Akademik Ziraat Dergisi 12(2): 187-198 (2023)
ISSN: 2147-6403 e-ISSN: 2618-5881 DOI: http://dx.doi.org/10.29278/azd.1333478

Araştırma
(Research)

Morphological Characteristics and Molecular Analysis of Newly Recorded *Longidorus moesicus* Lamberti, Choleva & Agostinelli, 1983 (Dorylaimida: Longidoridae) From Türkiye*

Serkan ÇELİK[1], Faruk AKYAZI[1*]

[1]Ordu Üniversitesi, Ziraat Fakültesi, Bitki Koruma Bölümü, Ordu/TÜRKİYE

*This article has been generated from the first author's master's thesis. We thank Ordu University for their support to this project (ODUBAP; Project No, BY-1713)

Alınış tarihi: 27 Temmuz 2023, Kabul tarihi: 4 Eylül 2023

Sorumlu yazar: Faruk AKYAZI, e-posta: farukakyazi@hotmail.com

Abstract

Objective: This study was conducted to determine the morphological, morphometric, and molecular characteristics of *Longidorus moesicus*, which was first recorded in Türkiye.

Materials and Methods: A nematode survey was conducted in rose cultivation greenhouses in 2017 in Yalova province, Türkiye. Nematodes were extracted from soil samples using Cobb's sieve method and centrifugal flotation technique. Morphological characters and morphometric measurements were used to identify the obtained nematodes. Additionally, identification was confirmed by molecular analyses of the 28S rRNA gene D2/D3 expansion region sequences.

Results: Needle nematodes in the genus *Longidorus* (Micoletzky, 1922) include many ectoparasitic nematode species that transmit nepoviruses as well as causing damage by direct feeding on root cells. A *Longidorus* species was found in the examined soils.

Conclusion: To our knowledge, this is the first report of *Longidorus moesicus* Lamberti, Choleva & Agostinelli, 1983 in Türkiye.

Keywords: Needle *nematodes*, *Longidorus* spp., *Longidorus moesicus, Rosa* spp.

Öz

Amaç: Bu çalışma, Türkiye'de ilk kayıt olan *Longidorus moesicus*'un morfolojik, morfometrik ve moleküler karakterlerlerinin belirlenmesi amacıyla yapılmıştır.

Matertal ve Yöntem: Yalova ili gül yetiştirilen seralarda 2017 yılında nematod sürveyi gerçekleştirilmiştir. Nematodlar, santrifüj ve Cobb elek yönteminin bir modifikasyonu ile topraktan ekstrakte edilmiştir. Elde edilen nematodların teşhisinde morfolojik karakterler ve morfometrik ölçümler kullanılmıştır. Teşhisler ayrıca 28S rRNA geni D2/D3 genleşme bölgesi dizilerinin moleküler analizleri ile doğrulanmıştır.

Araştırma Bulguları: *Longidorus* (Micoletzky, 1922) cinsi içerisinde yeralan iğne nematodları, bitki hücreleri ile doğrudan beslenerek zarar vermesinin yanısıra nepovirüsleri ileten çok sayıda ektoparazitik nematod türlerini içermektedir. İncelenen topraklarda *Longidorus* cinsi bir nematod türü tespit edilmiştir.

Sonuç: Bildiğimiz kadarıyla *Longidorus moesicus* Lamberti, Choleva & Agostinelli, 1983'ün Türkiye için ilk rapordur.

Anahtar kelimeler: İğne nematodları, *Longidorus* spp., *Longidorus moesicus, Rosa* spp.

Türkiye'den Yeni Bir Kayıt *Longidorus moesicus* Lamberti, Choleva & Agostinelli, 1983 (Dorylaimida: Longidoridae)'un Morfolojik Özellikleri ve Moleküler Analizi

Introduction

Longidorus spp., known as needle nematodes first described by Micoletzky in 1922, represent a significant group of plant parasites (Sirca and Urek, 2009).

Çelik, S. & Akyazı, F. (2023). Morphological Characteristics and Molecular Analysis of Newly Recorded *Longidorus moesicus* Lamberti, Choleva & Agostinelli, 1983 (Dorylaimida: Longidoridae) From Türkiye. *Akademik Ziraat Dergisi*, 12(2), 187-198.

The *Longidorus* genera encompass numerous large plant ectoparasitic nematode species that severely damage various host plants (Tzortzakakis et al., 2014). Apart from inducing direct damage through feeding, certain species within this genus are recognised for their capability to transmit plant viruses (Taylor, 1962; Harrison, 1964; Archidona-Yusta et al., 2019). This genus constitutes a large group of plant-ectoparasitic nematodes that are distributed nearly worldwide. The first description of *Longidorus moesicus* was made by Lamberti et al. (1983) from the Bulgarian rhizosphere of black currant (*Ribes nigrum* L.). Later, it was reported from numerous plant species, including *Populus* sp., *Trifolium* sp., pear and grapevine, *Urtica* sp., and *Rubus* sp., in various regions of Serbia (Barsi and Lamberti, 2004). *Longidorus moesicus* was recently discovered in a vineyard soil sample from Slovenia (Širca and Urek, 2009). This species was most recently found in the rhizosphere of grapevine in Crete Island in Greece (Tzortzakakis et al., 2014). The genus *Longidorus* consists of more than 176 valid species in the world (Gutiérrez et al., 2020; Archidona-Yuste et al., 2019) but, there were very limited reports of *Longidorus* species in Türkiye. Until now, four species have been reported from Türkiye: *Longidorus attenuatus* Hooper, 1961*, Longidorus elongatus* (de Man, 1876) Mycoletzky, 1922 on *Vitis vinifera* L. (Arınç, 1982; Elekçioğlu et al., 1992; Öztürk and Enneli, 1994, Kepenekci et al., 2006), *Longidorus goodeyi* Hooper, 1961 and *Longidorus leptocephalus* Hooper, 1961 on *Medicago sativa* L. (Öztürk and Enneli, 1994). A survey study was carried out to detect plant parasitic nematodes in cut flowers cultivation areas in Yalova province in Türkiye. Needle nematodes belonging to the genus of *Longidorus* were found in the soils taken from a rose greenhouses in Çiftlikköy, Yalova (N40°39'5.68";E 29°18'20.04"). *Longidorus* group nematodes are significant plant parasites because of their ability to transmit plant viruses. Therefore, it is essential that these species need to be clearly identified. Species identification is based primarily on morphometrics. However, variations within morphometrics increase the potential for misidentification among species (Ye & Robbins, 2004). In this research, we analysed the morphometric characteristics of *Longidorus* sp. and compared them with specific traits of previously identified species. Additionally, we employed a Scanning Electron Microscope (SEM) to visualise surface features, particularly the head structures of nematodes that are not easily observable with the Light Microscope. Additionally, molecular analysis was used to confirm morphological identification. Researchers have reported that employing a combination of morphology and molecular analysis in taxonomy is valuable for identifying *Longidorus* species (Archidona-Yuste et al., 2019; Cai et. al., 2020).

This study aimed at detailed morphological, morphometric, and molecular characterisation of the newly recorded species *L. moesicus* Lamberti et al., 1983, isolated from the rhizosphere soils of roses in greenhouses in Türkiye.

Materials and Methods

Needle nematode population

Soil samples with *Longidorus* population were collected from the rhizosphere of roses in a greenhouse in Çiftlikköy of Yalova, as a part of the survey to determine the plant-parasitic nematodes in cut flower growing areas in Yalova province in Türkiye in September 2017 (Figure 1).

Figure 1. A map of the location and greenhouse where roses are produced in Çiftlikköy, Yalova

Nematodes were extracted from the soil using Cobb's sieving method (Cobb, 1918) and sugar-flotation-sieving method (Byrd et. al.1966). The specimens were handpicked, heat-killed, fixed in TAF, and transferred to glycerin according to Seinhorst (1959).

Morphological and morphometrical characterization

For the morphological and morphometric analysis, female nematodes were used. The measurements and preparation of microphotographs were carried out using a Carl Zeiss Axio light microscope, which was equipped with a ZEISS Axiocam 105 digital camera. The morphometric measurements of nematodes were analysed using Microsoft Excel. The morphometric characteristics of *L. moesicus* detected in this study were compared with the original Bulgarian population measured by Lamberti et al, (1983). The morphometrics of the female was also compared with the previously identified Sirbian (Barsi & Lamberti, 2004), Slovenian (Širca & Urek, 2009), and Greece (Tzortzakakis et al., 2014) populations.

Scanning electron microscopy (SEM)

Females and fourth stage juveniles (J4) were prepared for SEM according to Eisenback's methods (Eisenback, 1985). Fixed nematodes were transferred to 0.2 M phosphate buffer for 1 hour at 4 °C (Seinhorst, 1959). Then, 6% glutaraldehyde was added three times every 30 minutes and stored in the refrigerator for 24 hours. The nematodes were once again transferred to 0.1 M phosphate buffer (pH 7.2), and a 2.0% aqueous osmium tetroxide solution was added. They were then left in this solution for 12 hours. The samples were washed in 0.1 M phosphate buffer as before, and then held in a series of 10% to 100% absolute ethanol. Nematodes were placed on double-sided carbon conductive tape and coated with 10 nm gold with a Spray Coater (SPC-900-C). The samples were scanned using Scanning Electron Mycroscope (Hitachi SU1510) at Ordu University.

Molecular characterization

A single mature female individual was used for genomic DNA extraction for molecular analyses. A single female was transferred and crushed in a 10 µl of extraction buffer (10 mM Tris-HCl, pH 8.8, 1 mM EDTA). Then, 0.1 % Triton X-100 (v/v) and 0.1 mg/ml proteinase K) were added to it and kept overnight at -20 °C (Pagan et al., 2015). At the end of the period, the mixture was incubated at 56°C for 1 hour, then at 95°C for 10 minutes. The mixture was used as a DNA template for polymerase chain reaction (PCR).

PCR amplification of D2/D3 28S rDNA was carried out with the following primers: D2A (5′-ACAAGTACCGTGAGGGAAAGTTG-3′), D3A (5′-GACCCGTCTTGAAACACGGA-3′), and D3B (5′-TCGGAAGGAACCAGCTACTA-3′) (Castillo et al. 2003; He et al. 2005; Palomares-Rius et al. 2008). The PCR was performed in a final volume of 25 µl (8.5 µl distilled water, 12.5 µl 2x Hot Start Master mix (New England BioLabs, Ipswich, MA), 1.25 µl of each primer (10 pMol/µl), and 1.5 µl of DNA template. The PCR reaction was conducted with a Veriti™ 96-Well Thermal Cycler (Singapore) and the program was as follows: an initial denaturation step at 95°C for 3 min, followed by 40 cycles of 30 s at 95°C, 45 s at 53°C, 1 min at 72°C with a final extension at 72°C for 10 min. DNA fragments were separated on 1.5% agarose gel in 1X Tris-acetate EDTA (TAE) buffer stained with ethidium bromide. The gel was run for 25 min at 150 V then visualized and photographed under UV light using ErBiyotek GEN-BOX imageER Fx. Sequence analysis of PCR products was performed at STAB VIDA in Portugal. Sequencing was conducted using an ABI 3730xl DNA Analyzer (Applied Biosystems). The obtained DNA sequences were used for BLASTN comparison against the GenBank sequence.

Phylogenetic analysis

Sequences of several species of the *Longidorus* genus used for phylogenetic analysis were obtained from the GenBank database of the NCBI (Table 1). For the phylogenetic analysis, a total of 33 D2 LSU rDNA sequences from previously identified *Longiorus* specimens were aligned with the sequences obtained in this study. The alignment was carried out using Clustal W in MEGA 7.0 software (Kumar et al., 2016). The evolutionary history was inferred using the Maximum Likelihood method, based on the Tamura-Nei model (Tamura and Nei, 1993). The phylogenetic tree of the D2 expansion segment of 28S rRNA was built using *Globodera rostochiensis* (KJ409633) as an outgroup, providing a basis for further exploration. A phylogram was constructed with data from 1000 bootstrap replicates, allowing for a detailed visual representation of the evolutionary history between species.

Results

Longidorus moesicus was reported for the first time in rhizosphere of rose in greenhouse in Yalova province in Türkiye.

Table 1. Nematodes Species and GenBank accession numbers used for the phylogenetic analysis.

Species	GenBank Accession number	Origin
L. moesicus	KJ802876	Greece
L. moesicus	KJ802875	Greece
L. moesicus	HM447029	Slovenia
L. iranicus	KP222294	Iran
L. iranicus	MK894273	Iran
L. iranicus	MK894272	Iran
L. pseudoelongatus	KJ802871	Greece
L. pseudoelongatus	KJ802870	Greece
L. pseudoelongatus	KJ802873	Greece
L. pauli	MW598386	Greece
L. pauli	MW598387	Greece
L. pauli	MW598384	Greece
Longidorus sp.	KF242334	Russia
L. proximus	MK894276	Iran
L. proximus	MT176055	Iran
L. proximus	MK894275	Iran
L. closelongatus	KJ802866	Greece
L. closelongatus	KJ802864	Greece
L. closelongatus	KJ802863	Greece
L. cretensis	KJ802869	Greece
L. cretensis	KJ802867	Greece
L. cretensis	KJ802868	Greece
L. attenuatus	KT755457	Ukraine
L. attenuatus	KR911851	Poland
L. attenuatus	AY601572	Germany
L.apulus	MK894282	Iran
L. apulus	MK894280	Iran
L. apulus	MK894278	Iran
L. iluturgenensis	MH430013	Spain
L. iluturgenensis	MH430012	Spain
L. azarbaijanensis	MG765547	Iran
L. azarbaijanensis	MF677863	Iran
Globodera rostochiensis	KJ409633	United Kingdom

Morphological characterization

Female: Female individuals obtained have curved bodies as a result of fixation. When killed females, the body ventrally curved in an open "C" shape (Figure 2C). The reproductive system displays amphidelphy, with both branches exhibiting nearly equal development. Odontostyle long and slender. Odonophore typical of the genus with basal slight swellings. The lip region frontally flattened and slight depression (Figure 4A). The tail is slightly ventrally curved, ending in a rounded terminus, and bears three caudal pores (Figure 5B). Obtained morphometric data of this species from Türkiye are presented in Table 1. Body length was characterised by high size (7.3 ± 1.1 mm). The maximum body diameter at the base of the pharynx is 52.5 ± 3.5 µm. Total stylet length 196.2 ± 3.5 µm, odontosytle length 128.5 ± 2.4 µm, odontophore length 67.7 ± 2.3 µm on average. The guide ring is prominent in the head part and its distance from the anterior is 32.8 ± 0.9 µm (Figure 4A). The vulva is located close to the middle parts of the body and its distance from the anterior and posterior are 3932.3±417.2 and 3452.4 ± 741.3 µm respectively. The tail is 51.3 ± 5.4 µm on average and the tail ends round (Figure 4D; 5B).

Male: Males were not found.

The morphological and morphometrical data of female, in this study were compared with previously identified species from several different countries. Morphometrics of females are given in Table 2 and photomicrographs are presented in Figure 4 and 5. Firstly, compared to the original Bulgarian population described by Lamberti et al. (1983), morphologically adult females from the Türkiye have similar characteristics. But, morphometric characters differ from the original population.The Turkish population of *L. moesicus* females differs from Bulgaria population by having longer body length (7.3 *vs* 7.2 mm), stylet length (196.2 *vs* 182 µm), odontostyle length (128.5 *vs* 119 µm), odonophore length (67.7 *vs* 63 µm), body diameter at lip region (12.4 *vs* 12 µm), body diameter at base of pharynx (52.5 *vs* 50 µm),

body diameter at vulva (63.2 *vs* 61 µm), body diameter at anus (50.3 *vs* 42 µm), tail length (51.3 *vs* 43 µm). However, the measurement of diameter at guide ring (24.3 *vs* 25 µm) was shorter than the original population described by Lamberti et al. (1983).

The main differences between Turkish population of *L. moesicus* and other populations of this species are longer body length (7.3 *vs* 6.5 mm (Serbian) (Barsi & Lamberti, 2004); 6.9 mm (Slovenian) (Širca & Urek, 2009); 5.3 mm (Greek) (Tzortzakis et al., 2014) and longer odontostyle (128.5 *vs* 114 µm) (Serbian); 107.1 µm (Slovenian); 100.5 µm (Greek)) and odonophore (67.7 *vs* 60.3 µm (Serbian); 48.7 µm (Slovenian); 53 µm (Greek)) and longer tail lenght (51.3 *vs* 39 µm (Serbian); 42.2 µm (Slovenian); 36 µm (Greek)). The Turkish population also differs from other populations by the smaller distance from anterior end to guide ring (32.8 *vs* 37.6 µm (Serbian); 35.6 µm (Slovenian) and the more narrow body diameter at guiding ring (24.3 vs 25.1 µm (Serbian); 26.6 µm (Slovenian); 32.3 µm (Greek)) and longer body diameter at base of pharynx, vulva and anus.

Consequently, the morphometrics obtained in this study show differences with the populations detected in previous studies, which may be due to the geographic intraspecies variability.

Table 2. Comparison of the morphological characteristics of *Longidorus moesicus* female with previously recorded world populations (All measurements are in µm (except L in mm) and expressed as means ± standard deviation (range).

Characters	**Türkiye** Present study	**Bulgaria** Lamberti et al. (1983)	**Serbia** Barsi & Lamberti(2004)	**Slovenia** Širca & Urek(2009)	**Greece** Tzortzakakis et al.(2014)
n	**5**	**10**	**12**	**17**	**2**
	Mean±SD (Min.-Max.)	Mean±SD (Min.-Max.)	Mean±SD (Min.-Max.)	Mean±SD (Min.-Max.)	Mean±SD (Min.-Max.)
L	**7.3**±1.1 (6.3-9.6)	**7.2** (6.4-8.0)	**6.5**±0.63 (5.7-7.56)	**6.9**±0.7 (5.9-8.4)	**5.3**±0.4 (5.1-5.6)
a	**115.4**±5.8 (106-122)	**120** (96-147)	**124.4**±6.46 (115.4-139.5)	**128.6**±7.4 (117-145.5)	**101.7**±6.0 (97.5-105.9)
c	**143.0** ±7.3 (135-156)	**170** (146-186)	**166.8**±15.28 (141-197)	**164.3**±18.0 (129-200.8)	**148.7**±16.6 (137.0-160.4)
c'	**0.8**±0.4 (0.0-1.0)	**1** (0.8-1.2)	**0.94**±0.09 (0.79-1.06)	**1.05**±0.08 (0.90-1.23)	**0.9**±0.03 (0.9-1.0
V %	**53.6** ±2.3 (49.1-55.7)	**53** (50-54)	**48.9**±1.73 (44.8-51.5)	**52.7**±1.4 (49.6-54.6)	**48.5**±0.7 (48-49)
Stylet lenght	**196.2**±3.5 (191.0-201.5)	-	**174.3**±5.05 (163.8-183.8)	**155.8**±5.9 (143.1-161)	-
Odontosytle	**128.5**±2.4 (124.5-132.1)	**119** (115-124)	**114**±5.60 (102.5-125)	**107.1**±4.4 (96.3-114.6)	**100.5**±4.9 (97-104)
Odontophore	**67.7**±2.3 (64.1-69.7)	**63** (59-66)	**60.3**±3.70 (55-67.5)	**48.7**±4.0 (42-56.4)	**53.0**±2.8 (51-55)
Vulva from anterior end	**3932.3±417.2** (3515.0-4728.8)	-	-	-	-
Vulva from posterior end	**3452.4±741.3** (2798.5-4900.7)	-	-	-	-
Oral aperture to guide ring	**32.8**±0.9 (32.0-34.5)	**34** (32-38)	**37.6**±1.80 (34.4-41.9)	**35.6**±1.6 (32.8-39.1)	-
Body diameter at lip region	**12.4**±0.5 (11.6-12.9)	**12** (11-13)	**13.1**±0.36 (12.8-13.8)	**12.4**±1.0 (10.5-13.9)	**11.0**±0.7 (10.5-11.5)
Body diameter at guide ring	**24.3**±1.3 (22.8-26.3)	**25** (24-26)	**25.1**±0.81 (23.4-26.3)	**26.6**±1.1 (24.2-28.4)	**32.3**±3.2 (30.0-34.5)
Body diameter at base of pharynx	**52.5**±3.5 (48.6-57.4)	**50** (45-61)	**44.4**±2.50 (40-49.7)	**46.0**±2.9 (41.9-50.8)	-
Body diameter at vulva	**63.2**±9.4 (56.5-81.9)	**61** (52-71)	**52.1**±3.04 (48.1-58.4)	**53.6**±3.7 (48.4-59.9)	-
Body diameter at anus	**50.3**±10.4 (43.9-70.9)	**42** (39-49)	**40.5**±1.67 (37.8-43.1)	**40.2**±3 (36.3-46.1)	-
Tail length	**51.3**±5.4 (45.7-61.7)	**43** (40-49)	**39**±2.45 (34.3-42.5)	**42.2**±3.8 (36.2-49.4)	**36.0**±1.4 (35-37)

Abbreviations: n: number of nematodes measured; L: body length; a: body length/maximum body diameter; c: body length/tail length; c': tail length/body width at anus; V%: distance of the vulva from anterior end expressed as a percentage of body length.

Scanning Electron microscopy (SEM)

Morphological features of *L. moesicus* based on SEM were also observed in this study. No SEM observations were found in previous *L. moesicus* identification studies. SEM observations of female showed that the oral opening was round and surrounded by six inner labial sensilla. The lip region is flat and slightly depressed (Figure 5).

Molecular characterization

The amplification of the D2-D3 expansion segments of the 28S rRNA gene resulted in a single fragment of approximately 782 bp for the D2A/D3B primer and 350 bp for the D3A/D3B primer (Figure 2). Based on the BLAST analysis, D2-D3 expansion segments of 28S rRNA of *L. moesicus* showed 99.6% similarity (with 100% query cover) with the samples of *L. moesicus* with accession number KJ802876 isolated from Harakas province in Greece and accession number HM447029 isolated from Slovenia.

Phylogenetic analysis

The Maximum Likelihood (ML) method was employed to generate a phylogenetic relationship based on the D2 region of the 28S rRNA gene, using corresponding nucleotide sequences from *L. moesicus* (Figure 3). The result of the phylogenetic analysis, in 28S phylogeny, *L. moesicus* formed a clade. According to the phylogenetic analysis, our specimen was found to be the sister species of the clade formed by *Longidorus* iranicus (KP222294). This clade, in turn, was the sister group of *Longidorus pseudoelongatus* from Greece. In the phylogenetic tree reconstructed based on 28S sequences, *L. moesicus* (Turkish population) formed a monophyletic group with three additional *L. Moesicus* sequences from Greece and Slovenia, with 68% bootstrap support (Figure 3).

Discussion

The *Longidorus* genus (Micoletzky, 1922) contains long ectoparasitic nematode species that are polyphageal in many plants, including various agricultural products, and transmit nepoviruses to the plant roots and damage directly by stem cells (Taylor and Brown, 1997). Studies have reported that clover, beet, lettuce, water lily, grape, strawberry, olive and citrus are among the hosts of *Longidorus* species (Norton and Hoffmann, 1975; Robbins and Brown, 1996). According to Buser (1990), it is seen that the nematodes carry the virus in their bodies for a while after ingesting it with their food. Approximately 170 *Longidorus* species have been identified, of which only 9 have been reported as virus vectors, and 7 of 38 nepoviruses have been reported to be transmitted by these species (Taylor and Brown, 1997; Decraemer and Robbins, 2007). In the most *Longidorus* species, the virus can be transported in less than three months. Hence, accurate identification of *Longidorus* species is essential for the implementation of appropriate control measures.

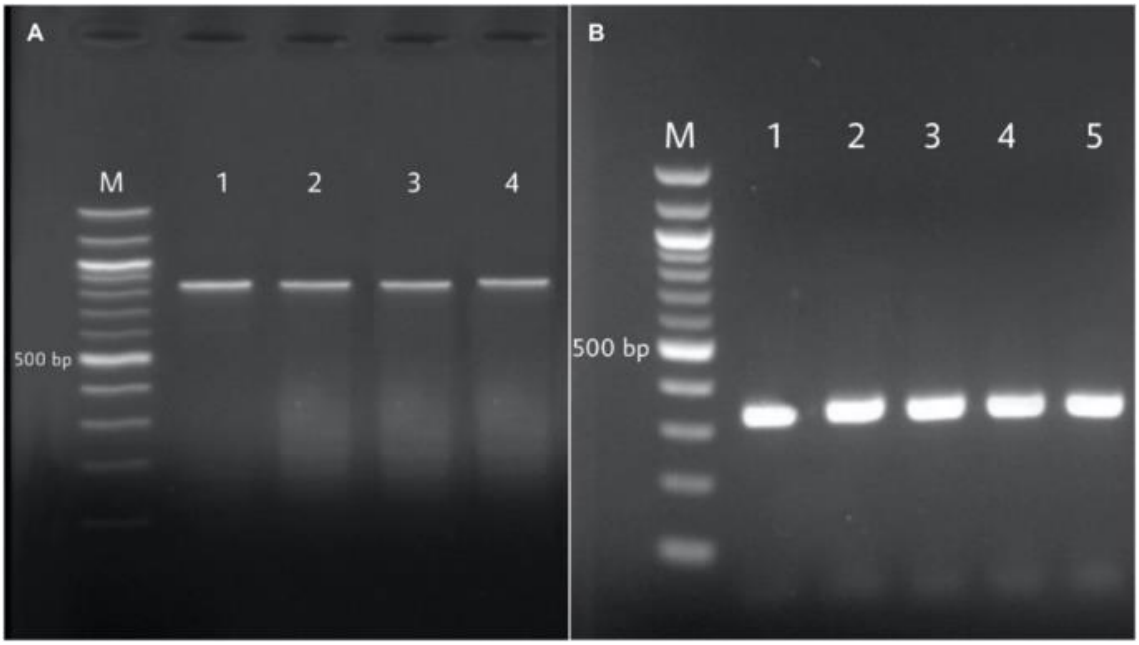

Figure 2. PCR products of *Longidorus moesicus* species. A: Fragments of D2/D3 region of 28S rDNA using D2A/D3B primers (Line1- 4); B: Fragments of D2/D3 region of 28S rDNA using D3A/D3B primers (Line1-5); M:100 bp DNA ladder.

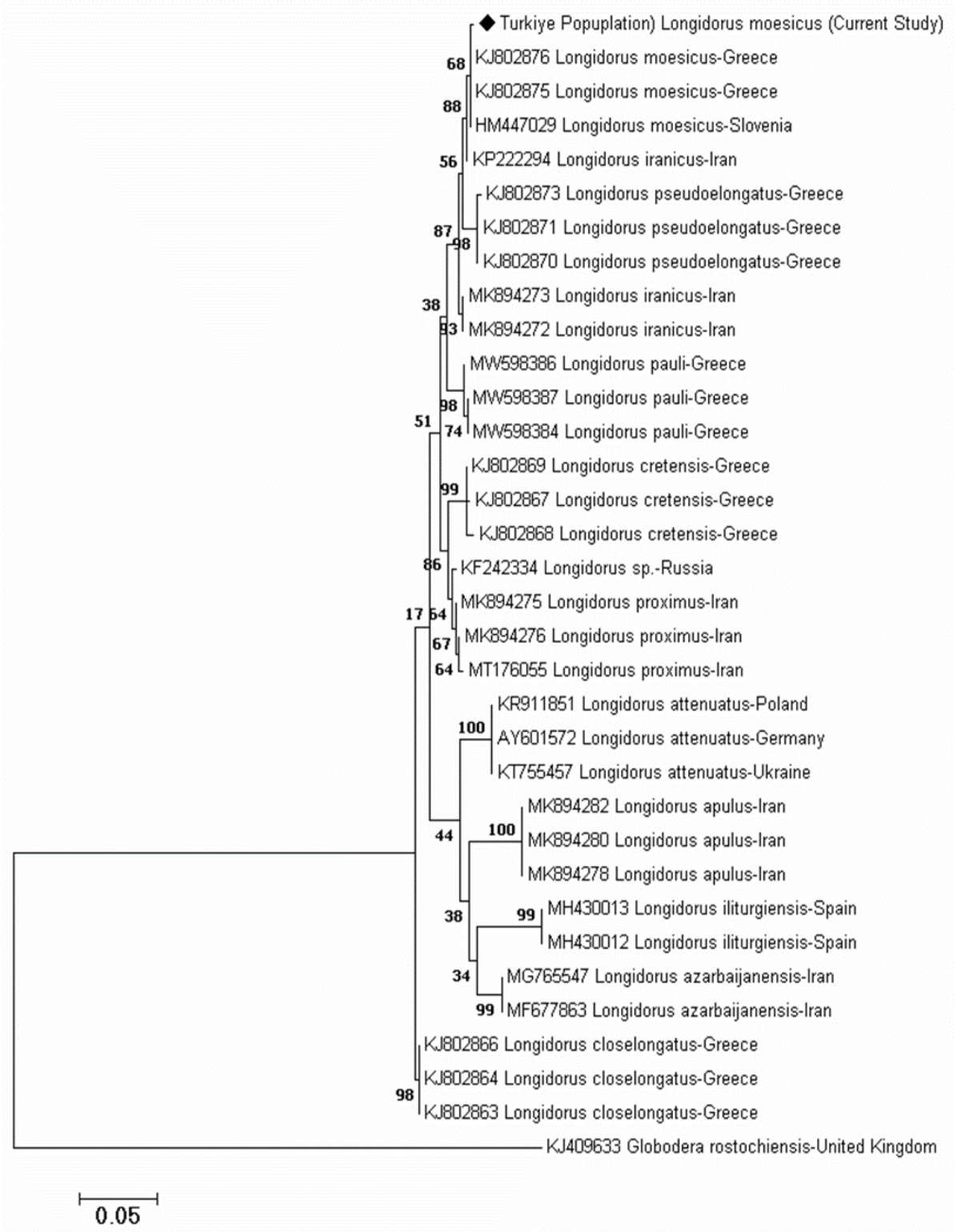

Figure 3. The phylogenetic relationship between *Longidorus* sp. species. Maximum Likelihood (ML) phylogenetic tree of *Longidorus moesicus* from Türkiye inferred D2-D3 of 28S rDNA region based on the Tamura-Nei model. *Globodera rostochiensis* was used as outgroup. The analysis was made using 1000 bootstrap replicates.

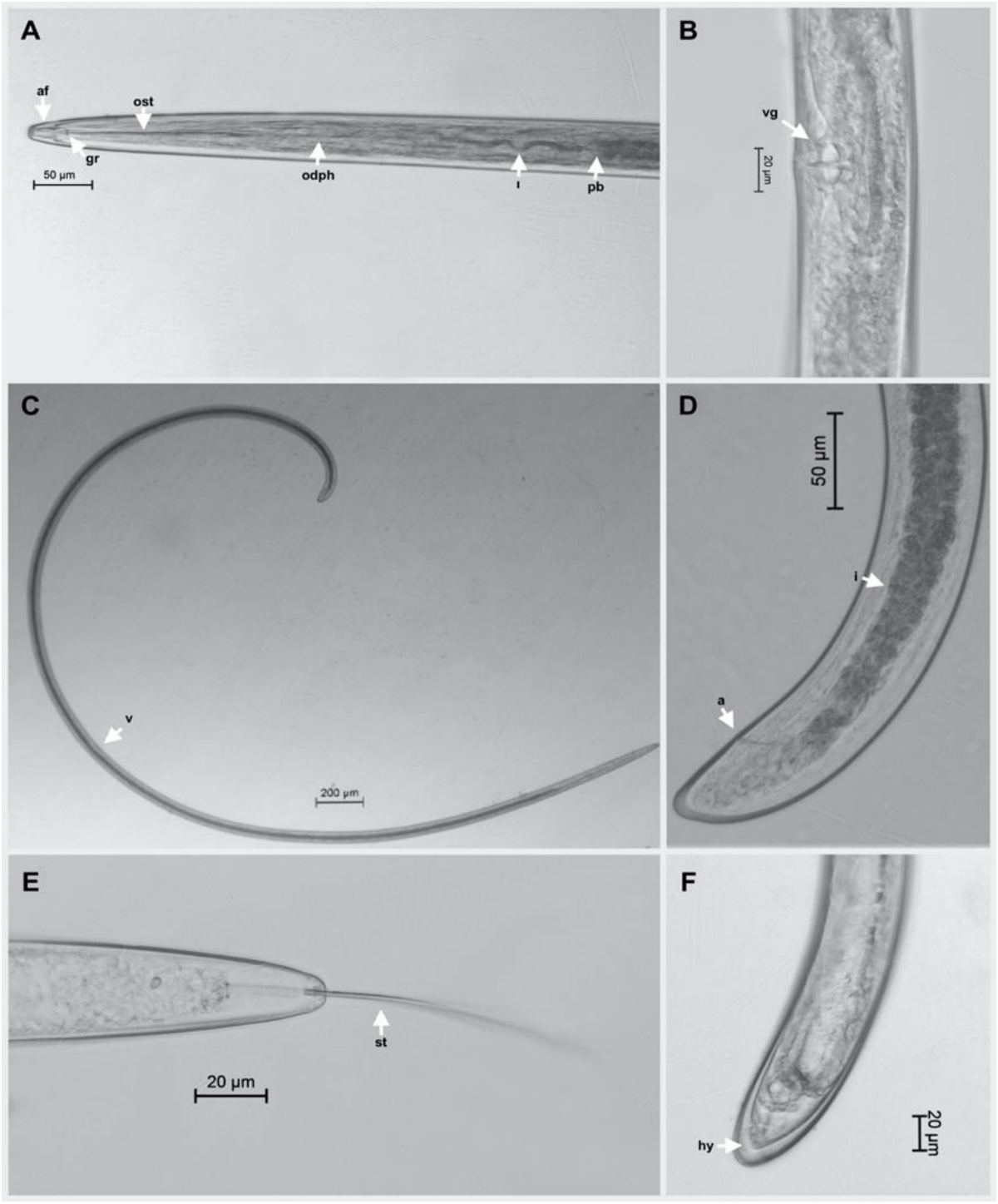

Figure 4. Light micrographs of *Longidorus moesicus* from Türkiye. A: Female anterior region, B: Vulval region, C: Whole body, D: Tail region, E: Larvae (4th) anterior region F: Larvae (4th) tail region. Abbreviations: **st**: stylet, **af**: amphidial fovea, **gr**: guiding ring; **ost**: odonthostyle; **odph**: odonophore, **l**: lumen, **lbp** = pharyngeal bulb, **vg**: vagina, **V**: vulva, **a**: anüs, **i:** intestine, **hy**: hyalin.

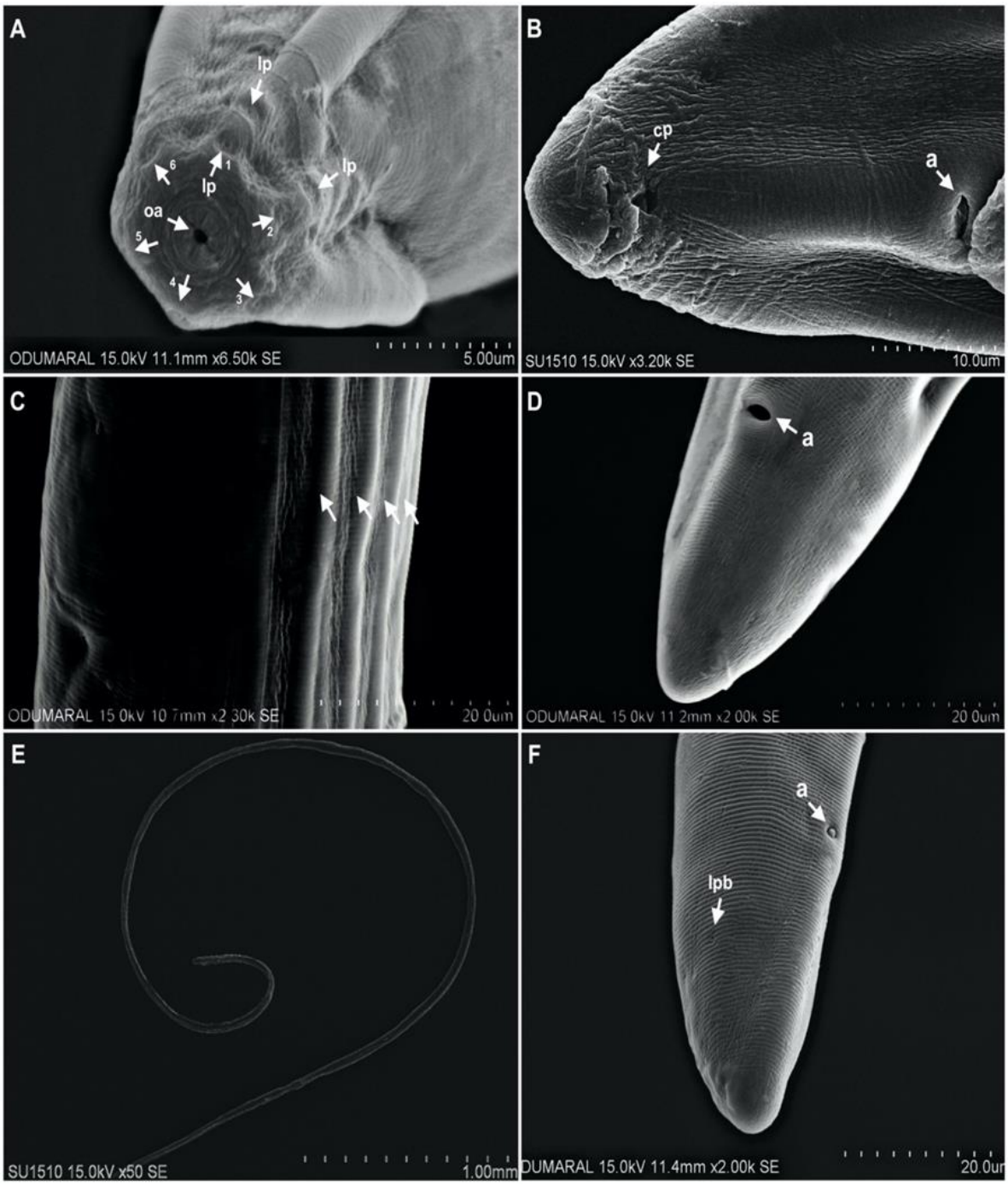

Figure 5. SEM photomicrographs of *Longidorus moesicus* female from Türkiye. A: Anterior region, B:Tail region, C: Mid-body, D: Tail region, E: Whole body, F: Tail. Abbreviations: **a**: anus; **oa**: oral aperture; **lp**: labial papilla; **cp**:caudal pore **lbp** = lateral body pore; **V**: vulva

Longidorus moesicus was first detected in black currants in Kostinbrod near Sofia, Bulgaria in the world (Lamberti et al., 1983). Other locations where it was found in the same study; D'Lgopol (apple), Dolna Banya (black currant), Krivnya (grapevine), Petrich (grapevine), Razgrad (grapevine), Shumen Dragoevo (grapevine), Travnovo (plum) and Varna (rose). This species was also reported in vineyards in Italy (Coiro et al., 1992), pear, *Populus* sp., *Rubus* sp., *Trifolium* sp., *Urtica* sp. and vine in Serbia (Barsi and Lamberti, 2004), vineyards in Slovenia (Sirca and Urek, 2009), and the island of Crete in Greece (Tzortzakakis et al., 2014). A study conducted in Türkiye show that, *Longidorus* nematodes have been reported in vineyards and olive fields in the Salihli district of Manisa (Aydeniz et al., 2018).

Longidorus elongatus was identified in soils in grape growing areas of the Thrace Region (Öztürk et al., 2017).

In this study, *L. moesicus* species was reported in a rose cultivated in greenhouse. Despite several *Longidorus* species has been identified in Türkiye, *Longidorus moesicus* was recorded for the first time. The morphological and morphometric measurements of the females of *L. moesicus* population in this study were similar to the Bulgarian population previously found by Lamberti et al. (1983).

Conclusion

Distinguishing *Longidorus* species is challenging due to their conserved and overlapping morphological and molecular characteristics. Therefore, a combination of morphological and molecular analyses together with scanning electron microscopy was used to identify *L. moesicus* from the rhizosphere of roses in Türkiye. The present study confirms the correct diagnosis of this species. This is the first report of this nematode in Türkiye and it represents an important reference for upcaming studies in the future**.**

Conflict of Interest

The authors state that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Author Contribution Statement

SC: The individual has contributed to the planning, execution and laboratory analyses and writing stages of the research.

FA: The individual has contributed to the planning, execution, laboratory analyses, data evaluation and writing stages of the research.

References

Archidona-Yuste, A., Cantalapiedra-Navarrete, C., Castillo, P., & Palomares-Rius, J. E. (2019). Molecular phylogenetic analysis and comparative morphology reveals the diversity and distribution of needle nematodes of the genus *Longidorus* (Dorylaimida: Longidoridae) from Spain. *Contributions to Zoology*, *88*(1), 1-41.

Arinç, Y. (1982). Ege Bölgesi Bağ Alanlarında Zararlı Olan *Xiphinema* Türleri (Nematoda: Longidoridae), Yayılışı, Konukcuları ve Zararları Üzerinde Araştırmalar. *Izmir Böl. Zir. Müc. Aras. Ens. Arastìrma Eserleri Serisi*, *41*, 83s.

Aydeniz, M. M., Özdemir, F. G. G., & Yaşar, B. (2018). Manisa ili Salihli ilçesinde meyve bahçelerinde bulunan bitki paraziti nematodların belirlenmesi. 6th ASM International Congress of Agriculture and Environment, 2018, Isparta, Türkiye (in Turkish with abstract in English), 68-75.

Byrd, D. W., JR., Nusbaum, C. J. & Barker, K. R. (1966). A rapid flotation-sieving technique for extracting nematodes from soil. *Plant Disease Reporter*, 50, 954-957.

Barsi, L., & Lamberti, F. (2004). Morphometrics of *Longidorus* juvenilis and first record of L. aetnaeus and L. moesicus (Nematoda: Dorylaimida) from Serbia. *Nematologia mediterranea*, *32*(1), 5-14.

Buser, A. 1990. Untersuchungen über die Pfeffingerkrankheit der Süsskirsche und deren Véklor *Longidorus macrosoma*. Eidgn. Techn. Hochschule Zürich, Switzerland, Dissertation No 9194, 205p.

Cai, R., Prior, T., Lawson, B., Cantalapiedra-Navarrete, C., Juan E. Palomares-Rius, Castillo, P. & Archidona-Yuste, A. 2020. An integrative taxonomic study of the needle nematode complex *Longidorus goodeyi* Hooper, 1961 (Nematoda: Longidoridae) with description of a new species. *European journal of Plant Pahology*, (158), 59-81.

Castillo, P., Vovlas, N., Subbotin, S., & Troccoli, A. (2003). A new root-knot nematode, Meloidogyne baetica n. sp.(Nematoda: Heteroderidae), parasitizing wild olive in Southern Spain. *Phytopathology*, *93*(9), 1093-1102.

Cobb, N. A. (1918). Agricultural technology circular. *Washington, DC: US Department of Agriculture, Bureau of Plant Industry*, (1), 48.

Coiro, M. I., Agostinelli, A., & Lamberti, F. (1992). I Longidoridae (Nematoda) nei vigneti della provincia di Verona. *Nematologia Mediterranea*, *20*, 87-95.

Decraemer, W., & Robbins, R. T. (2007). The who, what and where of Longidoridae and Trichodoridae. *Journal of Nematology*, *39*(4), 295.

Eisenback, J. (1985). Diagnostic characters useful in the identification of the four most common species of rooot-knot nematodes (*Meloidogyne* spp). En:Bartker, K.; Carter, C. y Sasser, J. (Ed). An advanced treatise on *Meloidogyne*. Vol. II, Methodology.North Carolina State University Graphics, p.95-112.

Elekcioglu, I. H. (1992). Untersuchungen Zum Auftreten und Zur Verbreitung Phytoparasitarer Nematogen in den Land Wirtschafflichen Hauptkulturen des Astmediterranen Gebretes der Turkei. Plits, *10*(5): 120 (in German with abstract in English).

Gutiérrez-Gutiérrez, C., Santos, M. T., Inácio, M. L., Eisenback, J. D., & Mota, M. (2020). Description of *Longidorus bordonensis* sp. nov. from Portugal, with systematics and molecular phylogeny of the genus (Nematoda, Longidoridae), Zoosystematics and Evolution, *96*(1): 175-193.

Harrison, B. D. (1964). Specific nematode vectors for serologically distinctive forms of raspberry ringspot and tomato black ring viruses. *Virology*, *22*(4), 544-550.

He, Y., Subbotin, S. A., Rubtsova, T. V., Lamberti, F., Brown, D. J., & Moens, M. (2005). A molecular phylogenetic approach to Longidoridae (Nematoda: Dorylaimida). *Nematology*, *7*(1), 111-124.

Hooper, D. J. (1961). A redescription of *Longidorus elongatus* (de Man, 1876) Thome & Swanger, 1936 (Nematoda, Dorylaimidae) and descriptions of five new species of Longidona from Great Britain. *Nematologica*, *6*(3), 237-257.

Kepenekci, İ., Evlice, E., & Toktay, H. (2006). Plant parasitic nematodes associated with Vineyards (Vitis vinifera L.) in the Central anatolia region of Turkey. In *28th Europen Society of Nematologist Congress, Blagoevgrad, Sofia, Bulgaria, Pensoft Publishers. Abstract*, 156 p.

Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular biology and evolution*, *33*(7), 1870-1874.

Lamberti, F. & Roca, F. (1987). Present status of nematodes vectors of plant viruses. In: Veech, J. A., Dickson, D.W. (Eds). Vistas on Nematology. Florida, De Leon Springs, pp. 321-328.

Lamberti, F., Choleva, B., & Agostinelli, A. (1983). Longidoridae from Bulgaria (Nematoda, Dorylaimida) with description of three new species of *Longidorus* and two new species of Xiphinema. *Nematologia mediterranea*, *11*(1): 49-72.

Micoletzky, H., (1922). Die freilebenden Erd-Nematoden mit besonderer Berücksichtigung der Steiermark und der Bukowina, zugleich mit einer Revision sämtlicher nichtmariner, freilebender Nematoden in Form von GenusBeschreibungen und Bestimmungsschlusseln. Archiv für Naturgeschichte *87*(9), 321–650.

Norton, C. D., & Hoffmann, J. K. (1975). *Longidorus breviannulatus* n. sp.(Nematoda: Longidoridae) associated with stunted corn in Iowa. *Journal of Nematology*, *7*(2), 168-171.

Öztürk, G., & Enneli, S. (1994). Distribution of plant parasitic nematodes in alfa growing areas in Central Anatolia Region of Turkey. In *9th Congress of the Mediterranean Phytopathological Union* (pp. 537-538).

Öztürk, L., Avcı, G. G., Behmand, T. & Elekcioğlu, H. I. (2017). Incidence of viruses and vector nematodes in Thrace vineyards, Turkey. International Journal of Agriculture and Environmental Research, 3 (6): 4078- 4089.

Pagan, C., Coyne, D., Carneiro, R., Kariuki, G., Luambano, N., Affokpon, A., & Williamson, V. M. (2015). Mitochondrial haplotype-based identification of ethanol-preserved root-knot nematodes from Africa. *Phytopathology*, *105*(3), 350-357.

Palomares-Rius, J. E., Subbotin, S. A., Landa, B. B., Vovlas, N., & Castillo, P. (2008). Description and molecular characterisation of *Paralongidorus litoralis* sp. n. and *P. paramaximus* Heyns, 1965 (Nematoda: Longidoridae) from Spain. *Nematology*, *10*(1), 87-101.

Robbins, R. T., & Brown, D. J. F. (1996). Descriptions of three new *Longidorus* species from Alaska (Nematoda: Longidoridae). *Journal of nematology*, *28*(1), 83-93.

Seinhorst, J. W. (1959). A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. *Nematologica*, *4*(1), 67-69.

Širca, S., & Urek, G. (2009). Morphological and molecular characterization of six *Longidorus* species (Nematoda: Longidoridae) from Slovenia. *Russian Journal of Nematology*, *17*(2), 95.

Tamura, K., & Nei, M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular biology and evolution*, *10*(3), 512-526.

Taylor, C. E., & Brown, D. J. F. (1997). *Nematode vectors of plant viruses* (CAB International). Wallingford, UK. pp. 286.

Taylor, G. E. (1962). Transmission of raspberry ringspot virus by *Longidorus elongatus* (de Man)(Nematoda: Dorylaimidae). *Virology*, *17*(3), 493-494.

Tzortzakakis, E. A., Archidona-Yuste, A., Cantalapiedra-Navarrete, C., Nasiou, E., Lazanaki, M. S., Kabourakis, E. M., Palomares-Rius, J. E. & Castillo, P. (2014). Integrative diagnosis and molecular phylogeny of dagger and needle nematodes of olives and grapevines in the island of Crete, Greece, with description of *Xiphinema cretense* n. sp. (Nematoda, Longidoridae). *European Journal of Plant Pathology*, *140* (3), 563-590.

Tzortzakakis, E. A., Clavero-Camacho, I., Cantalapiedra-Navarrete, C., Ralli, P., Palomares-Rius, J. E., Castillo, P., & Archidona-Yuste, A. (2021). Morphological and molecular characterisation of (Nematoda: Longidoridae), first report from Greece. *Journal of Nematology*, *53*(1), 1-10.

Waele, D. D., & Coomans, A. (1990). Occurrence and ecology of longidorid nematodes in Belgium. *Revue de nématologie*, *13*(2), 197-202.

Xu, Y., Guo, K., Ye, W., Wang, J., Zheng, J., & Zhao, Z. (2017). Morphological and molecular characterisation of *Longidorus juglans* sp. nov. and a sister species L. fangi Xu & Cheng, 1991 (Nematoda: Longidoridae) from China. *Nematology*, *19*(8), 951-970.

Ye, W., & Robbins, R. T. (2004). Cluster analysis of *Longidorus* species (Nematoda: Longidoridae), a new approach in species identification. *Journal of Nematology*, *36*(3), 207-218