

Some Plant Parasitic Nematodes Associated With Grapevines in Thrace, Turkey

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Abstract

Plant parasitic nematode species in vineyards in Thrace, Northwestern Marmara was determined in extensive research carried out between 2015 and 2018. A total of 274 soil samples were collected from Edirne, Kırklareli and Tekirdağ provinces, nematodes were isolated and identified on a species basis. *Rotylenchus cypriensis*, Antoniou, 1981 was recovered of 14.30% of all soil samples. The article includes detailed information about the morphological and morphometric characteristics of *R. cypriensis* and some other species. The species descriptions were supported with female nematode images. The other species described and illustrated were *Helicotylenchus Canadensis* Wasseem, *Pratylenchus thornei* (Sher and Allen 1953), (Sher, 1948), *Pratylenchus neglectus* (Rensch, 1924) Filipjev and Scuurmans Stekhoven, 1941, *Tylenchorrhynchus cylindricus* Cobb, 1913.

Introduction

Agriculture is the most important sector that produces food for the survival of human beings, creates employment for a large part of the population, and provides raw materials for industry. In many developed or developing countries, agricultural production is carried out in limited areas since lands opened up for agriculture are being lost day by day due to urbanization and drought. For this reason, in order to obtain the amount of agricultural production that will meet the demanded food of the population throughout the world, practices that promote productivity are carried out and efforts are made to eliminate yield losing factors.

Many abiotic and biotic factors are involved in decreasing agricultural yield, and one of these factors is plant parasitic nematodes (Luc et al., 2005b). Plant parasitic nematodes belong to phylum Nematoda, the most abundant group of invertebrates with about 30.000 known species. Based on feeding habitat nematodes are classified into 5 groups; fungivores that feed with fungi, bacterivores that feed with bacteria,

predators, omnivores and plant parasitic herbivores (Kennedy and Luna, 2004). Approximately 15% of identified nematodes are plant parasitic herbivores and these are the largest group with 250 nematode species from 43 genera harmful to plants and 126 species from 33 genera included in the quarantine list of several countries (Lambert and Bekal, 2002; Singh et al., 2013). Herbivore nematodes that feed on stem, leaf, flower, seed and root of plants were divided into 3 groups (ectoparasite, endoparasite, semi endoparasite) based on feeding behavior. Ectoparasite nematodes feed on an external surfaces without entering into plant roots. Endoparasites totally enters roots while semi endoparasites insert only the head region into root cells to feed (Decreamer and Hunt, 2006). Although many plant parasitic nematodes have been identified in agricultural areas the economically most harmful species include in genera of *Heterodera*, *Globodera*, *Meloidogyne*, *Pratylenchus* and *Ditylenchus* (Brown, 1984; Smiley, 2009; Janati et al., 2018).

Grapevine is the second most widely cultivated fruit crop in the world covering an area

of 8 million hectares (Vivier and Pretorius, 2000). Over 14.000 varieties grown throughout the world, produce fruits with rich mineral and vitamin content (Alleweld et al., 1990). The berries are consumed as raisins and fresh or processed into wine. It is grown in varied climatic conditions ranging from temperate to semi-tropic and tropics. Firstly grown in Anatolia about 7500 years ago, grapevine has always had an important place in the agricultural structure of Turkey. As a matter of fact, it ranks 4th in the world in terms of vineyard area and 6th in terms of fresh grape production.

Grapevine is a host to more than 300 plant-parasitic nematode species belonging to 35 genera (Singh et al., 2013) and in studies on the distribution of plant parasitic nematodes in different regions of Anatolian part of Turkey, nematode species belonging to the orders Tylenchida, Aphelenchida, Dorylaimida, Triplonchida have been identified. Since there are limited studies covering vineyards in European part of Thrace of Turkey a broad spectrum survey was carried out in Edirne, Kırklareli and Tekirdağ, three main grape growing provinces in order to identify associated plant parasitic nematode species.

After species identifications nematodes were divided to three orders Aphelenchida, Tylenchida and Dorylaimida. Here, a description of four nematode species, information about classification, morphometric lengths, habitat and prevalence were given. The comparisons of each specimens with same species from different countries and microscopic images of vulva, head, tail region were also included.

Material and Methods

During field studies plant parasitic nematodes described here were extracted from vineyard soils. *Rotylenchus cypriensis* was found in samples from Edirne (Keşan, Uzunköprü), Tekirdağ (Şarköy, Malkara, Süleymanpaşa) and Kırklareli (Merkez). *Helicotylenchus canadensis* was collected from Edirne (Keşan), Tekirdağ (Şarköy, Süleymanpaşa) and Kırklareli (Merkez). *Pratylenchus thornei* was found in Edirne (Uzunköprü), Tekirdağ (Şarköy, Malkara, Süleymanpaşa) and Kırklareli (Merkez). *Pratylenchus neglectus* was collected from Edirne (Uzunköprü), Tekirdağ (Şarköy, Süleymanpaşa)

Table 1. The definitions of ratios of De Man (1876) used to identify nematode species

n	number of measured female specimens
L	body length
V%	% distance of vulva from anterior
a	body length / the diameter of the widest part of the body
b	body length / the length between lip and esophago-intestinal valve
c	body length / tail length
c'	tail length / tail diameter at anus
s	stylet length

Scutellonemoides Eroshenko, 1984
Varotylus Siddiqi, 1986

and Kırklareli (Merkez). *Tylenchorrhynchus cylindricus* was recovered from Edirne (Keşan, Uzunköprü, Meriç, İpsala, Enez), Tekirdağ (Şarköy, Malkara, Süleymanpaşa), Kırklareli (Merkez). Individuals of species were extracted by centrifuge flotation method of Jenkins (1964).

Species identifications were carried out by morphometric and morphologic observations of female individuals from prepared nematode slides. In order to prepare slides extracted females were heat killed at 60°C for one minute, mounted in TAF solution and placed on slides by wax-ring method (Seinhorst, 1959).

The morphometrics of nematodes was measured with software of Leica DM1000 microscope and the images were taken with a Celestron camera. The morphometric parameter ratios and indices (Table 1) developed by De Man (1876) were used for characterization. The classifications was conducted based on Siddiqi (2000).

Results and Discussion

Rotylenchus cypriensis Antoniou, 1980

(Figure 1 A-B)

Measurements at Table 2.

Classification

Order: Tylenchida Thorne, 1949; **Infraorder:** Tylenchata (Thorne, 1949) M.R. Siddiqi, 2000; **Suborder:** Hoplolaimina Chizhov & Berezina, 1988; **Superfamily:** Hoplolaimoidea Filipjev,

1934 (Paramonov, 1967); **Family:** Hoplolaimidae Filipjev, 1934 (Wieser, 1953); Subfamily: Hoplolaiminae; **Genus:** *Rotylenchus* Filipjev, 1936; **Species:** *Rotylenchus cypriensis*

Synonym

Anguillulina (Rotylenchus) Filipjev, 1936
Gottholdsteineria Andrassy, 1958
Orientalus Jairajpuri and Siddiqi, 1977
Calvatylus Jairajpuri and Siddiqi, 1977
Interrotylenchus Eroshenko, 1984

Morphology

Female straight or C shaped when relaxed. Lip region hemispherical with 3-4 annuli, headset off from the rest of the body. Lateral field with four lines, isolated only in the oesophageal region. Cephalic framework was well developed. Median bulb is strongly well developed and oval shaped. Stylet strong with anteriorly flattened

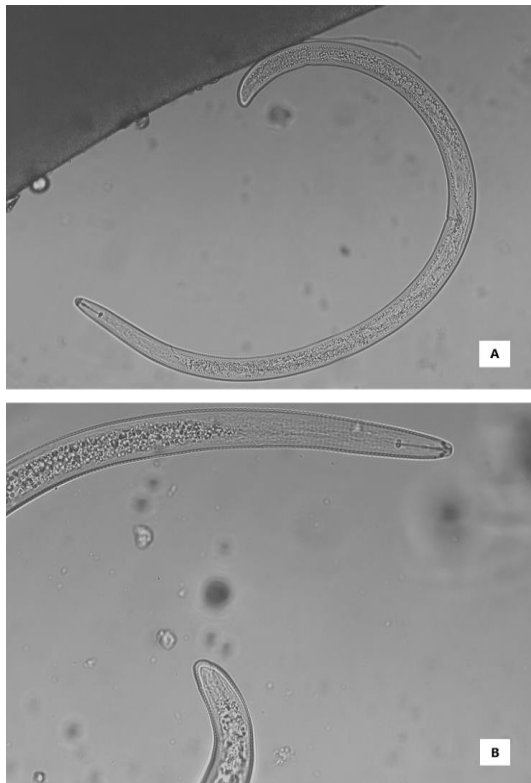


Figure 1. *Rotylenchus cypriensis* female A) Entire body B) Head and tail region.

Table 2. Morphometrics of *Rotylenchus cypriensis*. Measurements except L in μm

	Thrace	Antoniou (1980)	
		Holotype	Paratype
n	3	-	16
L (mm)	0.59 \pm 0.03 (0.55-0.63)	0,63	0.60 (0.50-0.69)
a	29.1 \pm 2.91 (25.2-32.1)	27	30 (25-39)
b	5.22 \pm 0.1 (5.11-5.36)	5.9	5.7 (5.3-6.4)
c	45.5 \pm 10 (41.1-48.4)	46	45 (40-49)
c'	1.06 \pm 0.01 (1.05-1.08)	1.4	1.2 (0.9-1.5)
Stylet (μm)	23.36 \pm 0.54 (22.6-23.8)	24	23 (21-24)
Tail (μm)	12.6 \pm 0.54 (12.2-13.4)	-	11-14
Vulva %	63 \pm 0.81 (62-64)	57	63 (67-66)

Mean \pm (SD) range

rounded knobs. Spermatheca small, sperms not observed. Two outstretched ovaries, oocytes lined in single row. Tail tip rounded. Phasmid located anterior to anus. Males not found.

Remarks

R. cypriensis has previously been isolated from grapevines in Southeastern Turkey (Kasapoğlu et al., 2012). The morphometrics of population from our study are similar with reports of Antoniou (1980).

Helicotylenchus canadensis Wasseem, 1961

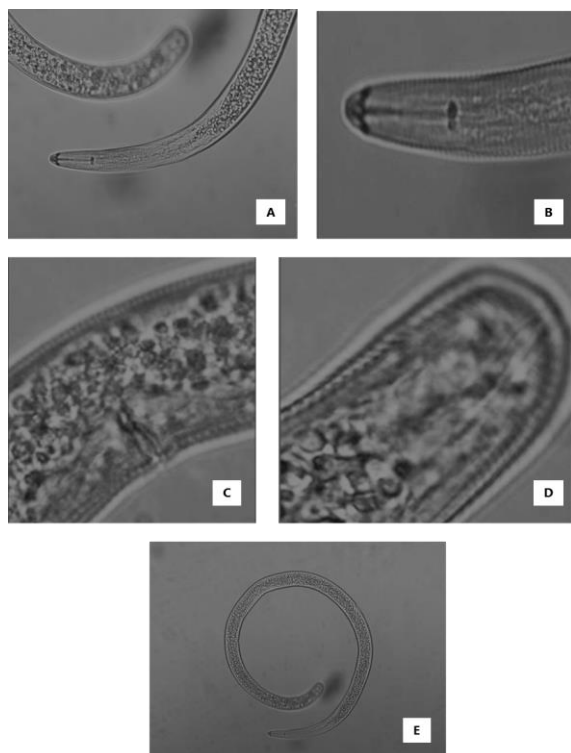
(Figure 2 A-B-C-D-E)
 Measurements at Table 3.

Classification

Order: Tylenchida Thorne, 1949; **Infraorder:** Tylenchata (Thorne, 1949) M.R. Siddiqi, 2000; **Suborder:** Hoplolaimina Chizhov & Berezina, 1988; **Super family:** Hoplolaimoidea Filipjev, 1934 (Paramonov, 1967); **Family:** Hoplolaimidae Filipjev, 1934 (Wieser, 1953);

Table 3. Morphometrics of *Helicotylenchus canadensis*. Measurements except L in μm

	Thrace	Yeates and Wouts (1992)	Firoza and Maqbool (1994)
n	7	10	18
L (mm)	0.85±0.57 (0.80-0.88) 31.9±0.06	0.74-0.85	0.68-0.97
a	(31.80-32.9)	25-31	20-34
b	6.32±0.65 (6.03-7.44)	5.9-7.0	-
c	58.5±9.64 (45.4-71.9)	45-60	38-51
c'	0.89±0.04 (0.84-0.94)	0.8-1.1	-
Stylet (μm)	26.3±1.19 (24.8-30)	28-32	28-30
Tail (μm)	14.01±1.11 (12.1-15.7)	13-19	10-12
Vulva %	61±0.01 (60-63)	59-63	61-66

Mean \pm (SD) range.**Figure 2.** *Helicotylenchus canadensis* female A) Head region B) Lip region and stylet C) Vulva D) Tail E) Entire body.

Subfamily: Rotylenchoidinae Whitehead, 1958
Genus: *Helicotylenchus* Steiner, 1945; **Species:** *Helicotylenchus canadensis* Wasseem, 1961

Synonym*Helicotylenchus cairnsi* Wasseem, 1961**Morphology**

Head killed female body forms open spiral shape. Lip region offset, strongly sclerotized and has five lip annules. The annules in the mid body and posterior part wider than neck region. Cuticle is transversely striate.

Excretory pore located anterior to level of the esophagus-intestinal valve. Lateral field with four visible incisures. Stylet well developed, knobs anteriorly concave. Hemizonid two annules long, located anterior to the excretory pore.

Medium bulb oval shaped. Reproductive system with two outstretched ovaries. Oocytes in single row position. Phasmids located anterior to anus level. Tail dorsally convex with annulations at ventral side. Males not observed.

Remarks

The morphology of Thrace populations resembles reports of Yeates and Wouts (1992), Firoza and Maqbool (1994).

The species has been reported from soil samples from apple, strawberry, kiwi, melon, watermelon, lentil, tea, potato, walnut, pear and almond growing areas in several location across Turkey (Kepenekci, 1999a; Kepenekçi and Akgül, 1999; Kepenekci and Öztürk, 1999; Akgül, 2004; Osmanoğlu, 2005; Karakaş, 2018; Tan et al., 2019).

***Pratylenchus neglectus* Filipjev & Stekhoven, 1941**

(Figure 3 A-B-C-D)

Measurements at Table 4.

Classification

Order: Tylenchida Thorne, 1949; **infraorder:** Tylenchata (Thorne, 1949) M.R. Siddiqi, 2000;
Suborder: Tylenchina Chitwood, 1950;

Super family: Tylenchoidea Örley, 1880; **Family:** Pratylenchidae Thome, 1949 (Siddiqi, 1963); Pratylenchinae Thorne, 1949; **Genus:** *Pratylenchus* (Filipjev, 1936); **Species:** *Pratylenchus neglectus* Filipjev & Stekhoven, 1941

Synonyms

Aphelenchus neglectus Rensch, 1924;

Table 4. Morphometrics of *Pratylenchus neglectus*. Measurements except L in μm

	Thrace	Kumari (2015)	Wu et al. (2013)
n	11	200	-
L (mm)	0.39±0.02 (0.34-0.45)	0.41-0.57	0.42-0.54
a	25.4±1.81 (24.8-28.3)	19.2-27.9	18.8-18.9
b	4.7±0.42 (4.09-5.5)	3.7-5	4.4-5.7
c	20.46 ±2.33 (15.4-23)	16-26.7	19.8-25.4
c'	1.78±0.08 (1.66-1.90)	1.6-2.7	4.2-5.5
Stylet (μm)	14±1.02 (12-15.1)	14-19	17.1-18.9
Tail (μm)	16,58±1,02 (15.7-19)	19-33	-
Vulva %	81.09±1.08 (81.6-82)	78-84	80.4-84.8

Mean ± (SD) range.



Figure 3. *Pratylenchus neglectus* female A) Entire body B) Lip region and stylet C) Vulva D) Tail.

Tylenchus neglectus (Rensch) Steiner, 1928
Anguillulina (Pratylenchus) neglecta (Rensch) W. Schneider, 1939
P. minyus Sher&Allen, 1953
P. capitatus Ivanova, 1968
P. noecapitatus Khan and Singh, 1975
P. similis Khan and Singh, 1975

Morphology

Female body C shaped when relaxed. Lip region rounded with 5 annuli, head slightly sclerotized. Head continuous with the rest of the body. Lateral field with six lines. Lines reduce to 4 at tail region.

Median bulb, oval shaped with valve. Cardia at the end of basal bulb. Basal bulb saccate occupying almost all the body width. Excretory pore posterior to isthmus, Hemizonid just posterior to isthmus. Stylet short, knobs rounded and posterior part flattened. Spermatheca rounded. Two outstretched ovaries, oocytes in single row. Tail conical and rounded. Phasmid is just anterior to mid-tail. Males not recovered.

Remarks

The morphology of Thrace populations was similar to reports of Kumari (2015) and Wu et al. (2013)

P. neglectus was found on different host plants in Turkey including corn, potato, pepper, wheat, strawberry, grapevine and walnut (Yüksel, 1974; Borazancı, 1977; Tunçdemir, 1983; Akgül, 1996; Kepenekçi, 1999a; Kovancı et al., 2000; İmren, 2007; Erdoğuş, 2009; Tan et al., 2018).

Pratylenchus thornei Sher and Allen, 1953

(Figure 4 A-B-C-D)
Measurements at Table 5.

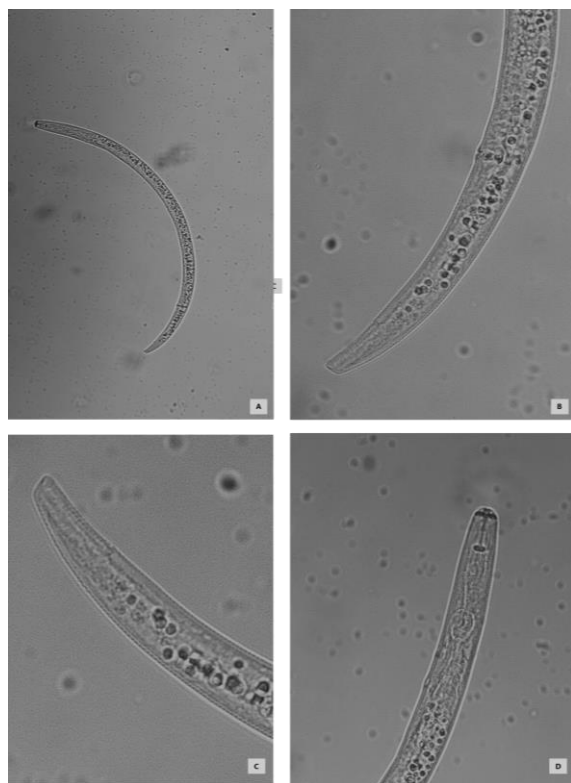
Classification

Order: Tylenchida Thorne, 1949; **Infraorder:** Tylenchata (Thorne, 1949) M.R. Siddiqi, 2000; **Suborder:** Tylenchina Chitwood, 1950; **Super family:** Tylenchoidea Örley, 1880; **Family:** Pratylenchidae Thome, 1949 (Siddiqi, 1963); Pratylenchinae Thorne, 1949; **Genus:** *Pratylenchus* (Filipjev, 1936);

Table 5. Morphometrics of *Pratylenchus thornei*. Measurements except L in μm

	Thrace	Troccoli et al. (1992)	Fayazi et al. (2012)
n	12	11	10
L (mm)	0.53±0.04 (0.49-0.57)	0.46-0.61	0.42-0.62
a	31.2±2.39 (26.7-35.7)	22.7-29.1	30-39.8
b	5.2±0.42 (4.76-5.92)	4.5-5.7	5.8-6.9
c	16.7±1.074 (15.1-18.4)	20-23.5	19.9-23.8
c'	2.52±0.02 (2.50-2.58)	1.8-2.4	1.6-2.1
Stylet (μm)	15.1±0.36 (14.2-15.5)	15-16	16-18
Tail (μm)	33±1.56 (31-35)	-	30
Vulva %	77±0.09 (77-78)	76.5-79	79-79.9

Mean± (SD) range.

**Figure 4.** *Pratylenchus thornei* female A) Entire body B) Vulva+tail C) Tail D) Head region.**Species:** *Pratylenchus thornei*, (Sher and Allen 1953), (Sher, 1948).**Morphology**

Female body slightly curved to ventral. Lip region straight, head slightly flattened. Head continuous with the rest of the body. Lip region with 3 annuli. Lateral field with four lines. Median bulb well developed, round shaped with valve. Isthmus is short and cylindrical.

Hemizonid two annuli long. Excretory pore located at the base of isthmus of just posterior to hemizonid. The style is well developed with rounded knobs. Post uterine sac as long as 1/5 body diameter. Spermatheca oval and without sperm. Two outstretched ovaries, oocytes in single row. Tail rounded, tail tip smooth. Phasmid is anterior to mid tail. Males were not observed.

Remarks

The morphology and morphometric parameters fit with Troccoli et al. (1992) and Fayezi et al. (2012).

Table 6. Morphometrics of *Tylenchorrhynchus cylindricus*. Measurements except L in μm

	Thrace	Lewis and Golden (1981)	Kepekçi (2012)
n	1	7	15
L (mm)	0.91	0.72-1.11	0.56-0.69
a	35	30.6-40.8	29.3-37.6
b	5.86	4.5-6.9	4.6-6.1
c	20.7	15.3-26.7	18.6-23.5
c'	2.46	-	2.4-3
Stylet (μm)	22	25.1-29.5	19-25
Tail (μm)	44.2	37.4-51.5	29-31
Vulva %	52	51-61	54-59.5

Mean± (SD) range.



Figure 5. *Thylenchorrhynchus cylindricus* female A) Entire body B) Vulva C) Head region D) Tail.

Other hosts addressed in Turkey were tomato, watermelon, melon, chickpea, phaseolus, eggplant, cotton, pepper, lentil, barley, poppy, anise, apple, pear and citrus (Elekçioğlu, 1992; Elekçioğlu and Uygun, 1994; Kepenekci and Ökten, 1999b; Kepenekci and Öztürk, 2002a; Kepenekci and Zeki, 2002; Kepenekci and Evlice, 2003; Tan, 2005; Evlice and Kepenekci, 2006; Mısırlıoğlu and Pehlivan, 2007; Evlice and Ökten 2008).

***Thylenchorrhynchus cylindricus*, Cobb,1913**

(Figure 5 A-B-C-D)

Measurements at Table 6.

Classification

Order: Tylenchida Thorne, 1949; **Infraorder:** Tylenchata (Thorne, 1949) M.R. Siddiqi, 2000;

Suborder: Tylenchina Chitwood, 1950; **Super family:** Tylenchoidea Örley, 1880; **Family:** Dolichodoridea Chitwood in Chitwood & Chitwood, 1950 (Siddiqi, 1986); **Subfamily:** Telotylenchinae Siddiqi, 1960; **Genus:** *Thylenchorrhynchus*, Cobb,1913; **Species:** *Thylenchorrhynchus cylindricus* Cobb, 1913

Synonyms

Tylenchus cylindricus (Cobb) Flipjev *Anguillulina cylindrica* (Cobb, Thorne).

Morphology

Female body slightly curved to ventral when heat killed. Body cylindrical.

Lip region rounded with 5 annuli, head slightly sclerotized. Head set off from the rest of the body. Lateral field with four lines, aerolated only in oesophageal region. Cephalic framework well developed. Median bulb well developed, oval shaped with valve. Basal bulb saccate with prominent cardia at the base. Hemizonid just anterior to excretory pore. Excretory pore located between median bulb and oesophagus base. Lateral field with four incisures. Stylet strong with knobs anterior side directed forward. Spermatheca rounded, filled with sperm. Two outstretched ovaries, oocytes in single row. Tail cylindrical and narrower at tail tip. Phasmid in the middle of the tail. Males not found.

Remarks

The body diameters and descriptions of Thrace populations correspond with reports of Lewis and Golden (1981) and Kepenekçi (2012). *T. cylindricus* has only isolated from vegetable gardens in Turkey (Ediz and Enelli, 1978).

Conflicts of Interest

The authors declare that there is no conflict of interest.

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