

## Diagnosis of *Trichodorus obtusus* and *Paratrichodorus minor* on Turfgrasses in the Southeastern United States

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### Introduction

Nematodes in the family Trichodoridae (Thorne, 1935) Siddiqi, 1961, commonly called "stubby-root" nematodes, are of agricultural importance both as plant-pathogens and as vectors for plant viruses. They are ectoparasites that feed primarily on meristematic cells of root tips. Feeding by these nematodes can cause cessation of root elongation and an abbreviated or "stubby" root system termed stubby root disease (3). The damaged root systems have reduced capacity to extract water and nutrients from soil and predispose turf to environmental stresses.

Two species of stubby-root nematode have been identified as pathogens on warm-season turfgrasses in the southern United States; these are *Paratrichodorus minor* (Figs. 1 and 2) and *Trichodorus obtusus* (Figs. 3 and 4) (4,13,14). During the past four years the University of Florida Nematode Assay Lab has conducted nematode diagnosis from approximately 20,000 turfgrass samples. From these turfgrass samples, all of the stubby-root nematodes identified have been either *T. obtusus* or *P. minor*. Both species have been demonstrated as pathogens of bermudagrass (*Cynodon dactylon* and *Cynodon* hybrids) and St. Augustinegrass (*Stenotaphrum secundatum*) in greenhouse experiments. However, research shows that *T. obtusus* is more damaging to both bermudagrass and St. Augustinegrass than is *P. minor* (4,14). Recently, the University of Florida Nematode Assay lab instituted separate action thresholds for *T. obtusus* and *P. minor* on warm-season turf grasses. In order to use separate action thresholds, methods for distinguishing these species were identified that do not require mounting of specimens and can be used with  $\times 100$  magnification or less.



Fig. 1. Female *Paratrichodorus minor*.

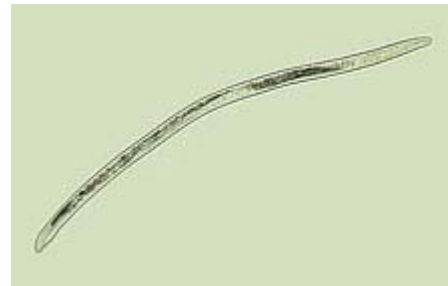


Fig. 2. Male *Paratrichodorus minor*.



Fig. 3. Female *Trichodorus obtusus*.

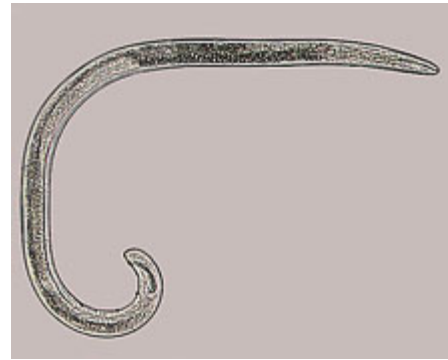


Fig. 4. Male *Trichodorus obtusus*.

**Disease:** Stubby root.

**Primary Hosts:** Bermudagrass [*Cynodon dactylon* (L.) Pers. and *Cynodon* hybrids] and St. Augustinegrass [*Stenotaphrum secundatum* (Walter) Kuntze].

**Pathogens:** *Trichodorus obtusus* Cobb (syn. *T. proximus*) and *Paratrichodorus minor* (Colbran) Siddiqi (syn. *T. minor*, *P. christiei*).

### Symptoms

Aboveground symptoms caused by stubby-root nematodes are similar to those caused by other root-feeding plant-parasitic nematodes on turfgrasses. These symptoms include irregularly shaped patches of wilting (Fig. 5), yellowing, and declining turf (16). In Florida, the turf decline often is accompanied by increased proliferation of weeds. Severe infestations combined with stress conditions can lead to death of the turf (Fig. 6).



Fig. 5. Wilting patches of St. Augustinegrass resulting from a high infestation of *Trichodorus obtusus*.



Fig. 6. Dying patches of St. Augustinegrass resulting from a high infestation of *Trichodorus obtusus* combined with drought stress.

Belowground symptoms are dark and abbreviated or "stubby" root systems (Fig. 7), hence the names stubby root disease and stubby-root nematode. However, these symptoms are indistinguishable from those caused by other virulent ectoparasitic nematodes such as sting nematode (*Belonolaimus longicaudatus*).



Fig. 7. Roots of St. Augustinegrass grown in soil inoculated with *Trichodorus obtusus* (left) and in uninoculated soil (right).

### Host Range

Known hosts of *T. obtusus* are: bermudagrass (4), St. Augustinegrass (4,14), and tomato (*Lycopersicon esculentum*) (9). It has been associated with big bluestem (*Andropogon gerardi*), sideoats grama (*Bouteloua curtipendula*), *Eucalyptus* sp., Kentucky bluegrass (*Poa pretensis*), rhododendron (*Rhododendron* sp.), sabal palm (*Sabal palmetto*), potato (*Solanum tuberosum*), littleleaf linden (*Tilia cordata*), sweetbay magnolia (*Magnolia virginiana*) (12), sorghum-sudangrass (*Sorghum bicolor* × *S. arundinaceum*) (9), and seashore paspalum (*Paspalum vaginatum*) (10).

*Paratrichodorus minor* has over 100 known hosts (5). Turfgrass hosts include bermudagrass, St. Augustinegrass, annual bluegrass (*Poa annua*), Italian ryegrass (*Lolium multiflorum*), perennial ryegrass (*L. perenne*) (2), and creeping bentgrass (*Agrostis palustris*) (15).

### Geographic Distribution

*Trichodorus obtusus* is only known to occur in the United States. A report of *T. proximus* (a synonym of *T. obtusus*) from Ivory Coast (1) was later determined to be a different species (6,8). Within the United States it has been reported in the states of Virginia, Florida, Iowa, Kansas, Michigan, New York, and South Dakota (6,12). The author found *T. obtusus* infesting St. Augustinegrass lawns near Dallas, Texas.

*Paratrichodorus minor* is spread around the globe, being reported in Afghanistan, Argentina, Belgium, Brazil, Canary Islands, Cuba, Egypt, Fiji, India, Israel, Italy, Ivory Coast, Japan, Java, Mauritania, Netherlands, New Zealand, Nicaragua, Philippines, Portugal, Puerto Rico, Russia, Senegal, Sweden, Switzerland, Taiwan, United States, Upper Volta, Venezuela, and West Germany (5). Within the United States it is widespread, being reported in most states (12).

### Pathogen Isolation

As ectoparasites, stubby-root nematodes are extracted from soil rather than plant tissue. The University of Florida Nematode Assay Lab has had good success extracting both *T. obtusus* and *P. minor* using either centrifugal-flotation or Baermann funnel techniques. However, any of the common methods for extracting nematodes from soil may probably be used (11).

### Pathogen Identification

The Trichodoridae are the only plant parasites in the order Triplonchida. Triplonchida have a two-part esophagus made up of a narrow anterior region expanding gradually to a swollen muscular base (Fig. 8). However, because the esophagus of trichodorid nematodes often overlaps the intestine and the basal portion expands gradually with no clear distinction between the anterior and basal regions, it is often difficult to distinguish the esophageal shape (Fig. 9).

The key morphological feature used to distinguish the Triplonchida from other orders, a six-layer cuticle, is typically not seen except with high-quality mounted specimens and is not a useful feature in most diagnostic settings.

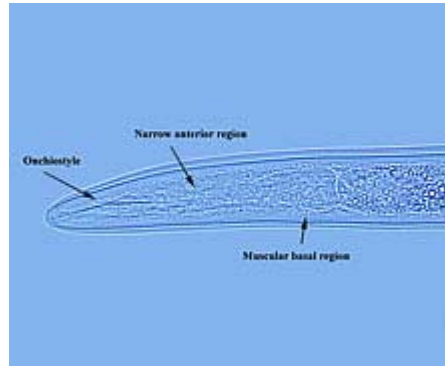


Fig. 8. The anterior region of *Trichodorus obtusus*. In this occurrence, the swollen muscular basal portion and narrow anterior region are easily observed. The curved onchiostyle also is clearly visible.

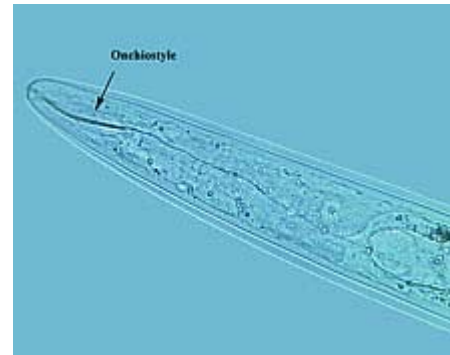


Fig. 9. The anterior region of *Paratrichodorus minor*. In this common occurrence, the esophageal shape of stubby-root nematodes is difficult to distinguish. However, the curved onchiostyle is clearly visible.

The most useful feature in the identification of Trichodoridae in diagnostic settings is their onchiostyle, a solid, dorsally curved stylet (Figs. 8 and 9) exclusive to the Trichodoridae that is clearly visible at  $\times 25$  magnification (Figs. 1 to 4). The only other stylet-bearing nematodes in the order Triplonchida are the fungivorous Ditherphoridae which have a convoluted or complex stylet (Fig. 10). Body shape is another useful morphological feature in identification of Trichodoridae. The body shape of the Trichodoridae is usually described as "cigar-shaped," meaning its body width to length ratio is high and is more or less rounded at both ends (Figs. 1 to 4).

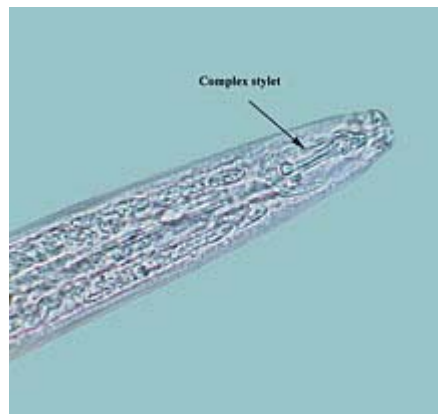


Fig. 10. The anterior region of *Diptherophora* sp. The stylet of *Diptherophora* is convoluted or complex.

The genera *Trichodorus* and *Paratrichodorus* are separated from the other genera in the Trichodoridae, *Monotrichodorus* and *Allotrichodorus*, by the number of gonads present in the females. In *Trichodorus* and *Paratrichodorus* females are amphidelphic, meaning they have two genital tracts facing in opposite directions (Figs. 11 and 12), whereas in *Monotrichodorus* and *Allotrichodorus* females are monodelphic-prodelphic, meaning there is a single genital tract stretching anteriorly.



Fig. 11. The female reproductive tract of *Trichodorus obtusus* is paired, with each pair facing opposite directions. The vagina is barrel-shaped and sclerotized.

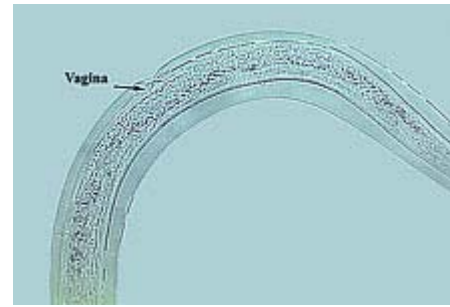


Fig. 12. The female reproductive tract of *Paratrichodorus minor* is paired, with each pair facing opposite directions. The vagina is oval in shape and not sclerotized.

In the case of warm-season turfgrasses in the southeastern United States the only species of stubby-root nematodes commonly associated with turf are *T. obtusus* and *P. minor*. Therefore, in a turfgrass diagnostic setting, distinguishing between these two species is all that is necessary in the majority of cases. Fortunately, *T. obtusus* and *P. minor* are easily distinguished from each other.

The presence or absence of males and the morphology of male sexual organs are the most useful features for separating *T. obtusus* from *P. minor* in a diagnostic setting. *Trichodorus obtusus* is an amphimictic species; both sexes are required for reproduction. Generally *T. obtusus* males are abundant in a given population. Contrarily, *P. minor* reproduces parthenogenetically, and since males are not needed for reproduction they are very rare. Males of *T. obtusus* have no bursa (caudal alae) and the spicules are large and curved (Fig. 13). If males of *P. minor* are present, a small bursa is present, and the spicules are thin and fairly straight (Fig. 14). Males of *T. obtusus* have 3 supplementary organs (precloacal ventromedian supplements) that are readily visible at  $\times 400$  or less (Fig. 13), whereas *P. minor* has only one that is so small it is not normally seen using light microscopy (Fig. 14).

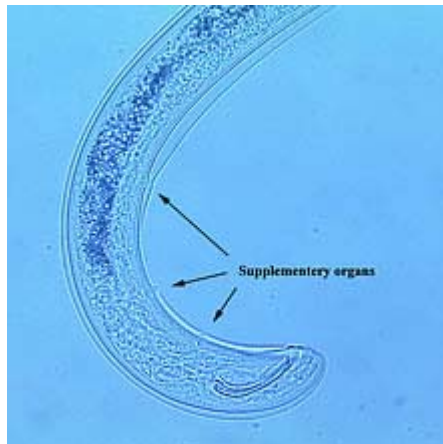


Fig. 13. The posterior of a male *Trichodorus obtusus*. The spicules are large and curved, and no bursa is present. Three supplementary organs are located anterior to the base of the spicules.

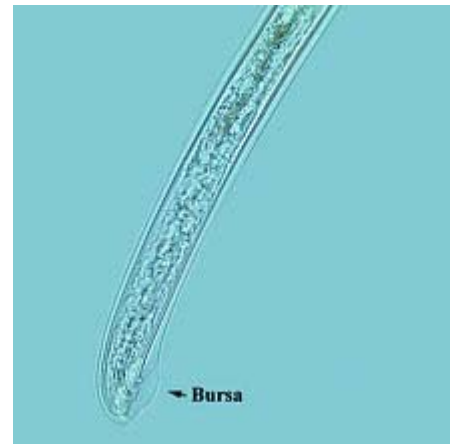


Fig. 14. The posterior of a male *Paratrichodorus minor*. The spicules are reduced and a bursa is present. Supplementary organs are not visible.

Body length also is a very useful characteristic that can be used to separate *T. obtusus* from *P. minor*. *Trichodorus obtusus* is a larger nematode than *P. minor*. *Paratrichodorus minor* has female body lengths close to 500  $\mu\text{m}$  (10), whereas female body lengths of *T. obtusus* are 1100 to 1500  $\mu\text{m}$  (4). Female sexual organs also may be used in distinguishing *T. obtusus* from *P. minor* but are less useful in diagnostic settings than are male characteristics. The vulva of *T. obtusus* is barrel-shaped when relaxed, sclerotized, and easily seen (Fig. 11). The vulva of *P. minor* is oval-shaped, less sclerotized, and not seen as easily (Fig. 12).

Occasionally other *Trichodorus* or *Paratrichodorus* species may be present in turfgrass samples, making it necessary to separate *T. obtusus* or *P. minor* from other nematodes within the same genus. *Trichodorus obtusus* is distinguished from other *Trichodorus* spp. found in the United States by having a single ventromedian cervical pore located slightly anterior to the excretory pore in the esophageal region of the male nematode (Fig. 15), other species in the United States have multiple ventromedian cervical pores. All three supplementary organs of *T. obtusus* males are typically located anterior to base of the spicules (Fig. 13), other *Trichodorus* spp. found in the United States have either no supplementary organs or at least one that is not anterior to the base of the spicules. Females of *T. obtusus* typically have a barrel-shaped vagina when relaxed (Fig. 11), while vagina of other species are shaped otherwise. *Paratrichodorus minor* is distinguished from other *Paratrichodorus* spp. species by the females having no lateral body pores, the vulva a transverse slit, and an oval-shaped vagina (Fig. 12). Some useful diagnostic keys to species of *Trichodorus* and *Paratrichodorus* are available by W. Decraemer (5,7).

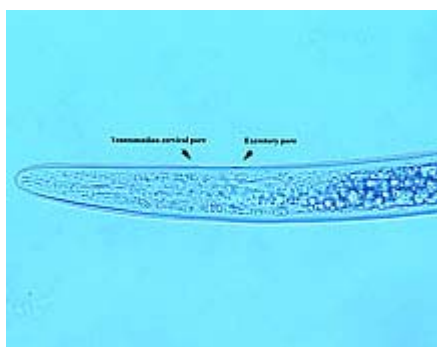


Fig. 15. A male *Trichodorus obtusus*. A single ventromedian cervical pore is located anterior to the excretory pore.

In summary, stubby-root nematodes are identified primarily on the presence of a curved onchiostyle and "cigar-shaped" body. On warm-season turfgrasses in the southeastern United States *T. obtusus* and *P. minor* are the most common species of stubby-root nematodes, and may be separated based on the following features. *Trichodorus obtusus* is generally > 1 mm long and has abundant males with large curved spicules, 3 supplementary organs anterior to the spicule base, no bursa, and a single ventromedian cervical pore immediately anterior to the excretory pore. *Paratrichodorus minor* is close to 0.5 mm long, mostly females, and males (if present) have a small bursa, straight spicules, and no visible supplementary organs or ventromedian cervical pore.

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