the portion of the body anterior to the tail. B) Posterior and anterior body of a coiled *T. palustris* specimen. Note in the posterior body the rectum (R) and the short tail containing cluster of fat globules > 2 µm diam and > 3 µm diam, compared to that of *T. graminis*. C) Posterior and anterior body of a coiled female *T. semipenetrans* specimen. Note in the posterior body the rectum (R) and the cluster of fat globules > 2 µm diam and > 3 µm diam occupying about 2/3 of the tail. In all figures H = nearly hyaline portion of the posterior body without fat globules > 2 µm diam. Scale bar for A, B, and C = 7.5 µm.

anus, only live specimens were used in this study. The length of the nearly hyaline portion of the posterior body without fat globules > 2 µm diam also was measured in live and healthy specimens of selected populations from Florida (TG1, TG2, TP1, TP2, TS2, TS5, TS6). The nearly hyaline portion of posterior body without fat globules > 2 µm diam for *T. graminis, T. palustris,* and female *T. semipenetrans* J2 is shown in Figs. 2 and 3. This parameter was used because, according to the original description of *T. graminis* (3), it allows the separation of this species from *T. palustris* and *T. semipenetrans.* We added another class of measurements related to the length of the nearly hyaline portion of the posterior body without fat globules > 3 µm diam to minimize the overlap of low range values observed in *T. graminis* with the



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upper range values observed in the other two species. This class of measurements encompassing those of the previous class (without fat globules > 2 µm diam) was taken in the populations TG1, TP1 and TS2. Values of tail length and the two parameters relative to the length of the nearly hyaline portion of the posterior body were subjected to the canonical discriminant analysis (6,7,10) to interpret morphological comparisons.

RESULTS

The rectum and anus were detected in live J2 of all populations of *T. graminis* and *T. palustris* and also in *T. semipenetrans* females (Figs. 2,3). These morphological features were visible only in live specimens placed in the lateral position on the water agar surface and observed under oil immersion. The anus and rectum were not clearly distinguishable in specimens mounted in ventral and dorsal positions.

In all the populations examined, *T. palustris* J2 had shorter tails than those of *T. graminis* and *T. semipenetrans* females (Table 2). Tail length values were ≤ 54 µm in *T. palustris* and ≥ 55 µm in *T. graminis* and *T. semipenetrans*. The canonical discriminant analysis for this parameter allowed the separation of the four *T. palustris* populations from those of *T. graminis* and *T. semipenetrans*, which all congregated in the same cluster (Fig. 4A).

Tail length values of male *T. semipenetrans* J2 obtained from TSI populations from Florida were 50.3 µm (47.0–53.0) (SD = 8.1; n = 13). These values were < 55 µm and similar to those derived from drawings and c ratio values of male *T. semipenetrans* J2 reported by Van Gundy in California, 51.7–57.0 µm (9). The anus and rectum in *T. semipenetrans* J2 males were located in the clear and square-like area of the posterior body.

The nearly hyaline portion of the posterior body without fat globules > 2 µm diam was longer in *T. graminis* J2 than in those of *T. palustris* and *T. semipenetrans* (Table 3) (3). Ranges in values for this class of measurements were 59.0–75.0 µm in *T. graminis* and were 24.5–59.0 and 35.0–60.0 µm in the populations of *T. palustris* and *T. semipenetrans* (Table 3), respectively, indicating slight overlap between the low range values of *T. graminis* and the upper range values of *T. palustris* and *T. semipenetrans*. Measurements of the nearly hyaline portion of the posterior body without fat globules > 3 µm diam were also greater in *T. graminis* J2 compared to those of *T. palustris* and *T. semipenetrans*. There was less overlap between the low range values of *T. graminis* (64.0– 78.0 µm) and the upper range values of *T. palustris* and *T. semipenetrans* (42.0–59.0 and 40.0–64.0 µm, respectively) (Table 3).

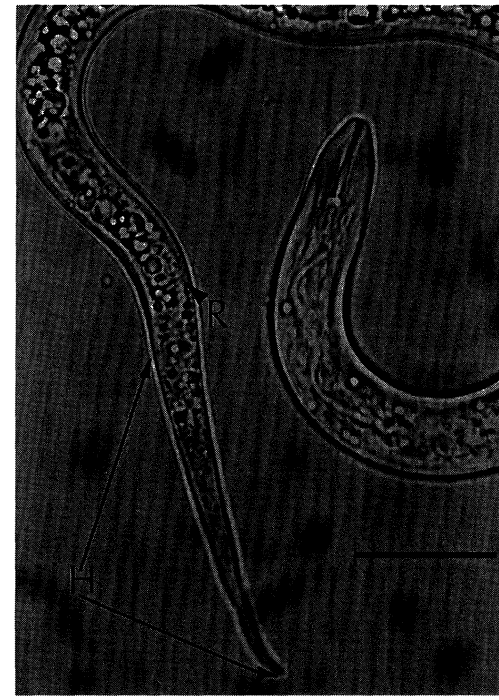
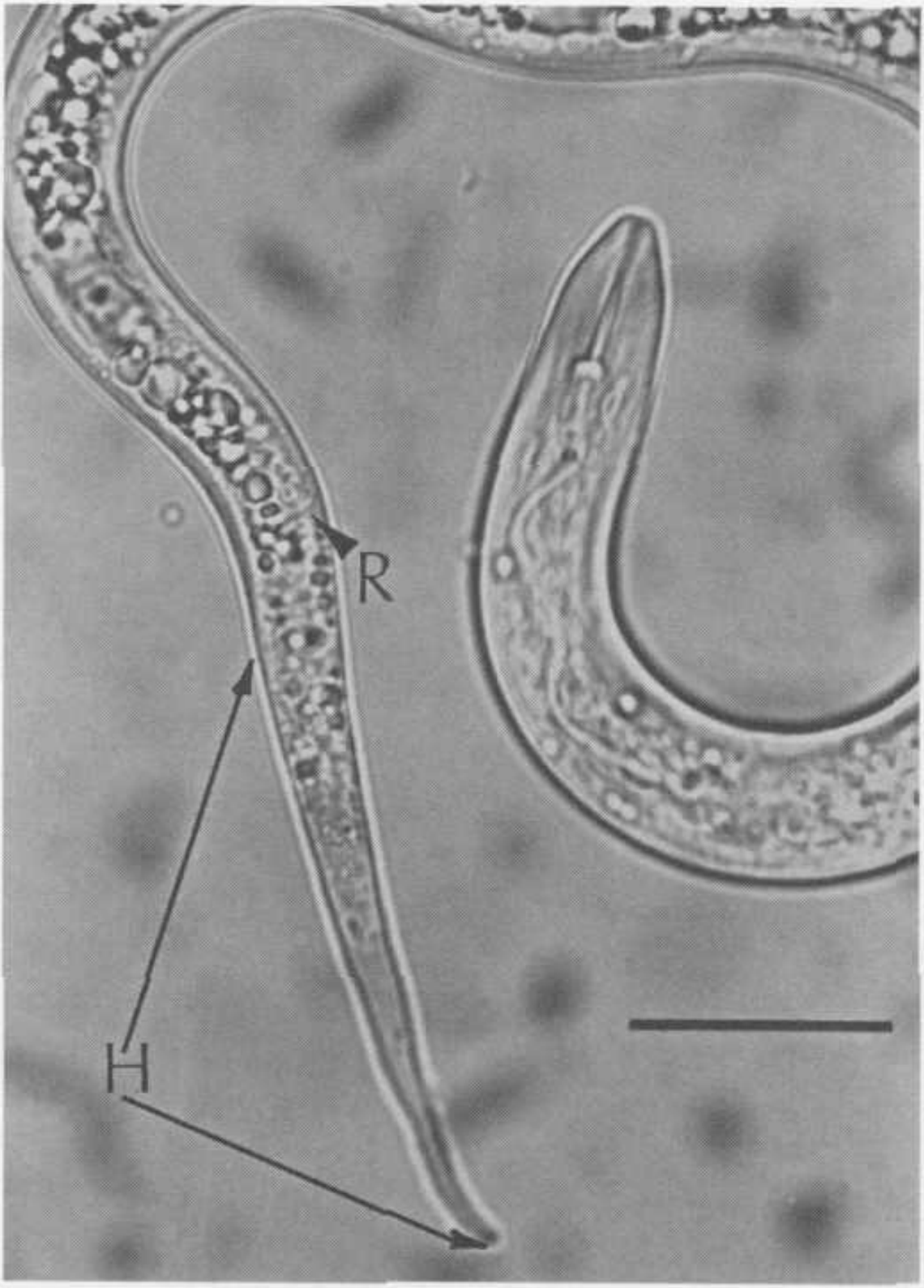


Fig. 3. Photomicrograph of female *Tylenchulus semipenetrans* J2 (citrus race) collected from an olive tree in Italy. Note the rectum (R) and the clusters of fat globules in the tail. H = nearly hyaline portion of the posterior body without fat globules > 2 µm diam. Scale bar = 20 µm.



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Fat globules > 2 µm diam and > 3 µm diam are rarely observed in the tail of *T. graminis* J2, whereas they are common in the tails of *T. palustris* and *T. semipenetrans* (Figs. 2,3).The dendrogram for these two classes of measurements and tail length separated the two populations of *T. graminis* from those of *T. palustris* and *T. semipenetrans*, which all congregated in the same cluster (Fig. 4B).

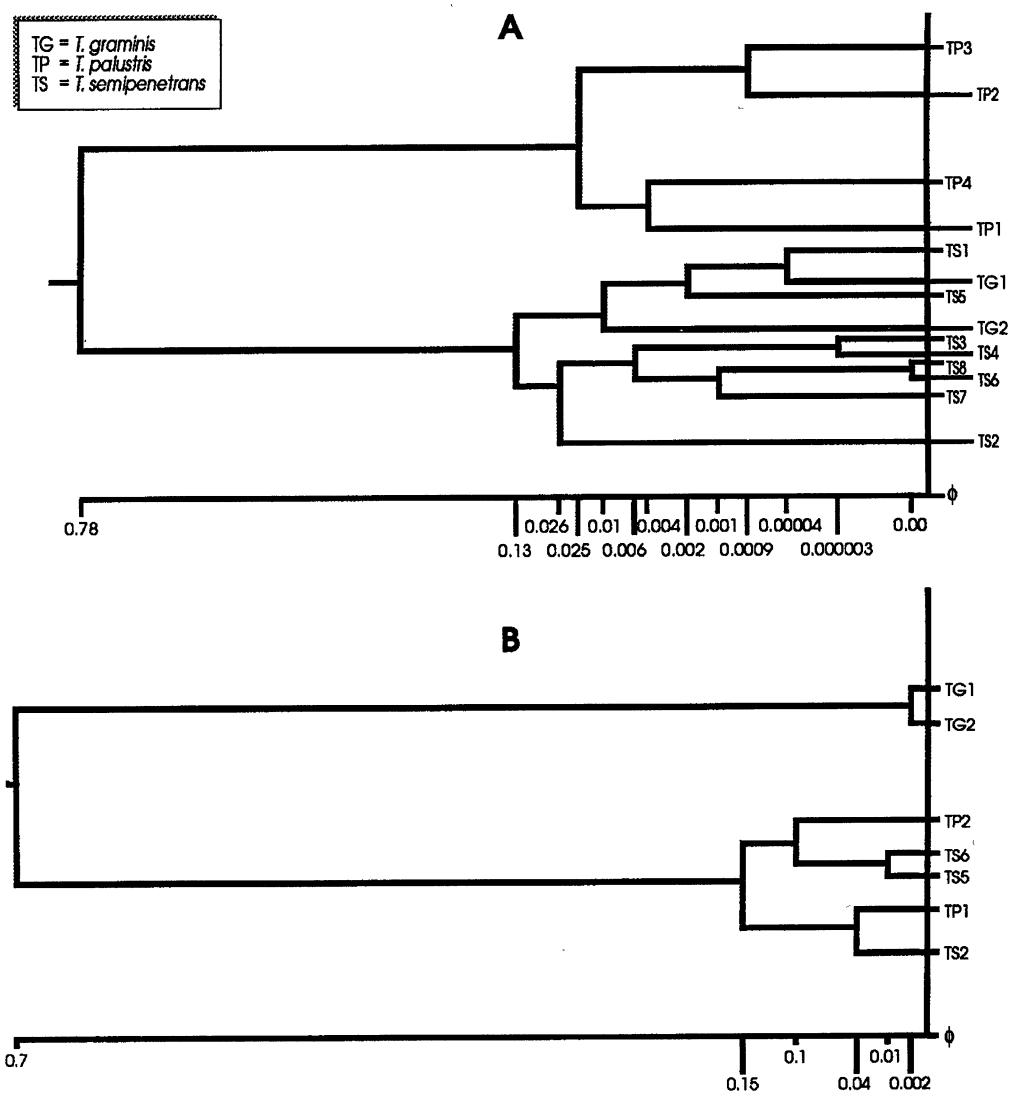

Fig. 4. Dendrograms showing the relationship among populations of *Tylenchulus graminis* (TG), *T. palustris* (TP), and *T. semipenetrans* (TS) J2 (see Table 1). A) Relationship based on tail length. Note separation of *T. palustris* populations from those of *T. graminis* and *T. semipenetrans*, which did not differ for this character and congregated in the same cluster. B) Relationship among selected populations from Florida based on the length of the posterior body without fat globules > 2 µm diam and > 3 µm diam. Note the separation of *T. graminis* population from those of *T. palustris* and *T. semipenetrans*, which did not differ for these characters and congregated in the same cluster. In both diagrams the average distances among clusters and nematode populations are reported in X and Y axes, respectively.

Table 3. Measurements of the nearly hyaline portion of the posterior body of *Tylenchulus graminis* (TG), *T. palustris* (TP), and *T. semipenetrans* (TS) populations from Florida on the basis of size of fat globule content.

Nematode population	Length of the hyaline portion without fat globules > 2 µm diam (µm)			Length of the hyaline portion without fat globules > 3 µm diam (µm)		
	Range	Mean (n = 20)[y]	SD[z]	Range	Mean (n = 20)	SD
TG1	60.0–75.0	68.0	409	64.0–78.0	70.8	4.1
TG2	59.0–74.0	69.0	4.5			
TP1	24.5–53.0	36.0	6.4	42.0–59.0	48.0	3.3
TP2	28.5–59.0	50.0	6.7			
TS2	48.0–60.0	55.0	3.5	40.0–64.0	52.3	5.3
TS5	38.0–56.0	46.0	4.6			
TS6	35.0–57.0	42.0	6.2			

[y] n = number of specimens measured.
[z] SD = standard deviation.

DISCUSSION

Second-stage juveniles of *Tylenchulus* species so far described can be distinguished as follows: *Tylenchulus furcus* J2 differ from those of all other *Tylenchulus* in having a furcate tail tip, unlike the tapered tails of the remaining *Tylenchulus* species. *Tylenchulus graminis* J2 differ from those of *T. palustris* and *T. semipenetrans* by having a longer hyaline portion of the posterior body without fat globules > 2 µm diam (59.0–75.0 versus 24.5–59.0 and 40.0–64.0 µm, respectively), as well as a longer hyaline portion of the posterior body without fat granules > 3 µm diam. *Tylenchulus graminis* J2 also have longer tails than those of *T. palustris* (59.5–72.5 versus 42.0–54.0 µm). *Tylenchulus palustris* J2 differ from those of *T. graminis* and *T. semipenetrans* by having shorter tails (42.0–54.0 versus 59.5–72.5 and 55.0–70.0 µm). *Tylenchulus semipenetrans* J2 differ from those of *T. graminis* in having a shorter hyaline portion of the posterior body containing fat globules > 2 µm diam and 3 µm diam (35.0–60.0 and 40.0–64.0 versus 59.0–75.0 and 64.0–78.0 µm). They differ from those of *T. palustris* in having longer tails (55.0–70.0 versus 42.0–54.0 µm).

Populations of *T. graminis* with shorter tails than those of the two populations listed in Table 2 have been detected in northern Florida. In one of these populations, tail-length values ranged 56.0–62.0 µm (n = 10). Tail length is a better defined character than length of the hyaline portion of the posterior body. The lack of overlap in the range of tail-length values between the populations of *T. palustris* and those of *T. graminis* and *T. semipenetrans* facilitates the separation of *T. palustris* J2 from those of the other two species, which do not differ for this character.

About 30% of *T. semipenetrans* J2 are males in the populations of this species; occasionally J2 males can represent 50% of *T. semipenetrans* J2. This male portion of *T. semipenetrans* J2 can be separated from *T. graminis*, *T. palustris*, and female *T. semipenetrans* J2 by the presence in the posterior body of a clear, square-like area, which is absent in the J2 of *T. graminis*, *T. palustris*, and *T. semipenetrans* females (3,9). How-

ever, *T. semipenetrans* J2 males cannot be separated from *T. palustris* J2 on the basis of tail length because *T. semipenetrans* J2 males have tail length values < 55 µm as do *T. palustris* J2.

The occurrence of overlap between the low end of the range of length values for the hyaline portion of the posterior body of *T. graminis* and the upper end of the range of corresponding values for *T. semipenetrans* (Table 3) may complicate the differentiation process of the J2 of these two species in mixed populations or in small collections of specimens. In this case, additional material and information about the nematode hosts (citrus or grasses) present at the collection site are essential for the correct identification of the J2 of the two species.

It is desirable that at least 10 specimens of each *Tylenchulus* species be available to separate the three species. It is also essential that the specimens are alive because in dead or fixed specimens the rectum and anus are not distinguishable. Long exposure of J2 to sugar solution during sugar flotation can alter size and arrangement of body fat globules and the length of the nearly hyaline portion of the posterior body. Baermann funnel and root incubation techniques are preferable to sugar flotation for these morphological studies. The rectum and anus are easier to detect in living starved specimens due to depletion of the fat globules obscuring the rectum and anus. However, fat globule depletion can alter the length values of the nearly hyaline portion of the posterior body. In spite of the limitations in the use of this separation method, it provides a reliable means to identify these *Tylenchulus* species in the J2 stage.

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