

## CHAPTER 14

### Trichodorid Nematodes in Portugal

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#### I. INTRODUCTION

Nematodes of the family Trichodoridae are plant parasites, directly damaging plants by their feeding on plant root cells. Of particular importance is their ability to transmit tobraviruses which infect a wide range of plants.

As for other plant parasitic nematodes it is important to accurately identify the species included in Trichodoridae. Species determination is not always easy and several authors have discussed the importance of taxonomic features (Allen, 1957; Bird, 1967a,b; Bird & Mai, 1968; Wyss, 1970; Esser, 1971; Loof, 1973, 1975; Siddiqi, 1974; Rodriguez-M. & Bell, 1978, Rodriguez-M. *et al.*, 1978; Shishida, 1979; Decraemer, 1980; Andrassy, 1989).

Decraemer (1988a,b, 1989) studied extensively the morphological and morphometric variability and the value of characters used in the identification of these nematodes.

It was only about thirty years ago that nematology research began in Portugal. However, detailed knowledge about the occurrence and distribution of trichodoridae nematodes in Portugal has only recently become available. In order to facilitate the identification of the Trichodoridae present in Portugal we present simple keys based on morphological characters of males and females.

## II. OCCURRENCE OF TRICHODORIDS IN PORTUGAL

Although the earliest reference to *Trichodorus* s.l. (*sensu lato*) in Portugal was published in 1963 (Macara, 1963; Table I) only relatively few reports of species of Trichodoridae occurring in Portugal are available (Sturhan, 1973; Siddiqi, 1974; Craveiro & Santos, 1984; Santos *et al.*, 1984; Almeida & Santos, 1987, 1988 a,b; Sturhan, 1989; Almeida *et al.*, 1989).

From the available information it is known that representatives of both genera of Trichodoridae occurring in Europe have been found in Portugal: *Trichodorus* Cobb, 1913 and *Paratrichodorus* Siddiqi, 1974. To date, the following species have been reported from Portugal: *T. azorensis* Almeida, De Waele, Santos & Sturhan, 1989, *T. beirensis* Almeida, De Waele, Santos & Sturhan, 1989, *T. lusitanicus* Siddiqi, 1974, *T. primitivus* (De Man, 1880) Micoletzky, 1922, *P. acutus* (Bird, 1967a) Siddiqi, 1974, *P. allius* (Jensen, 1963) Siddiqi, 1974, *P. anemones* (Loof, 1965) Siddiqi, 1974, *P. minor* (Colbran, 1956) Siddiqi, 1974, *P. nanus* (Allen, 1957) Siddiqi, 1974, *P. pachydermus* (Seinhorst, 1954) Siddiqi, 1974 and *P. porosus* (Allen, 1957) Siddiqi, 1974.

During a survey begun in 1982, which involved the collection of a total of 370 soil samples from various types of vegetation in different localities, Trichodoridae were found in 100 of the samples. Single and mixed populations of species of *Trichodorus* and/or *Paratrichodorus* were found (Table II).

Determination of the species is based on the study of various taxonomical features. The characters considered important to identify species of Trichodoridae nematodes have been reviewed by Decraemer & Almeida (See Chapter 8).

TABLE I  
PLANTS AND LOCALITIES WHERE *Trichodorus* s.l. POPULATIONS HAVE BEEN FOUND

Vegetation	Locality	Reference
<i>Allium cepa</i> L.	Cantanhede	Abrantes <i>et al.</i> , 1978
<i>A. porrum</i> L.	Cantanhede	Abrantes <i>et al.</i> , 1978
<i>Asparagus densiflorus</i> (Kunth) Jessop cv. Sprengeri	Moita	Reis, 1985b
<i>A. setaceus</i> (Kunth) Jessop	Moita	Reis, 1985b
<i>Beta vulgaris</i> L.	Bolão, Coimbra; Tocha	Abrantes <i>et al.</i> , 1978
<i>Buxus sempervirens</i> L.	Queluz	Reis, 1985b
<i>Chrysanthemum morifolium</i> Ramat.	Lamaçães, Moita	Reis, 1985b
<i>Citrus deliciosa</i> Ten.	Tovim de Baixo, Coimbra	Abrantes <i>et al.</i> , 1978
<i>Dahlia coccinea</i> Cav.	Moita	Reis, 1985b
<i>Dianthus caryophyllus</i> L. cvs. Lena, Scannia	Moita	Reis, 1985b
<i>Galinsoga parviflora</i> Cav.	Bolão, Coimbra	Abrantes <i>et al.</i> , 1978
<i>Humulus lupulus</i> L. "Brewer's Gold"	Braga; Bragança	Reis <i>et al.</i> , 1986
<i>Lycopersicon esculentum</i> Mill.	Bolão, Coimbra	Abrantes <i>et al.</i> , 1978
<i>Musa paradisiaca</i> L.	Madeira	Reis & Faria, 1990
<i>Nicotiana tabacum</i> L.	Caria; Coruche; Deveza; Idanha-a-Nova; Leiria	Reis, 1985a
<i>Olea europaea</i> L.	Bencanta, Coimbra; Montinho, Cantanhede; Soure	Abrantes, 1980
<i>Phaseolus vulgaris</i> L. and <i>Brassica napus</i> L.	Arazede	Abrantes <i>et al.</i> , 1978
<i>Pyrus malus</i> L., var. Golden	Viseu (Escola Agrícola)	Macara, 1963
<i>Saccharum officinarum</i> L.	Lisboa (Jardim do Ultramar)	Macara, 1963
<i>Solanaceae</i>	Caldas da Rainha	Reis, 1985c
<i>Solanum tuberosum</i> L.	Guarda	Abrantes <i>et al.</i> , 1978
<i>S. tuberosum</i> L., <i>Malus domestica</i> Borkh., and <i>Pyrus communis</i> L.	Vilarelho, Caminha	Abrantes <i>et al.</i> , 1978
<i>Strelitzia reginae</i> Ait.	Moita	Reis, 1985b
<i>Zea mays</i> L.	Louriçal, Pombal; Vila Nova de Anços, Soure	Abrantes <i>et al.</i> , 1978

TABLE II

SOIL SAMPLES CONTAINING SINGLE OR MIXED POPULATIONS  
OF *Trichodorus* AND/OR *Paratrichodorus* SPECIES  
(DATA BASED ON CONTINENTAL PORTUGUESE POPULATIONS)

Occurrence (%)	<i>Trichodorus</i> spp. <sup>a</sup>	<i>Paratrichodorus</i> spp. <sup>a</sup>
41	+	-
26	-	+
2	++	-
25	+	+
2	++	+
3	+	++
1	-	++

<sup>a</sup> +, presence of one species; ++, presence of two different species; -, absence.

During our observations of Portuguese populations we have found some variability, even for relatively stable differential characters such as the spicule shape of males or the shape of the vaginal sclerotizations and vagina in females. Morphological variability in these features is illustrated for several populations of some continental species: *T. beirensis*, *T. lusitanicus* and *T. primitivus* and *T. azorensis* from the Azores (Figs. 1-8). Distortion of the vaginal sclerotizations due to the intrusion of a substance of unknown origin in the vagina can occur relatively frequently, especially within *Trichodorus* spp. populations (Almeida *et al.*, 1989). In such circumstances, in lateral view, some difficulty can arise when distinguishing general patterns of the vaginal sclerotizations of females from related species.

In order to assist with the identification of the trichodorids which occur in Portugal simple keys for males and females are presented.

Some morphological features for distinguishing *Trichodorus* and *Paratrichodorus* genera and species are illustrated in Figure 9 and in Figures 10 and 11, respectively.

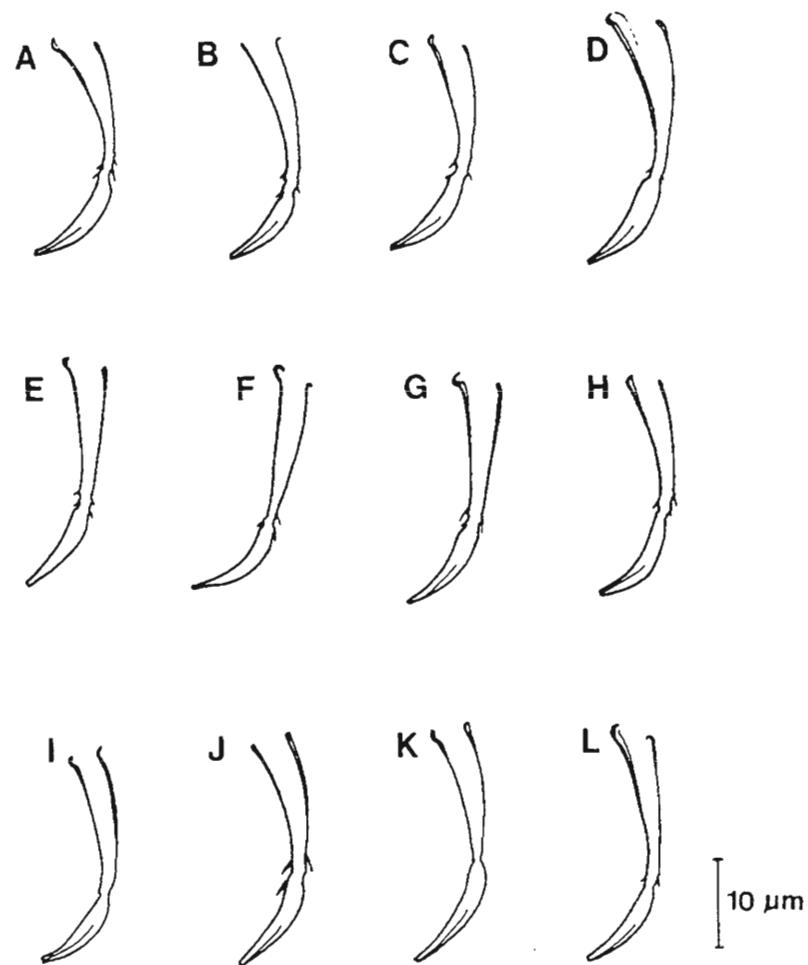
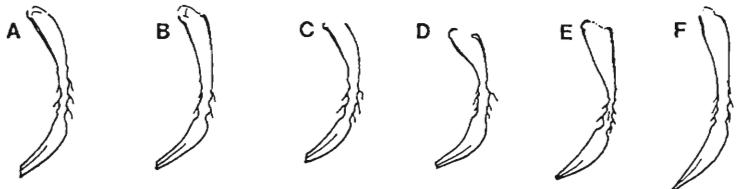
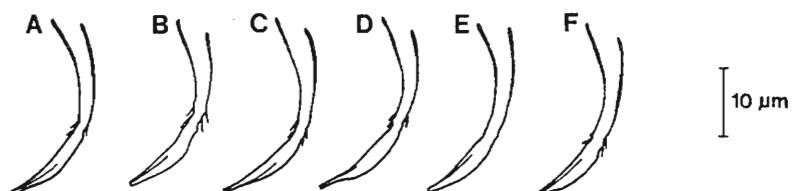


FIG. 1. Variability of spicules in males of *T. beirensis*: (A-H) paratypes, Tapada do Salgueiro population; (I-L) Ínsua do Pontão population.



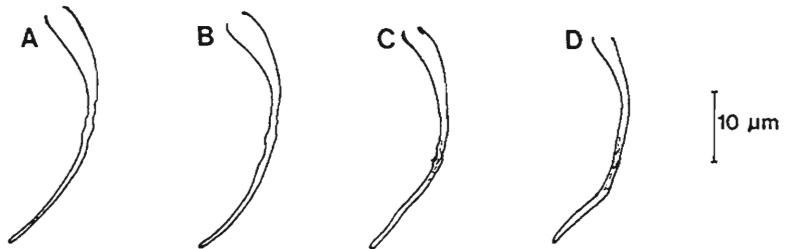
[10 µm]

FIG. 2. Variability of spicules in males of *T. lusitanicus*: (A-C) Coruche population; (D-F) Ponte de Sor population; (G) Figueira dos Cavaleiros population; (H,I) Salvaterra de Magos population; (J,K) Alcácer do Sal population.



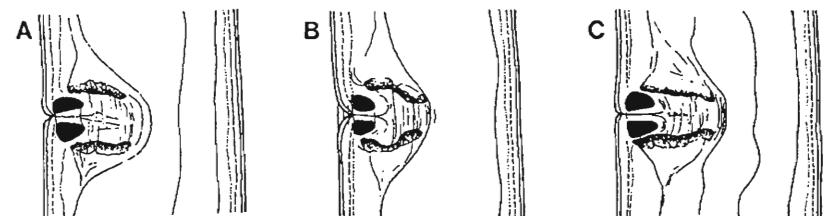
[10 µm]

FIG. 3. Variability of spicules in males of *T. azorensis*: (A-D) paratypes, Lagoa das Furnas, Azores population; (E,F) Mata da Serreta, Azores population.



[10 µm]

FIG. 4. Variability of spicules in males of *T. primitivus*: (A) Bencanta population; (B) Horta, Azores population; (C,D) Condeixa population.



[10 µm]

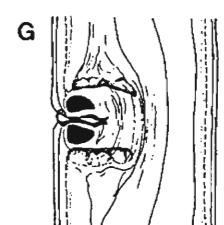
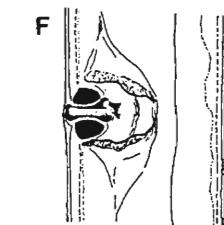
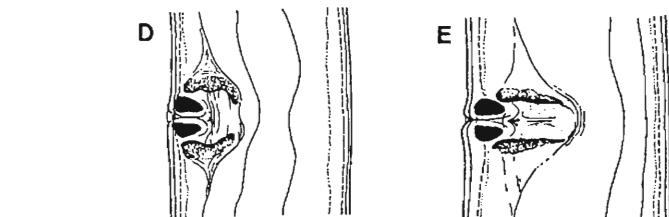


FIG. 5. Variability of vaginal region in females of *T. beirensis*: (A-C) paratypes, Tapada do Salgueiro population; (D-G) Ínsua do Pontão population.

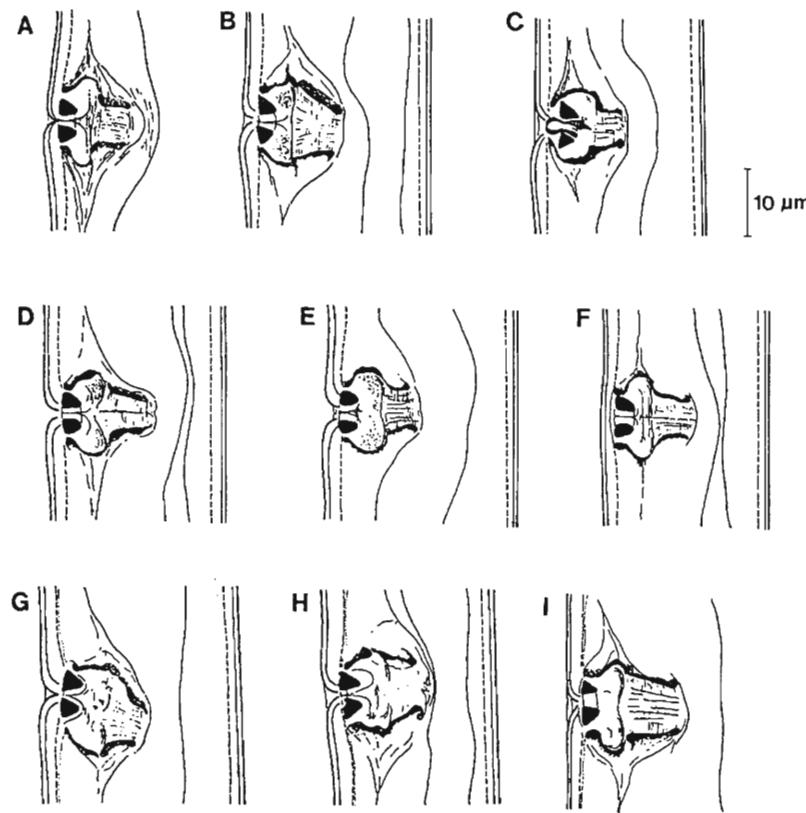


FIG. 6. Variability of vaginal region in females of *T. lusitanicus*: (A-D) Coruche population; (E) Figueira dos Cavaleiros population; (F) Salvaterra de Magos population; (G-I) Alcácer do Sal population.

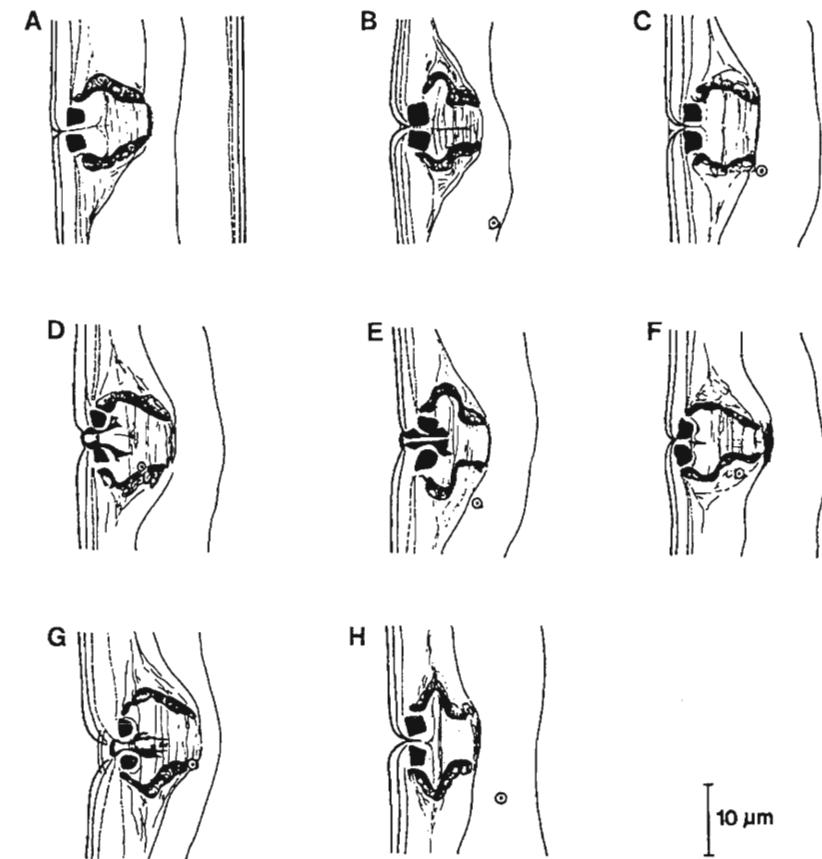


FIG. 7. Variability of vaginal region in females of *T. azorensis*: (A) holotype, Lagoa das Furnas, Azores population; (B-G) paratypes, Lagoa das Furnas, Azores population; (H) Mata da Serreta, Azores population.

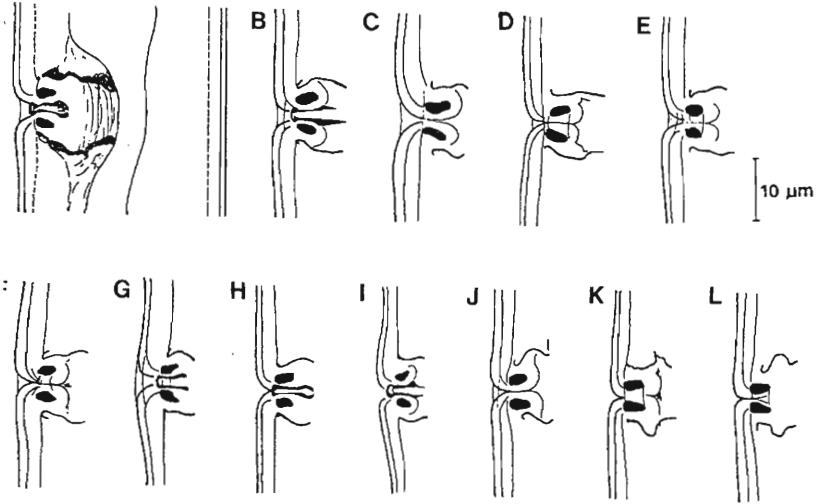


FIG. 8. Variability of vaginal region in females of *T. primitivus*: (A-E) encanta population; (F-I) Condeixa population; (J-L) Cadaixo population.

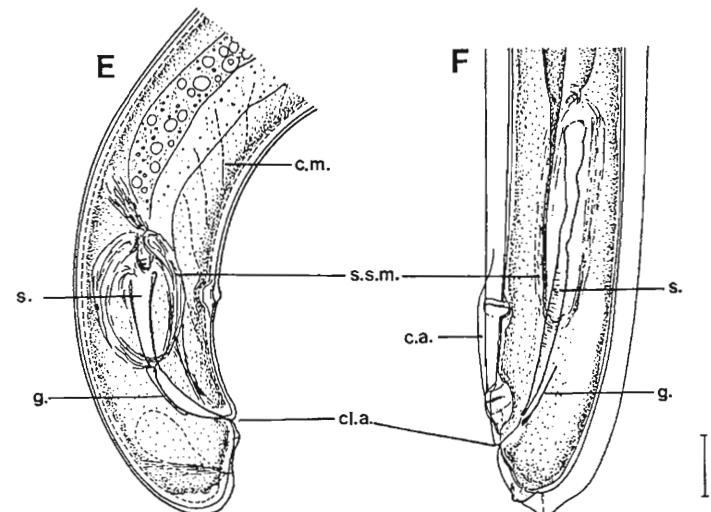
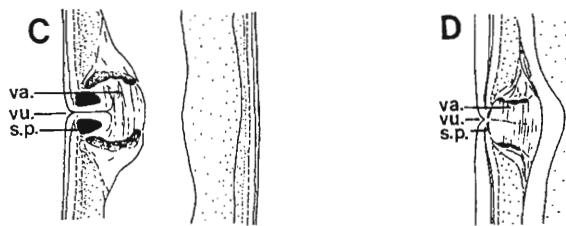
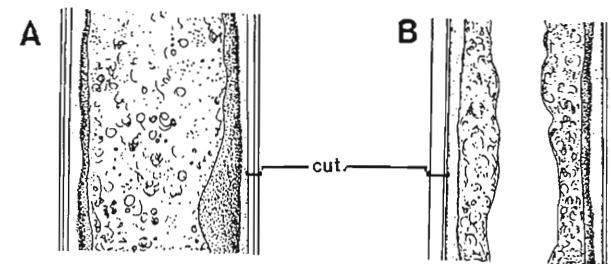


FIG. 9. Structure of *Trichodorus* (A,C,E) and *Paratrichodorus* (B,D,F) species. Top row, mid body region; second row, female vaginal region, lateral view; bottom, posterior region of males, lateral view: (A) *T. beirensis*, male; (B) *P. allius*, male; (C) *T. beirensis*; (D) *P. minor*; (E) *T. beirensis*; (F) *P. anemones*. c.a., caudal alae; cut., cuticle; c.m., copulatory muscles; cl.a., cloacal aperture; g., gubernaculum; s., spicule; s.s.m., spicular suspensor muscles; s.p., sclerotized pieces; va., vagina; vu., vulva.

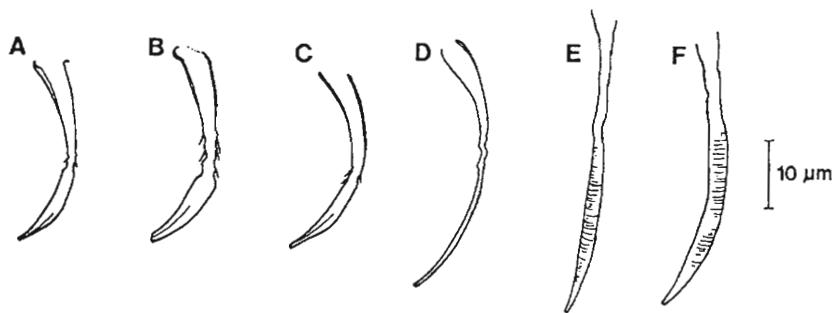


FIG. 10. Variability of spicules in males of *Trichodorus* (A-D) and *Paratrichodorus* (E,F) species from Portugal: (A) *T. beirensis*; (B) *T. lusitanicus*; (C) *T. azorensis*; (D) *T. primitivus*; (E) *P. anemones*; (F) *P. pachydermus*.

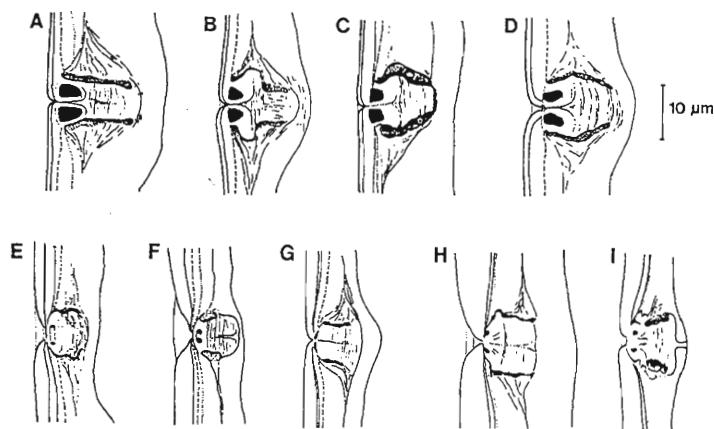


FIG. 11. Variability of vaginal region in females of *Trichodorus* (A-D) and *Paratrichodorus* (E-I) species from Portugal: (A) *T. beirensis*; (B) *T. lusitanicus*; (C) *T. azorensis*; (D) *T. primitivus*; (E) *P. allius*; (F) *P. nanus*; (G) *P. minor*; (H) *P. pachydermus*; (I) *P. anemones*.

### III. KEYS TO THE (PARA)TRICHODORUS SPECIES OCCURRING IN PORTUGAL

#### MALES

1. Body posteriorly ventrally curved; cuticle not-swollen (Fig. 9A); anterior intestinal or posterior oesophageal gland overlap absent or negligible; spicules arcuate; copulatory muscles evident and suspensor muscles conspicuous; caudal alae absent (Figs. 9E; 10A-D) ..... *Trichodorus* spp.-2
- Body straight; cuticle swollen (Fig. 9B); anterior intestinal and/or posterior oesophageal gland overlap usually present; spicules straight, striated; suspensor muscles poorly developed; caudal alae present (Figs. 9F; 10E,F) ..... *Paratrichodorus* spp.-5
2. Spicules with distal region quite slender, smooth or setosae at middle (Fig. 10D); gubernaculum mostly appearing ventral to spicules ..... *T. primitivus*
- Spicules with distal region anteriorly widening and gradually tapering posteriorly, setosae always at middle; gubernaculum not ventral to spicules ..... 3
3. Proximal region of spicules mostly longer than distal region; first precloacal supplement (SP1) usually lying within retracted spicules region ..... 4
- Proximal region length of spicules almost equal to distal region; mid corpus of spicule, strongly irregular (Fig. 10B); SP1 usually lying anterior to head of retracted spicules ..... *T. lusitanicus*
4. Spicules with a very narrow constriction, irregular in a short distance, with distal region usually appearing grooved, gradually tapering (Fig. 10A); gubernaculum spatulate ... *T. beirensis*
- Constriction of the spicules not so narrow and more slightly irregular; distal region not always grooved, usually tapering suddenly and dorsally plain (Fig. 10C) ..... *T. azorensis*
5. Males absent ..... *P. acutus*
- Males abundant or rare ..... 6
6. Males rare, with straight spicules; caudal alae inconspicuous ..... *P. porosus*
- Caudal alae more evident ..... 7
7. One ventromedian cervical papilla (CP) present ..... 8
- CP absent, males absent or rare ..... 10
8. Males rare, with 2 or 3 SP ..... *P. allius*
- Males abundant, with 3 SP ..... 9
9. Tail short; spicules mostly slender, proximally irregular and narrower than distally, excepting terminal end (Fig. 10E) ... *P. anemones*

- Tail long, more or less trapezoid; spicules different, broader, not narrowing proximally (Fig. 10F) ..... *P. pachydermus*
10. Caudal pores present; usually with intestinal overlapping by esophageal glands ..... *P. minor*
- Caudal pores lacking; absent or very slight intestinal overlapping by oesophageal glands ..... *P. nanus*

#### FEMALES

1. Body ventrally curved; cuticle not-swollen; vagina about or up to one half body width long; vaginal sclerotizations well developed, conspicuous in lateral view (Figs. 9C;11A-D) ..... *Trichodorus* spp.-2  
Body straight; cuticle swollen; vagina less than half one body width long; vaginal sclerotizations small in lateral view (Figs. 9D; 11E-I) ..... *Paratrichodorus* spp.-5
2. Vaginal sclerotizations separated elongated rods or oval pieces, parallel or almost parallel; lateral body pores present; vagina elongated to rhomboid (Fig. 11D) ..... *T. primitivus*  
Vaginal sclerotizations different, oval, triangular or square shaped; lateral body pores present or absent ..... 3
3. Lateral body pores present; vaginal sclerotizations triangular or square shaped ..... 4  
Lateral body pores absent; vaginal sclerotizations, large rounded triangular or oval pieces; vagina, barrel shaped (Fig. 11A); vulva, a small transverse slit in ventral view ..... *T. beirensis*
4. Vaginal sclerotizations regular triangular; vagina almost mushroom shaped, sometimes with spherical masses (Fig. 11B); vulva, a pore in ventral view ..... *T. lusitanicus*  
Vaginal sclerotizations as two square-shaped pieces; vagina rhomboid-shaped (Fig. 11C) ..... *T. azorensis*
5. Sperm cells large ..... 6  
Sperm cells small ..... 8
6. Ventromedian body pores present ..... *P. porosus*  
Ventromedian body pores absent ..... 7
7. Spermathecae present; vaginal sclerotizations small, triangular (Fig. 11I); vagina more or less trapezoid; lateral body pores varying from 0-6 ..... *P. anemones*  
Sperm distributed through uterus; vagina more or less cylindrical (Fig. 11H) ..... *P. pachydermus*
8. Vulva, a longitudinal slit in ventral view; caudal pores, present; vaginal sclerotizations as well separated, small dots (Fig. 11E); lateral body pores absent; males absent or rare ..... *P. allius*

- Vulva, a transverse or longitudinal slit in ventral view; caudal pores absent or rare ..... 9
9. Tail shape conoid with acute terminus; lateral and caudal pores absent ..... *P. acutus*  
Tail shape hemispherical ..... 10
10. Basal region of oesophageal bulb not overlapping or ventrally overlapping intestine; excretory pore usually opposite to base of oesophageal bulb; vaginal sclerotizations as slender drop-like, triangular or rod-like pieces, almost parallel to the cuticle (Fig. 11G) ..... *P. minor*  
Basal region of oesophageal bulb not overlapping intestine; excretory pore more posterior, opposite to anterior part of intestine; vaginal sclerotizations as broad rounded pieces (Fig. 11F) ..... *P. nanus*

#### IV. DISTRIBUTION

Information about the hosts, habitat and the geographical distribution of plant parasitic nematodes may contribute to the identification process (Eisenback, 1988). The sample locality and associated plants of (*Para*)*Trichodorus* species occurring in Portugal are given in Table III and the distribution of these species is illustrated in Figures 12 and 13.

#### V. CONCLUSIONS

To assist with the characterization and identification process, we suggest that the following aspects should be taken into account: 1) mixed populations occur relatively frequently therefore, whenever possible, at least ten specimens should be examined; 2) existing taxonomical studies indicate that morphological variability should be studied and should be presented in any report; 3) the occurrence of distortion of the vaginal sclerotizations in females in lateral view should be examined and evaluated. The identification keys presented here should be considered simply as working instruments which are open to improvement. Results from future

TABLE III

OCCURRENCE AND DISTRIBUTION OF *Trichodorus* AND *Paratrichodorus* SPECIES IN PORTUGAL

Species	Locality	Vegetation	Reference
<i>T. azorensis</i>	Lagoa das Furnas, São Miguel, Azores	Bushland with <i>Ulex europaeus</i> L., <i>Plantago lanceolata</i> L., <i>Rubus</i> sp., Leguminosae, grass, moss	Almeida <i>et al.</i> , 1989
	Mata da Serreta, Terceira, Azores	Forest with <i>Myrica faya</i> Ait., <i>Pittosporum</i> sp., <i>Pteridium aquilinum</i> (L.) Kühn, <i>Selaginella kraussiana</i> (G. Kunze) A. Braun, <i>Senecio</i> sp., grass	
<i>T. beirensis</i>	Ínsua do Pontão and Tapada do Salgueiro, Celorico da Beira	<i>Secale cereale</i> L. and <i>Lolium multiflorum</i> Lam.	Almeida <i>et al.</i> , 1989
<i>T. lusitanicus</i>	Lagoalva de Cima, Alpiarça Paul de Arzila, Coimbra	<i>Lycopersicon esculentum</i> Mill. <i>Eucalyptus globosa</i> Labill. and <i>Pinus pinaster</i> Ait.	Siddiqi, 1974 Almeida & Santos, 1987
	Cadaixo, Miranda do Corvo Alcácer do Sal; Conuche; Figueira dos Cavaleiros; Gavião; Pinhal do Norte, Carrazeda d'Anciães; Ponte de Sor; Salvaterra de Magos; Vendas Novas	<i>Olea europaea</i> L. <i>Quercus suber</i> L.	Almeida <i>et al.</i> , 1989

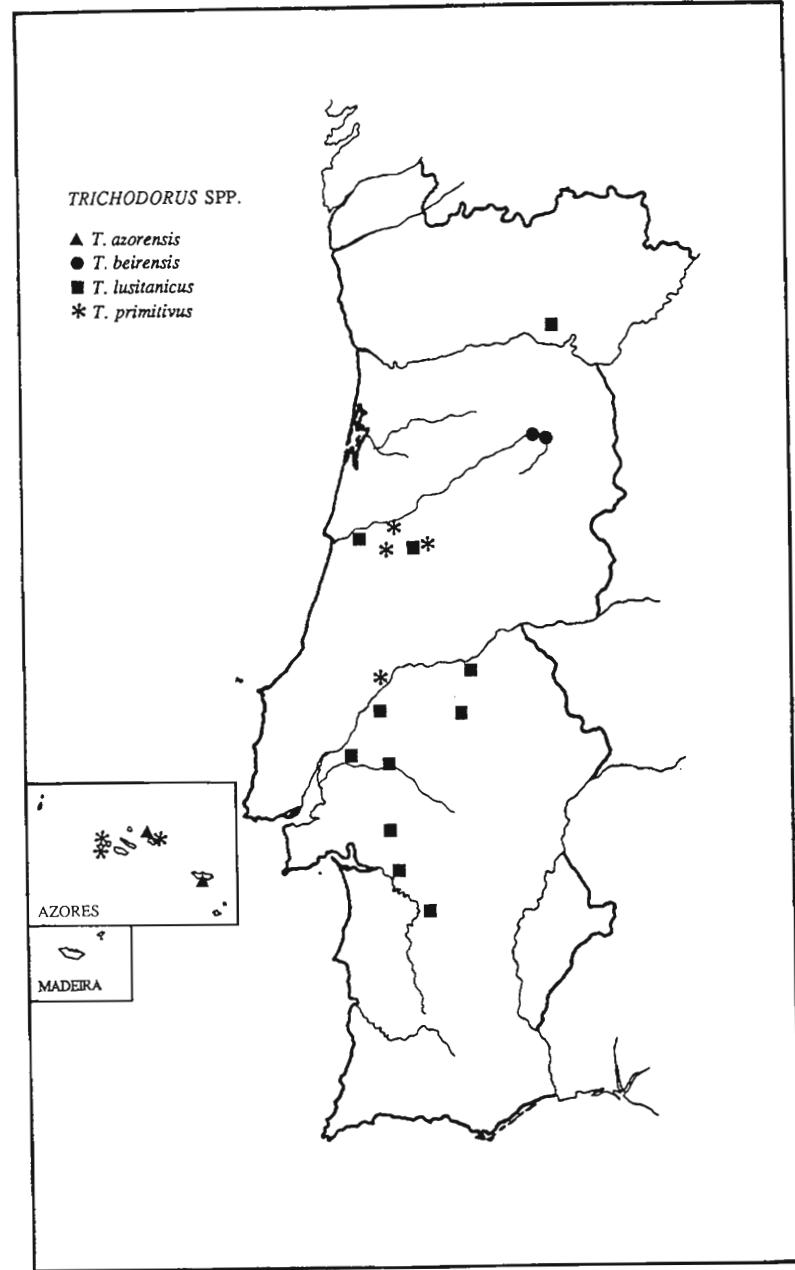
(continued)

TABLE III (continued)

Species	Locality	Vegetation	Reference
<i>T. primitivus</i>	Bencanta, Coimbra; Cadaixo, Miranda do Corvo; Condeixa; Golegã Capelo, Faial, Azores	<i>O. europaea</i> L. Grassy vegetation with <i>Ulmus procera</i> Salisb., <i>Solanum tuberosum</i> L. and <i>Citrus</i> sp.	Almeida <i>et al.</i> , 1989
	Horta, Faial, Azores	Meadow with <i>Poa trivialis</i> L., <i>Plantago lanceolata</i> L., <i>Mentha</i> sp., <i>Trifolium pratense</i> L., <i>Rumex</i> sp., etc.	
	Mata da Serreta, Terceira, Azores		
<i>P. acutus</i>	Santana, Madeira	Grass	D. Sturhan, pers. comm.
<i>P. allius</i>	Salvaterra de Magos Chão, Deserta, Madeira Deserta Grande, Madeira	<i>Q. suber</i> L. <i>Jasminum odoratissimum</i> L. a	Almeida & Santos, 1988b Sturhan, 1989 D. Sturhan, pers. comm.
<i>P. anemones</i>	Areias de Vilar, Barcelos Tapada do Salgueiro, Celorico da Beira	<i>Humulus lupulus</i> L. <i>S. cereale</i> L. and <i>L. multiflorum</i> Lam.	Craveiro & Santos, 1984

TABLE III (continued)

Species	Locality	Vegetation	Reference
<i>P. minor</i>	Gafanha da Boavista, Ílhavo Quijos, Figueira da Foz	<i>Brassica oleracea</i> L., <i>Phaseolus vulgaris</i> L. <i>L. esculentum</i> Mill.	Almeida & Santos, 1987 Craveiro & Santos, 1984
	Madeira	a	D. Sturhan, pers. comm.
<i>P. nanus</i>	Lagoalva de Cima, Alpiarça	a	Siddiqi, 1974
<i>P. pachydermus</i>	Pregal, Póvoa de Lanhoso	<i>H. lupulus</i> L.	Craveiro & Santos, 1984
<i>P. porosus</i>	São Miguel, Açores Ribeira Brava, Madeira	<i>Musa paradisiaca</i> L. <i>Saccharum officinarum</i> L.	Sturhan, 1973

<sup>a</sup> UnknownFIG. 12. Distribution of *Trichodorus* species in Portugal.

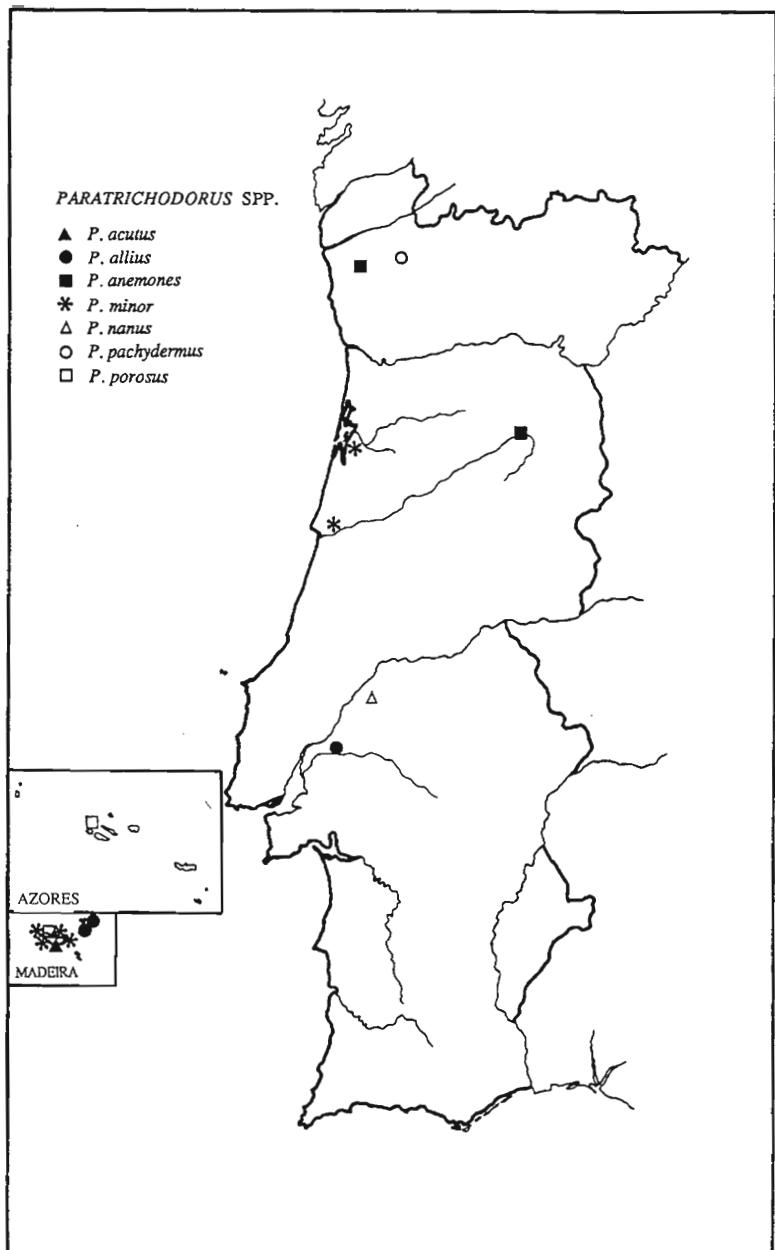


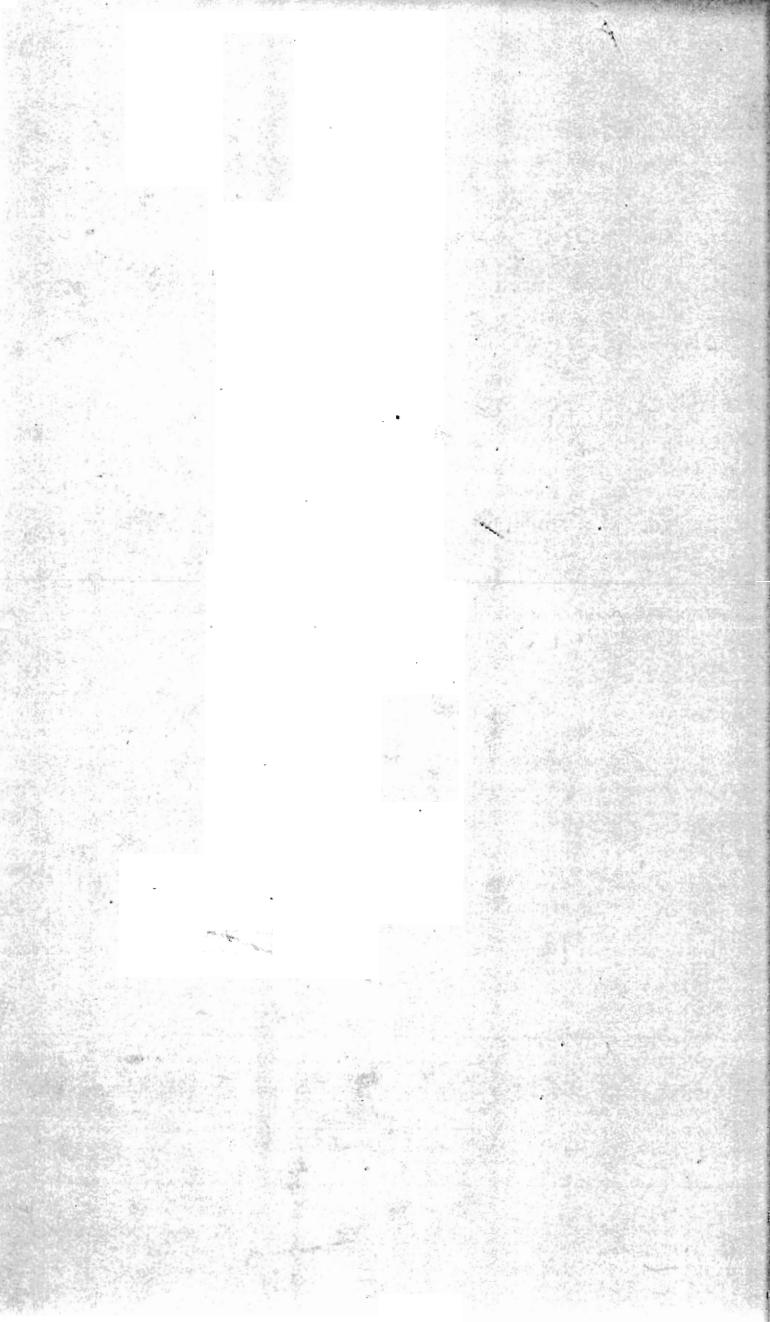
FIG. 13. Distribution of *Paratrichodorus* species in Portugal.

studies should be included to maintain and increase the effectiveness of the keys. From the relatively little information available concerning the occurrence and distribution of Trichodoridae in Portugal it seems that much remains to be learnt about these nematodes and especially their association with tobraviruses.

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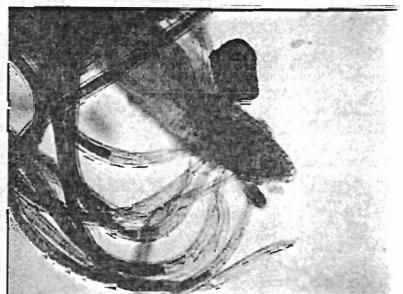
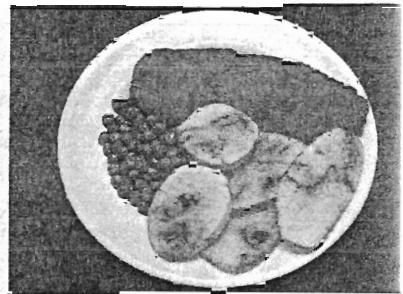
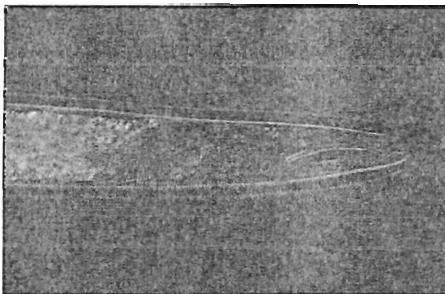


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# AN INTRODUCTION TO VIRUS VECTOR NEMATODES AND THEIR ASSOCIATED VIRUSES

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