

# Parasitic Habits of *Trophotylenchulus floridensis* (Tylenchulidae) and its Taxonomic Relationship to *Tylenchulus semipenetrans* and Allied Species<sup>1</sup>

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**Abstract:** Parasitism by *Trophotylenchulus floridensis* Raski, 1957 was studied on roots of sand pine (*Pinus clausa* [Chapm.] Vasey). Different life stages of the nematode were observed to be covered by dark, spherical, brittle, capsule-like structures which protruded from the root surface. The mature capsule enveloped a single sedentary female with a gelatinous matrix containing an average of 76 (44–117) eggs. The capsule was composed of a single layer of cells which appeared to be of plant origin. The anterior end of the nematode was embedded superficially in the root tissue where it created a feeding site comprised of a small number of discrete stelar parenchyma cells with dense cytoplasm and enlarged nuclei and nucleoli. The nematode also infected slash pine, *Pinus elliottii*, loblolly pine, *P. taeda*, red oak, *Quercus falcata*, post oak, *Q. stellata*, and sweet gum, *Liquidambar styraciflua*, in four different locations in central Florida. The taxonomic relationship between *Tr. floridensis* and *Tylenchulus* spp. is discussed. Based on differences in the tail and lip regions, position of the excretory pore, type of obesity and especially in the mode of plant parasitism, the genus *Trophotylenchulus* Raski, 1957 is upheld, and the transfer of *Tylenchulus clavicaudatus* Colbran, 1966, *Ty. mangeloti* Luc, 1957, and *Ty. obscurus* Colbran, 1961 to *Trophotylenchulus* is proposed. **Key words:** pine, citrus nematode, histopathology, taxonomy.

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Since its description more than 26 years ago, *Trophotylenchulus floridensis* Raski, 1957 has received little attention. Maggenti (9) compared the excretory system of this species to that of *Tylenchulus semipenetrans* Cobb, 1913 and concluded that the two nematodes were congeneric. Within Florida, the nematode has been seen occasionally in routine survey samples (R. P. Esser, personal communication), but no

further investigations on its biology have been conducted. To date, the only population of *Tr. floridensis* described outside of Florida is from Kerala, India, where it was recently reported to parasitize cultivated black pepper, *Piper nigrum* L. (11). However, a number of species closely resembling *Tr. floridensis* have been described from different parts of the world, and their taxonomic relationships to *Ty. semipenetrans* and *Tr. floridensis* have aroused interest, and some contention (13).

Our investigations focused primarily on the mode of parasitism of *Tr. floridensis* in its natural habitat; however, the question of its taxonomic relationship to *Ty. semipenetrans* and allied species is also considered.

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## MATERIALS AND METHODS

A number of forest tree species from undisturbed field sites in central Florida were sampled for *Tr. floridensis* parasitism. Unless otherwise stated, all the following reports refer to studies made on sand pine (*Pinus clausa* [Chapm.] Vasey) roots, which is a previously unrecorded host of *Tr. floridensis*.

*Trophotylenchulus floridensis*-infected roots of oak and pine were periodically collected at Sanlando Park, Altamonte Springs, Florida. Living and fixed (10% alcoholic formalin) roots and nematodes were observed with dissecting and compound light microscopes.

Sections (12  $\mu$ m thick) of infected root segments were made from tissue fixed in 10% alcoholic formalin, dehydrated through a tert-butyl alcohol series, and em-

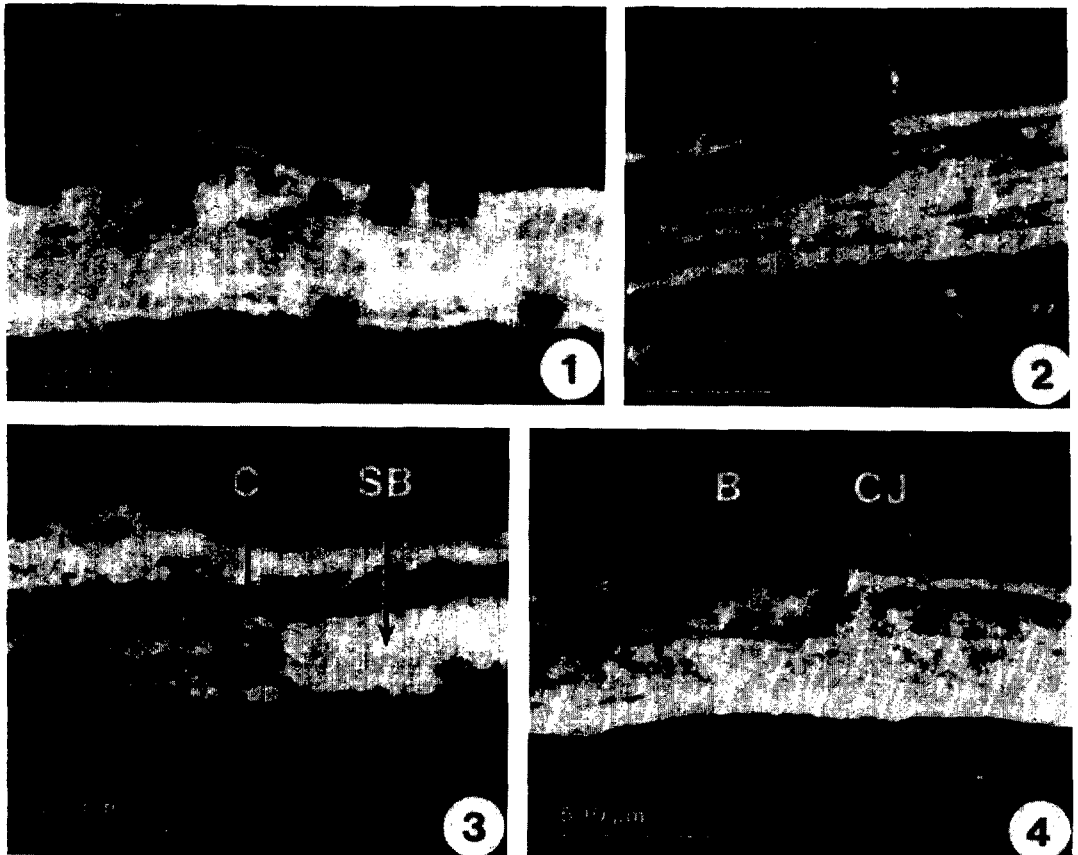
bedded in paraffin at 59 C. Sections were stained with safranin fast green, examined, and photographed with a photomicroscope.

For scanning electron microscope (SEM) observations, infected root segments were fixed in 3.5% glutaraldehyde, postfixed in 1% osmium tetroxide, critical point dried, mounted on stubs, and coated with carbon. The specimens were viewed with a JEOL JSM-35 scanning electron microscope.

Specimens of *Ty. semipenetrans* used for comparative studies were obtained from a greenhouse culture on rough lemon (*Citrus limon* [L.] Burm. f.) roots.

## RESULTS AND DISCUSSION

*Mode of parasitism:* An outstanding feature of the parasitic habit of *Tr. floridensis* is the round, brittle, capsule-like structure which always covers the nematode externally (Figs. 1-4). Capsule dimensions



Figs. 1-4. Encapsulated *Trophotylenchulus floridensis* on roots of forest trees. 1) Capsules on thin woody root of sand pine, *Pinus clausa*. 2) Detail of single capsule on sand pine root. 3) Capsule (C) in area with sloughed bark (SB) on thick woody root of post oak, *Quercus stellata*. 4) Capsules around a meristem with bud-like appearance (B) of post oak root. C.J. indicates a capsule from which a motile J-2 was dissected.

were dependent on nematode age (Fig. 4). Capsule size increased, sometimes three or more times, from an immature to an adult female with an egg-filled matrix. Small capsules covered single motile J-2s, and in one case even a single adult male was detected within a small capsule. Capsules were typically brown to dark brown, sometimes black. Empty capsules were always black and were often attached to roots.

Nematode capsules were observed on roots of different sizes—thin young roots (Figs. 1–2) and thicker older ones (Fig. 3)—but infected roots were generally woody, often with little or no cortex or bark. Capsules seemed to be particularly concentrated near and around budding root primordia, hereafter referred to as buds (Fig. 4).

Large, mature intact capsules were easily separated from roots. The mature capsule contained a single adult female (often intact, but sometimes with the head broken off), a mass of colorless gelatinous matrix, eggs, motile juveniles, and adult males (Figs. 5–10). Dislodged mature capsules ( $n = 20$ ), contained an average of 76 (range = 44–117) total offspring per capsule/female, of which 62% were in the form of intact eggs and 38% were vermiform stages. Of the latter, 61% were juveniles and 39% were adult males. This reproductive pattern is similar to that of *Ty. semipenetans*. Appearance, biometric dimensions, and morphology of all the nematode stages generally agreed with the descriptions and figures of Raski (12).

**Origin and formation of capsule:** Raski (12) reported that females of *Tr. floridensis* occur “individually in small darkened galls composed of host root material apparently two cells or more in thickness.” Subsequent descriptions of new species closely resembling *Tr. floridensis*, have interpreted the nature of the capsule differently. Luc (8), in describing *Ty. manganoti* from Ivory Coast, reported that the sedentary adult female was surrounded by a brown, smooth, hard “kind of shell (‘matrix’).” Colbran de-

scribed *Ty. obscurus* (3) and *Ty. clavicaudatus* (4) from Australia as being embedded under a cover of “small, dark, brittle scales,” which he believed to be a hardened exudate produced by the mature female. Similar “hard, dark scale” covers, thought to be produced by the adult female in the form of a structural hardening of the matrix, were more recently reported by Samsoen and Ali (13) in populations identified as *Ty. obscurus* from Kenya and Cameroon. Due to its significance in the taxonomy of the Tylenchulidae (4, 13), the structure and origin of this scale, or, as we prefer to term it, capsule, warrants further elucidation.

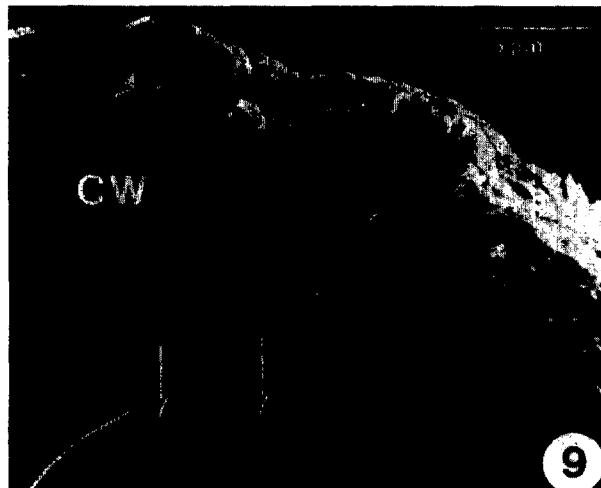
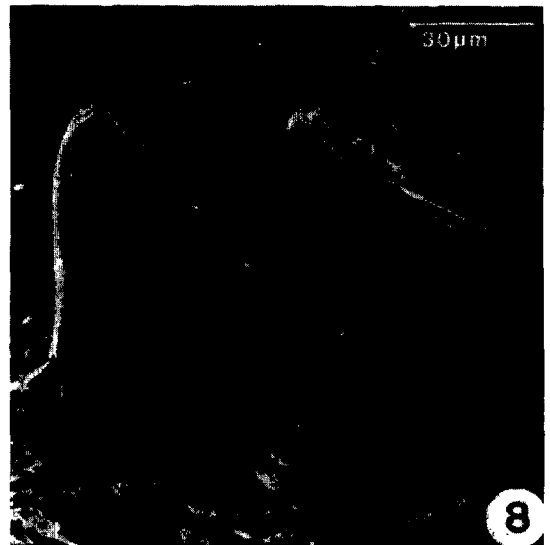
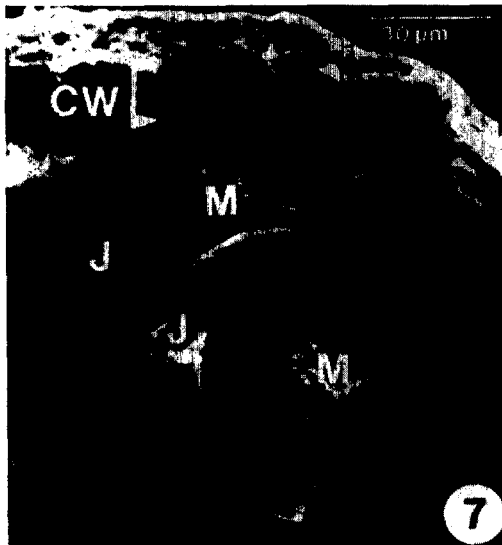
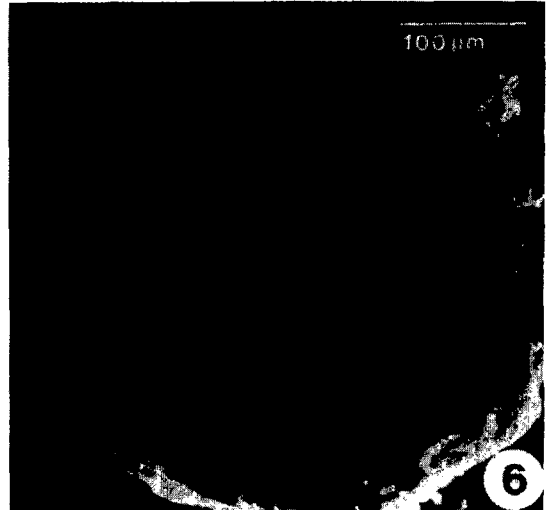
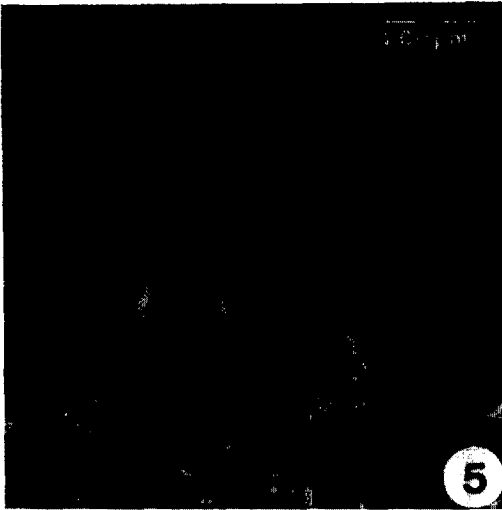
From our observations it appears that the capsule *Tr. floridensis* was not formed by a hardening of the matrix, since it surrounded immature life stages (juveniles, young females) which do not produce a matrix and even a male. Moreover, despite its external, somewhat amorphous appearance (Figs. 5–10), in cross-section it consistently appeared as a single layer of amber colored cells which were often contiguous with an adjacent similarly stained layer within the plant tissue (Figs. 11–13). Furthermore, in young roots with intact cortical tissues, capsules containing young females were sometimes observed to be totally embedded in the cortex (Fig. 14). Therefore, it would appear that the capsule in *Tr. floridensis* is of plant origin.

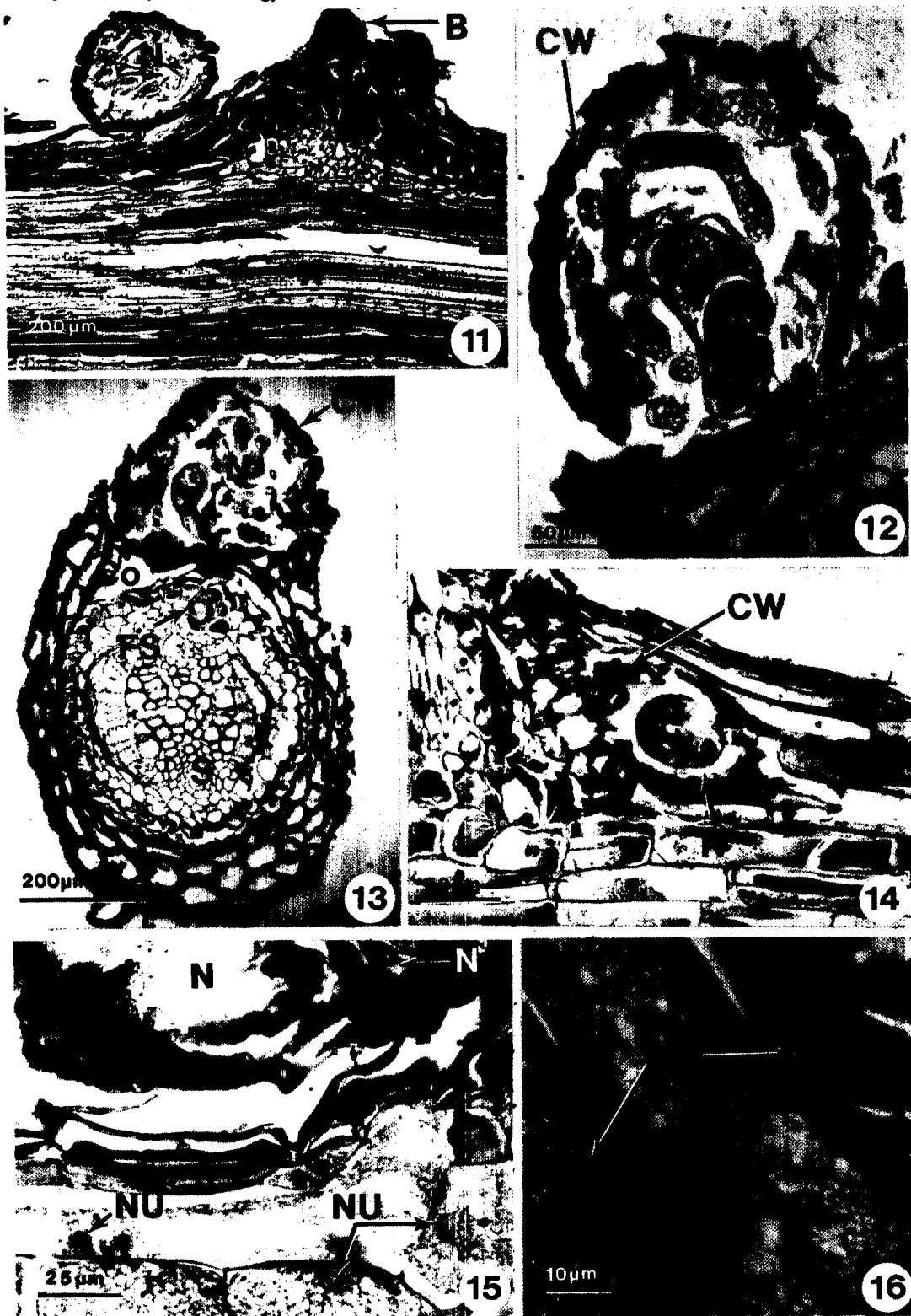
The capsule may arise when the infective J-2 stage initiates feeding in the stelar zone of the root. Feeding might induce a response in the formative plant tissue (pericycle?) which envelopes the invading nematode. With time, nematode development, and plant growth, the typically shaped capsule would then be formed. This process develops within the cortex and the mature complete capsules become exposed only after the cortex sloughs off.

**Histopathology:** While the encapsulated nematode is located outside of the stele, it



Figs. 5–10. SEM of capsule and contents of *Trophotylenchulus floridensis* on *Pinus clausa*. 5) Capsules on root surface. 6) Teased capsule with view of contents. 7) Interior of capsule showing thick wall (CW), juveniles (J), and matrix (M). 8) Interior of capsule showing egg (E), dense matrix (M), and juvenile (J). 9) Detail of capsule wall (CW) and partial view of juvenile showing four incisures in lateral field (arrows), in contrast to only two incisures reported for this species by Maggenti (9). 10) View of empty capsule with contents removed.





Figs. 11-16. Cross sections of encapsulated *Trophotylenchulus floridensis* and roots of *Pinus clausa*. 11) Nematodes (N) in capsule on thick woody root near a meristem with a bud-like appearance (B) (X = xylem). 12) Detail of differentially stained nematode (N) and capsule wall (CW) attached to root. 13) Encapsulated nematode (N) in cortex (Co) of young root with feeding site (FS) formed in stelar (S) parenchyma region. Note apparent contiguity of capsule wall layer with cortical tissue layer of root. 14, 15) Young nematode (N) within capsule wall and feeding site comprised of discrete stelar parenchyma cells with enlarged nuclei (NU). 16) Detail of cells within feeding site showing granulated cytoplasm (G) and nuclear hypertrophy (NU).

parasitizes tissues within the stele (Fig. 13). Penetration of its anterior end is only superficial (1-4 plant cell layers), and a small feeding site, composed of 3-6 stelar parenchyma cells in cross-section, is established (Fig. 15). The affected "nurse" cells in the feeding site remain discrete, without conspicuous cell wall thickening; contain dense, granulated cytoplasm; and exhibit marked nuclear and nucleolar hypertrophy (Fig. 16). Hence, at the cellular level, *Tr. floridensis* feeding induces a host response similar to that of *Ty. semipenetrans* (2), differing from the latter primarily in that it is distinctly a stelar, rather than a cortical, parasite.

**Host range and distribution:** In addition to sand pine, *Tr. floridensis* was found infecting roots of slash pine, loblolly pine, red oak, post oak, and sweet gum. All except red oak (its type host) constitute new host records for this nematode (Table 1). On all these hosts the nematode was observed exclusively in the encapsulated condition and produced the typical stelar feeding site, as described on sand pine.

An updated summarized host list of *Tr. floridensis* is presented in Table 1. Black pepper (*Piper nigrum*) was recently reported as a host (11) and it is included in the list, but we feel that the nematode's identification, at the specific level, requires confirmation.

A survey of nematode occurrence was not undertaken; however, *Tr. floridensis*-infected pine and/or oak roots were recovered in four out of seven undisturbed natural sites sampled within central Florida (Lake Alfred, Altamonte Springs, Clermont,

and Gainesville areas). It seems likely that this nematode is fairly common throughout the state but may be overlooked because of its unusual parasitic life habits (i.e., the encapsulated state and occurrence on woody roots). Indeed, in searching for *Tr. floridensis* at the outset of our study, we failed to recognize populations on roots subsequently found to be infected.

**Taxonomic relationship to *Ty. semipenetrans* and allied species:** Raski (12) distinguished between the genera *Tylenchulus* and *Trophotylenchulus* on the basis of differences in the lip region (strongly developed submedian lobes or "circumoral elevation" in *Trophotylenchulus*) and the position of the excretory pore (more anterior in *Trophotylenchulus*). However, Allen (1), Maggenti (9), and Geraert (5), while acknowledging these differences, did not consider them adequate for generic differentiation. On the other hand, these criteria were acceptable to Thorne (14), Goodey (7), and Mai and Lyon (10), who kept the two genera separate. Golden (6) also recognized *Trophotylenchulus*, which he separated from *Tylenchulus* because of the anterior position of the excretory pore and the posterior position of the orifice of its dorsal esophageal gland (DEG). Recently, Samsoen and Ali (13) identified some additional differences between the genera, but did not consider the differential position of the DEG outlet as valid and rejected generic status for *Trophotylenchulus*. Nevertheless, Mohandas and Ramana (11) considered the genus valid.

The major differences which separate *Ty. semipenetrans* from *Tr. floridensis* are:

Table 1. Host list of *Trophotylenchulus floridensis*.

Common name	Scientific name	Reference
Persimmon	<i>Diospyros</i> sp.	(12)
Sweetgum	<i>Liquidambar styraciflua</i> L.	n.r.*
Magnolia	<i>Magnolia</i> sp.	(12)
Sand pine	<i>Pinus clausa</i> (Chapm.) Vasey	n.r.
Slash pine	<i>P. elliotii</i> Engelm.	n.r.
Loblolly pine	<i>P. taeda</i> L.	n.r.
Black pepper	<i>Piper nigrum</i> L.†	(11)
Red oak	<i>Quercus falcata</i> Michx.‡	(12)
Post oak	<i>Q. stellata</i> Wang.	n.r.

\*n.r. = new record.

†Population from Kerala, India.

‡Type host.

- *Position of the excretory pore (PE)*: As emphasized by earlier workers and illustrated in Figs. 17–22, this difference is prominent and consistent (PE = 80–90% in *Ty. semipenetrans*; 45–55% in *Tr. floridensis*). A possible difference in function between the excretory pores of the two species exists; specimens of *Ty. semipenetrans* whose excretory pore has been shown to produce the matrix (9) invariably had globules of excretion around the pore opening, whereas that of *Tr. floridensis* did not (Fig. 19–22).
- *Obesity*: As discussed at length by Samsoen and Ali (13), after maturation the two species differ markedly in their mode of obesity; in contrast to *Ty. semipenetrans* (Figs. 17–18), adult females of *Tr. floridensis* consistently appear coiled.
- *Tail length and shape*: The tail region (postvulval curvature) of *Tr. floridensis* is coiled, compared to the outstretched tail end of *Ty. semipenetrans*, and is two to four times the length of the tail region of *Ty. semipenetrans* (Figs. 23–24).
- *Anus*: Present, albeit obscure, in *Tr. floridensis* (9); absent in *Ty. semipenetrans*.
- *Lip region morphology*: All stages of *Tr. floridensis* bear a pronounced elevated, almost buttonlike, head cap formed by the fusion of the lips, which is well set off from the rest of the head region (Fig. 25). This is lacking in *Ty. semipenetrans* which has a low, non-protruding head cap (Fig. 26). This feature, also variably called a circumoral elevation (12) and prominent submedian lobes (13), has been recognized by earlier workers as a major differentiating character between the two species at the generic level (10).
- *Outstanding differences in mode of parasitism*:
  - (a) Exposed body protruding from root in *Ty. semipenetrans*; encapsulated condition in *Tr. floridensis*.
  - (b) Fifty percent penetration of body in host tissue by *Ty. semipenetrans*; superficial (less than 10%) penetration by *Tr. floridensis*. (Fig. 17, 18).

- (c) *Ty. semipenetrans*, cortical feeder; *Tr. floridensis*, stelar feeder.

We consider the characteristics listed above to be sound, consistent, and of valid diagnostic value at the generic level. We particularly stress the importance of the biological differences between the two genera (encapsulation and feeding habits). Hence, we propose the genus *Trophotylenchulus* Raski, 1957 be retained.

From information available in the literature, it appears that three other species of *Tylenchulus*—*Ty. clavicaudatus*, *Ty. mangeloti*, and *Ty. obscurus*—share most, and perhaps all, of the described characters of *Tr. floridensis* (13). Hence, Goodey (7) transferred *Ty. mangeloti* to the genus *Trophotylenchulus*. Fixed encapsulated specimens of *Ty. mangeloti* on roots of its type host, *Dorsteria embergia* G. Mangelot, were made available to us by Dr. M. Luc, M. M. H. N., Paris, and were found to be structurally similar to capsules of *Tr. floridensis*. All these forms were described from natural vegetation in Africa, Australia, and North America, and the fact that they exist in such varied habitats alongside populations of *Ty. semipenetrans*, from which they can be readily separated, would further corroborate the validity and consistency of the differential characteristics listed above. Clearly, then, these four species belong in the same genus, and are here considered to be congeneric. The following classification of the genus, *Trophotylenchulus*, is proposed:

Genus:

*Trophotylenchulus* Raski, 1957

Type species:

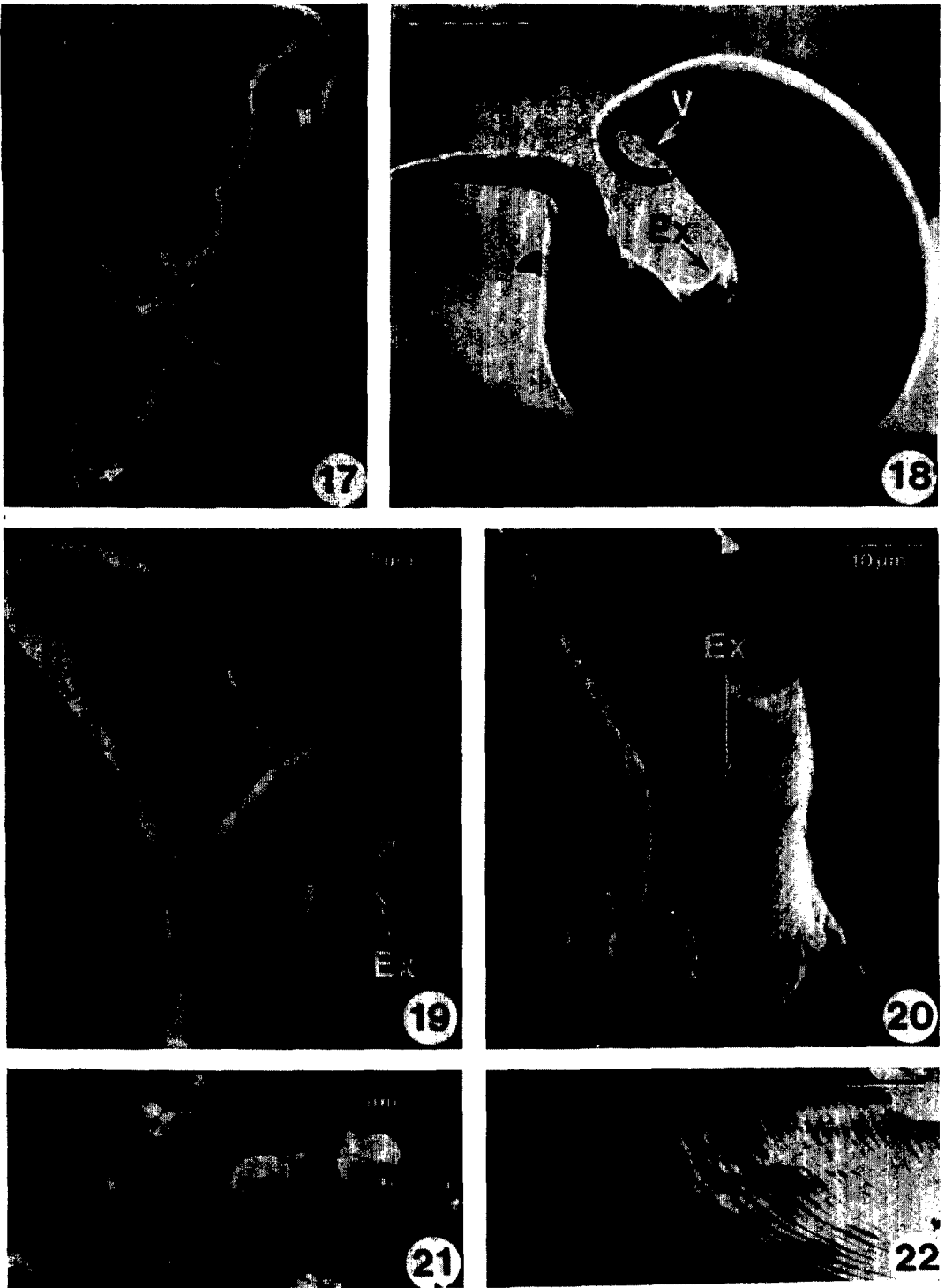
*Trophotylenchulus floridensis* Raski, 1957 syn. *Tylenchulus floridensis* (Raski, 1957) Maggenti, 1962

Other species:

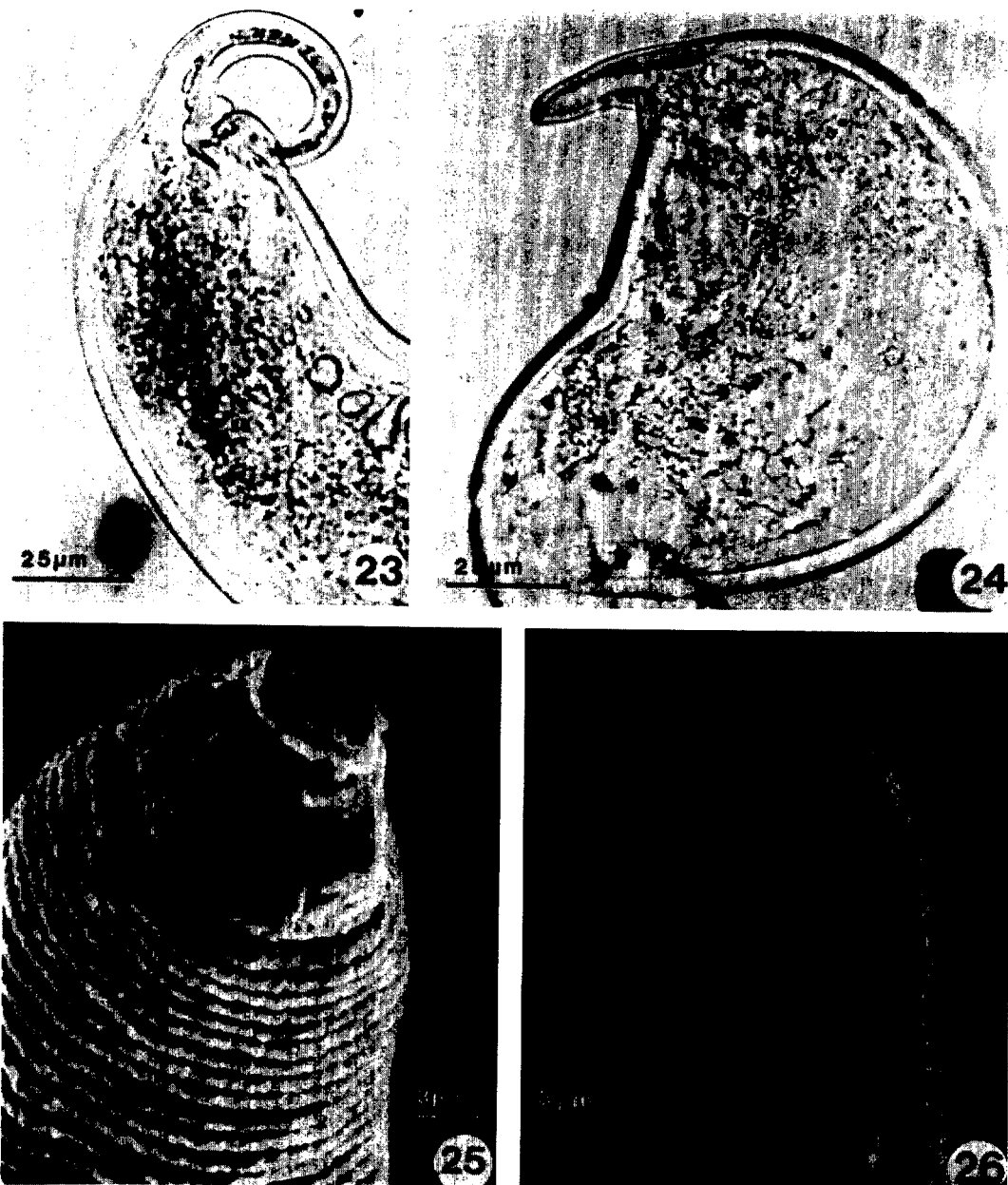
*Trophotylenchulus clavicaudatus* (Colbran, 1966) n. comb. *Tylenchulus clavicaudatus* (Colbran, 1961) n. syn.

*Trophotylenchulus mangeloti* (Luc, 1957) Goodey, 1963 syn. *Tylenchulus mangeloti* Luc, 1957.

*Trophotylenchulus obscurus* (Colbran, 1961) n. comb. *Tylenchulus obscurus* (Colbran, 1961) n. syn.



Figs. 17-22. SEM of some characters differentiate adult females of *Trophotylenchulus floridensis* and *Tylenchulus semipenetrans* from roots of *Pinus clausa* and *Citrus limon*, respectively. 17, 18) Appearance of *Ty. semipenetrans* (17) and *Tr. floridensis* (18) dissected from roots shows different modes of obesity, position of vulvas (V) and excretory pores (EX), and depth of root penetration at anterior end (broken line). 19) Posterior region of *Ty. semipenetrans* shows vulva (V) and excretory pore (EX). 20) Mid-body region of *Tr. floridensis* shows position of excretory pore (EX). 21) Detail of excretory pore of *Ty. semipenetrans*. Note globules of excretum. 22) Detail of excretory pore of *Tr. floridensis*.



Figs. 23-26. Head and tail regions of *Trophotylenchulus floridensis* and *Tylenchulus semipenetrans*. 23) LM of coiled elongate postvulval section of *Tr. floridensis* adult female. 24) LM of straight, short postvulval section of *Ty. semipenetrans* adult female. 25) SEM of elevated head cap ("circumoral elevation") of *Tr. floridensis* J-2. 26) SEM of low head cap of *Ty. semipenetrans* J-2.

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