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Original article (Orijinal araştırma)

Identification and distribution of root-knot nematode species (*Meloidogyne* spp.) in vegetable growing areas of Lakes Region in Turkey[1]

Türkiye Göller Bölgesi sebze üretim alanlarında kök-ur nematodu türleri (*Meloidogyne* spp.)'nin tanılanması ve yaygınlıkları

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Summary

In this study, the distribution and characterization of root-knot nematode species collected from intensively vegetable growing areas of Lakes Region were determined by morphological, molecular and North Carolina Differential Host Test between September 2014 and December 2015. A total of 160 samples were collected and 83 (51.8%) were found to be infested with root-knot nematodes. Each population was cultured from a single egg mass taken from galled roots and multiplied on the susceptible tomato cv. Tueza F1. Sixty-eight populations were morphologically identified based on perineal patterns and morphometrics of second stage juveniles, and molecularly determined using species specific primers. Of the 68 populations analyzed, 66 were identified as *Meloidogyne incognita* (25), *M. hapla* (22), *M. javanica* (18) and *M. arenaria* (1), and two populations were not identified. The incidence of *M. incognita*, *M. hapla*, *M. javanica* and *M. arenaria* was 36.7, 32.3, 36.5 and 1.5%, respectively. According to the differential host test, *M. incognita* races 2, 4 and 6 and *M. javanica* races 1 and 3 were determined. This was the first detection of *Meloidogyne javanica* race 3 in Turkey. Eighty four percent of the *M. incognita* populations were found in microclimatic areas with altitudes of up to 800 m, while 16% were found at altitudes between 800 and 1035 m. Some *M. javanica* populations (17%) were found in high plateau fields in this region, whereas most (83%) were found in lowlands. In contrast, the large majority of *M. hapla* populations (91%) of were found in cool, high altitude areas with sandy soils, whereas only 9% of *M. hapla* populations were in lowlands.

Keywords: Lakes Region, *Meloidogyne* spp., molecular identification, morphological identification, PCR, race

Özet

Çalışmada, Göller Bölgesi'nde yoğun sebze üretimi yapılan alanlarda, Eylül 2014 – Aralık 2015 yılları arasında toplanan kök-ur nematodu türleri morfolojik, moleküler ve Kuzey Karolina Konukçu Testi yöntemleri kullanılarak karakterize edilmiş ve yayılışları belirlenmiştir. Toplam 160 adet örnek alınmış ve 83 tanesinin (%51.8) kök-ur nematodu ile bulaşık olduğu bulunmuştur. Urlu kök örneklerinden tek yumurta paketi alınarak duyarlı 'Tueza F1' domates çeşidinde her popülasyonun saf kültürleri oluşturulmuş ve kitle üretimleri yapılmıştır. Altmış sekiz popülasyonun tür tanımlamaları morfolojik olarak dişi bireylerin perineal bölge modelleri ve ikinci dönem larva ölçümlerinden ve moleküler olarak türe özgü spesifik primerler kullanılarak yapılmıştır. Tanımlanan 68 kök-ur nematodu popülasyonundan 25 adedi *M. incognita*, 22 adedi *M. hapla*, 18 adedi *M. javanica* ve 1 adedi de *M. arenaria* olarak tespit edilmiş, iki popülasyonun ise tanılamaları yapılamamıştır. Türlerin yaygınlık oranları sırasıyla, %36.7, %32.3, %26.5 ve %1.5 olarak belirlenmiştir. Konukçu testine göre, *M. incognita*'nın ırk 2, ırk 4 ve ırk 6, *M. javanica*'nın ise ırk 1 ve ırk 3'ü belirlenmiş ve *M. javanica* ırk 3 Türkiye'de ilk kez rapor edilmiştir. *Meloidogyne incognita* popülasyonlarının %84'ü 800 m yükseltiye sahip mikroklima bölgelerde bulunurken, %16'sı 800-1035 m yükseltiye sahip örtü altı alanlarda tespit edilmiştir. Bazı *M. javanica* populasyonları (%17) yüksek yayla bölgelerinde tespit edilirken, çoğu (%84) alçak yükseltiye sahip sebze alanlarında bulunmuştur. Buna karşılık *M. hapla* popülasyonlarının büyük çoğunluğu (%91) daha yüksek serin bölgelerde ve kumsal toprak yapısına sahip alanlarda bulunurken, sadece %9 'u alçak yükseltiye sahip bölgelerde bulunmuştur.

Anahtar sözcükler: Göller Bölgesi, *Meloidogyne* spp., moleküler tanılama, morfolojik tanılama, PCR, ırk

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Introduction

Root-knot nematodes are one of the most devastating plant parasitic nematodes, affecting yield and quality of many crops, particularly causing economically significant losses in vegetable production. Blok et al. (2008) reported that the annual damage to vegetables from root-knot nematodes amounts to more than €80 billion. Lamberti (1978) and Davis & May (2005) reported that in tropical and subtropical climatic regions, root-knot nematodes caused yield loss of 47 29, 23, 22 and 15% in tobacco, tomato, eggplant, okra and pepper, respectively. Similarly, Netscher & Sikora (1990) reported that root-knot nematodes caused yields losses of 42-54% in tomatoes and 30-60% in eggplants. These nematodes also cause significantly economic loses in Turkey. Ağdacı (1978) found root-knot nematodes causing 17-47% loss in cucumber greenhouses in Antalya and Adana. Söğüt & Elekcioğlu (2007) reported that yield losses of about 80% in pepper greenhouses in Adana.

More than 90 root-knot nematode species have been identified across the world (Hunt & Handoo, 2009; Moens et al., 2009). Johnson & Fassuliotis (1984) that across 75 countries, 1000 root-knot nematode populations were 52% *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949, 30% *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949, 8% *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949, 8% *Meloidogyne hapla* Chitwood, 1949 and the remaining 2% other species. Similarly, root-knot nematodes are one of main groups of pests in the vegetable growing areas of the Mediterranean, Aegean and Black Sea Regions of Turkey. *Meloidogyne incognita* and *M. javanica* were dominant species in coastal regions of Turkey. Also, *M. arenaria, M hapla , Meloidogyne chitwoodi* Golden, O'Bannon, Santo & Finley, 1980, *Meloidogyne ethiopica* Whitehead, 1968, *Meloidogyne artiellia* Franklin, 1961 and *Meloidogyne exigua* Goeldi, 1892 have been found in Turkey (Elekcioğlu et al., 1994; Mennan & Ecevit, 1996; Kaşkavalcı & Öncüer, 1999; Söğüt & Elekçioğlu, 2000; Devran & Söğüt, 2009; Özarslandan et al., 2009; Akyazı & Ecevit, 2010; Yıldız & Elekçioğlu, 2011; Aydınlı et al., 2013; İmren et al., 2014; Kepenekçi et al., 2014). In the recent years, *M. chitwoodi* has been identified in potato producing areas in the Central Anatolia Region as an invasive species and *M. ethiopica* was identified in vegetable crops in the Black Sea Region (Aydınlı & Mennan, 2016; Evlice & Bayram, 2016). Particular *Meloidogyne* species have races distinguishable by a standardized set of differential hosts according to North Carolina Differential Host Test (Sasser & Triantaphyllou, 1977). This differential host test distinguishs four races of *M. incognita,* two races of *M. javanica* and two races of *M. arenaria* (Hartman & Sasser, 1985; Decker & Fritzsche, 1991). Carneiro et al. (2004) and Robertson et al. (2009) found new races of these common species in Brazil and Spain. Devran & Söğüt (2011) determined the new races of *M. incognita* (race 6) and *M. arenaria* (races 2 and 3) in the western coastal areas of the Mediterranean Region of Turkey.

Accurate identification of root-knot nematode species and races is important for management and control of these pests through host resistance, biological management and crop rotation. The Lakes Region of Turkey has agricultural areas with different geographic characteristics and altitudes from 275 to 1430 m in Burdur and Isparta Provinces. Vegetables can be grown twice a year in locations such as Çandır, Yeşilyurt, Elsazı and Çamlık with relatively low altitudes. Vegetables are also grown in other microclimatic areas at higher altitudes. The total production of vegetables, including bean, cucumber, eggplant, okra, sweet pepper and tomato, is about 283 kt/yr (TUIK, 2014). Thus, this region has a significant role for vegetables production in Turkey, particularly in of summer and early autumn. Root-knot nematodes infest crops in the region and are a major concern for farmers.

The purpose of study was to identify root-knot nematode species in vegetable growing areas of Lakes Region of Turkey using morphology, molecular methods and differential host test and to determine their distribution.

Material and Methods

Sampling and culturing of root-knot nematode populations

A total of 160 samples were collected from vegetable growing areas of Lakes Region between September and October, 2014. Samples consisted of about 19% open fields and 81% greenhouses. Between five and 20 plants were uprooted and average five root systems and soil samples were collected at each location. The number of samples each town were determined according to the size of the vegetable production areas reported by TUIK (2014). Locations infested with root-knot nematodes host plants, geographic coordinates, and altitudes are given in Table 1.

Table 1. Root-knot nematode populations and their host plants, production system, location, coordinates and altitudes from samples collected in Lakes Region of Turkey

Code	Host Plant	Production system	Location	Latitude (N)	Longitude (E)	Altitude (m)
B6	Eggplant (*Solanum melongena* L.)	Greenhouse	Askeriye/Burdur	37° 45' 54.2"	30° 21' 22.3"	960
B7	Pepper (*Capsicum annuum* L.	Greenhouse	Askeriye/Burdur	37° 45' 30.5"	30° 19' 54.0"	923
B10	Tomato (*Lycopersicon esculentum* Mill.)	Greenhouse	Askeriye/Burdur	37° 45' 18.2"	30° 19' 30.9"	925
B11	Pepper (*C. annuum*)	Greenhouse	Askeriye/Burdur	37° 45' 24.2"	30° 17' 21.4"	877
B12	Eggplant (*S. melongena*)	Greenhouse	Askeriye/Burdur	37° 45' 24.4"	30° 17' 19.2"	875
B13	Tomato (*L. esculentum*)	Greenhouse	Askeriye/Burdur	37° 45' 24.4"	30° 17' 19.2"	875
B15	Pepper (*C. annuum*)	Field	Elsazı/Burdur	37° 26' 34.0"	30° 47' 10.5"	276
B16	Cucumber (*Cucumis sativus* L.)	Greenhouse	Elsazı/Burdur	37° 26' 34.0"	30° 47' 10.5"	276
B18	Cucumber (*C. sativus*)	Greenhouse	Elsazı/Burdur	37° 26' 35.5"	30° 47' 09.6"	275
B19	Eggplant (*S. melongena*)	Field	Elsazı/Burdur	37° 27' 14.2"	30° 48' 46.4"	308
B22	Cucumber (*C. sativus*)	Greenhouse	Elsazı/Burdur	37° 26' 51.8"	30° 47' 12,8"	276
B23	Cucumber (*C. sativus*)	Greenhouse	Elsazı/Burdur	37° 26' 54.4"	30° 48' 32.5"	284
ISP28	Eggplant (*S. melongena*)	Greenhouse	Elsazı/Burdur	37° 27' 55.8"	30° 47' 45.1"	288
ISP30	Eggplant (*S. melongena*)	Greenhouse	Elsazı/Burdur	37° 26' 55.8"	30° 48' 31.8"	287
B24	Tomato (*L. esculentum*)	Greenhouse	Söğüt/Burdur	37° 01' 13.9"	29° 49' 13.1"	1430
B25	Tomato (*L. esculentum*)	Greenhouse	Söğüt/Burdur	37° 01' 03.9"	29°49' 24.6"	1433
B26	Tomato (*L. esculentum*)	Greenhouse	Söğüt/Burdur	37° 01' 05.4"	29°49' 20.5"	1434
B27	Tomato (*L. esculentum*)	Greenhouse	Söğüt/Burdur	37° 00' 54.0"	29° 49' 24.5"	1436
Ç4	Cucumber (*C. sativus*)	Greenhouse	Çamlık/Burdur	37° 29' 24.3"	30° 45' 26.4"	366
Ç5	Cucumber (*C. sativus*)	Greenhouse	Çamlık/Burdur	37° 29' 08.4"	30° 45' 30.4"	350
Ç7	Eggplant (*S. melongena*)	Field	Çamlık/Burdur	37° 29' 00.6"	30° 45' 37.8"	341
Ç8	Bean (*Phaseolus vulgaris* L.)	Greenhouse	Çamlık/Burdur	37° 28' 19.8"	30° 45' 34.7"	327
Ç9	Lettuce (*Lactuca sativa* L.)	Greenhouse	Çamlık/Burdur	37° 28' 38.3"	30° 44' 57.2"	360
Ç11	Cucumber (*C. sativus*)	Greenhouse	Çamlık/Burdur	37° 29' 01.0"	30° 45' 46.0"	338
Ç12	Cucumber (*C. sativus*)	Greenhouse	Çamlık/Burdur	37° 28' 57.9"	30° 45' 53.1"	339
ISP29	Tomato (*L. esculentum*)	Greenhouse	Çamlık/Burdur	37° 28' 37.5"	30° 45' 39.5"	325
ISP31	Cucumber (*C. sativus*)	Greenhouse	Çamlık/Burdur	37° 28' 18.5"	30° 45' 17.3"	353
ISP32	Tomato (*L. esculentum*)	Greenhouse	Çamlık/Burdur	37° 28' 55.7"	30° 45' 36.3"	350
E1	Tomato (*L. esculentum*)	Field	Eğirdir/Isparta	37° 55' 14.1"	30° 46' 25.0"	930
ISP1	Pepper (*C. annuum*)	Field	Yeşilyurt/Isparta	37° 31' 57.2"	30° 51' 42.1"	602
ISP3	Bean (*P. vulgaris*)	Greenhouse	Yeşilyurt/Isparta	37° 31' 58.8"	30° 51' 42.0"	607
ISP5	Eggplant (*S. melongena*)	Greenhouse	Yeşilyurt/Isparta	37° 31' 54.6"	30° 51' 46.7"	595
ISP6	Okra (*Abelmoschus esculentus* (L.) Moench)	Field	Yeşilyurt/Isparta	37° 31' 54.6"	30° 51' 45.2"	595
ISP40	Pepper (*C. annuum*)	Greenhouse	Yeşilyurt/Isparta	37° 29' 14.7"	30° 52' 44.1"	451

Table 1. (Continued)

Code	Host Plant	Production system	Location	Latitude (N)	Longitude (E)	Altitude (m)
ISP44	Eggplant (*S. melongena*)	Greenhouse	Yeşilyurt/Isparta	37° 32' 00.5"	30° 51' 46.7"	676
ISP55	Tomato (*L. esculentum*)	Greenhouse	Yeşilyurt/Isparta	37° 31' 54.6"	30° 51' 46.7"	596
ISP77	Cucumber (*C. sativus*)	Greenhouse	Yeşilyurt/Isparta	37° 31' 53.3"	30° 51' 31.8"	599
ISP78	Tomato (*L. esculentum*)	Greenhouse	Yeşilyurt/Isparta	37° 31' 53.3"	30° 51' 31.8"	600
ISP15	Cucumber (*C. sativus*)	Greenhouse	Çandır/Isparta	37° 27' 12.4"	30° 53' 12.3"	289
ISP16	Tomato (*L. esculentum*)	Greenhouse	Çandır/Isparta	37° 26' 13.6"	30° 53' 35.7"	275
ISP17	Cucumber (*C. sativus*)	Greenhouse	Çandır/Isparta	37° 26' 13.6"	30° 53' 35.7"	275
ISP18	Okra (*A. esculentus*)	Greenhouse	Çandır/Isparta	37° 26' 01.3"	30° 53' 40.4"	286
ISP21	Okra (*A. esculentus*)	Field	Çandır/Isparta	37° 26' 02.3"	30° 53' 40.0"	286
ISP22	Pepper (*C. annuum*)	Field	Çandır/Isparta	37° 26' 03.5"	30° 53' 44.7"	302
ISP151	Pepper (*C. annuum*)	Greenhouse	Çandır/Isparta	37° 27' 12.4"	30° 53' 12.3"	357
ISP11	Eggplant (*S. melongena*)	Greenhouse	Şeyhler/Isparta	37° 28' 05.2"	30° 52' 43.0"	321
ISP14	Eggplant (*S. melongena*)	Greenhouse	Şeyhler/Isparta	37° 27' 49.4"	30° 53' 00.2"	303
ISP141	Tomato (*L. esculentum*)	Greenhouse	Şeyhler /Isparta	37° 27' 49.4"	30° 53' 00.2"	359
ISP41	Cucumber (*C. sativus*)	Greenhouse	Şeyhler/Isparta	37° 27' 49.4"	30° 53' 00.2"	303
ISP42	Tomato (*L. esculentum*)	Field	Kuleönü/Isparta	37° 53' 06.8"	30° 38' 53.4"	941
ISP43	Tomato (*L. esculentum*)	Field	Atabey/Isparta	37° 56' 19.1"	30° 40' 50.3"	1036
ISP45	Eggplant (*S. melongena*)	Greenhouse	Atabey /Isparta	37° 56' 19.1"	30° 40' 50.3"	993
ISP47	Eggplant (*S. melongena*)	Field	Atabey /Isparta	37° 56' 24.9"	30° 40' 04.4"	993
ISP23	Tomato (*L. esculentum*)	Greenhouse	Deregümü/Isparta	37° 46' 55.8"	30° 30' 26.6"	1110
DR2	Tomato (*L. esculentum*)	Greenhouse	Deregümü/Isparta	37° 47' 49.4"	30° 30' 35.6"	1080
DR8	Tomato (*L. esculentum*)	Greenhouse	Deregümü/Isparta	37° 47' 35.5"	30° 30' 16.9"	1077
DR14	Tomato (*L. esculentum*)	Greenhouse	Deregümü/Isparta	37° 47' 49.4"	30° 30' 35.6"	1066
DR15	Tomato (*L. esculentum*)	Greenhouse	Deregümü/Isparta	37° 47' 46.6"	30° 30' 40.1"	1074
DR16	Tomato (*L. esculentum*)	Greenhouse	Deregümü/Isparta	37° 47' 42.9"	30° 30' 43.5"	1075
DR17	Eggplant (*S. melongena*)	Greenhouse	Deregümü/Isparta	37° 47' 43.5"	30° 30' 47.2"	1075
DR20	Eggplant (*S. melongena*)	Field	Deregümü/Isparta	37° 47' 35.6"	30° 30' 59.5"	1074
DR21	Tomato (*L. esculentum*)	Greenhouse	Deregümü/Isparta	37° 47' 35.6"	30° 30' 59.5"	1074
DR23	Tomato (*L. esculentum*)	Greenhouse	Deregümü/Isparta	37° 47' 31.6"	30° 31' 06.7"	1071
DR29	Tomato (*L. esculentum*)	Greenhouse	Deregümü/Isparta	37° 47' 41.7"	30° 31' 26.3"	1055
DR30	Tomato (*L. esculentum*)	Greenhouse	Deregümü/Isparta	37° 47' 28.1"	30° 30' 57.9"	1076
DR31	Tomato (*L. esculentum*)	Greenhouse	Deregümü/Isparta	37° 47' 30.0"	30° 30' 25.5"	1090
DR33	Tomato (*L. esculentum*)	Greenhouse	Deregümü/Isparta	37° 47' 27.8"	30° 30' 24.5"	1090
DR35	Pepper (*C. annuum*)	Field	Deregümü/Isparta	37° 47' 39.4"	30° 30' 30.3"	1083

Galled roots were gently washed with tap water and an egg mass was collected using needle and placed in Eppendorf tubes under a stereomicroscope. These egg masses were surface-sterilized for a short period in 0.5% NaOCl, rinsed in tap water three times and prepared for inoculation.

Susceptible tomato seedlings with 5-6 true leaves (cv. Tueza F1; Multi Tohum, Antalya Turkey) were transplanted into 250 ml pots containing a mixture of 68% sand, 21% silt and 11% clay soil autoclaved at 121°C for 40 min. Single egg masses were inoculated in to a hole 2-3 cm deep near each tomato seedlings five days after the transplantation. The assay was conducted at 25±1°C and 65±5% RH, with a 16:8 h L:D photoperiod in a controlled environment chamber. Five single-egg-mass cultures were established for each population. Eight weeks after the inoculation, plants were uprooted and the most developed selected for multiplication in pure culture.

Morphological identification

Perineal patterns: Mature root-knot nematode females were removed from galled tomato roots using needles and forceps under a stereomicroscope. Perineal regions were cut in 45% lactic acid and permanently mounted in glycerin (Hooper, 1986). Species level identification was made by one of us (İHE) according to Jepson (1987) and Karssen (2002).

Morphometric measurements of second stage juveniles: Second stage juveniles (J2) from the pure culture populations were fixed in TAF fixative and made permanently mounted according to Seinhorst (1959). About 15-20 J2s were placed on each slide and measured morphometrically according to Karssen (2002).

Microscopic examination and image analysis were done with a Leica DM 2500 light microscope and Leica Application Suite Software Version 4.1.0 program.

Differential host test: The host races was determined according to North Carolina Differential Host Test (Sasser & Triantaphyllou, 1977). Each of five standardized host cultivars were inoculated with an average of 2000 J2 and eggs from each pure cultured population: *Nicotiana tabacum* L. cv. NC 95; *Gossypium hirsutum* L. cv. Deltapine 61, *Capsicum frutescens* L. cv. California Wonder, *Arachis hypogaea* L. cv. Florunner and *Lycopersicon esculentum* Mill. cv. Tueza F1. Experiments were conducted with four replicates of each differential host. The plants were maintained in a growth room at 25±1°C with a 16:8 h L:D photoperiod for after 60 days from inoculation. Plants were harvested and roots washed with tap water. Galls and egg mass indices were determined on a 0 to 5 scale according to Hartman & Sasser (1985). Each host cultivar was classified as resistant or susceptible, when the average number of galls and egg masses per root system was 0-2 or 3-5, respectively.

Molecular identification

DNA isolation of root-knot nematode populations was obtained from twenty egg masses using a DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany). The final DNA samples extracted from each sample were in 100 µl of AE buffer and kept -20°C until PCR procedures for identification.

For molecular identification of root-knot nematode species, the primer pairs INCK 14R/INCK 14F, FJAV/RJAV, FAR/RAR were optimized by Devran & Söğüt (2009) with major root-knot nematode populations in the western Mediterranean Region of Turkey, and *M. hapla* specific primers, JMV1, JMV2 and JMV hapla, were used for *M. incognita*, *M. javanica*, *M. arenaria* and *M. hapla*, respectively (Table 2). A total of 25 µl PCR reaction was conducted by thermocycler (Veriti Thermal cycler, Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA). Reaction mixture consisted of 10 ng DNA (5 µl), PCR buffer (2.5 µl), 2 mM $MgCl_2$ (1 µl), 0.2 mM dNTP (1 µl), 10 mM Primer F (1 µl), 10 mM Primer R (1 µl), 1 unit Taq DNA polymerase (GenEon, San Antonio, TX, USA) (0.25 µl) and ddH_2O (13.25 µl).

The PCR cycles are described in Table 3. PCR products were visualized by agarose electrophoresis in 2% gel (Agarose Type I, Sigma-Aldrich, St. Louis, MO, USA) using EtBr as a fluorescent dye at 90 V for 2 h. The size of PCR fragments giving the DNA bands corresponding to different *Meloidogyne* spp. are listed in Table 2.

Table 2. Species specific primers of root-knot nematodes (*Meloidogyne* spp.) for molecular identification in the study

Species	Primers	Primer sequences (5'-3')	Fragments (bp)	Reference
M. arenaria	Far	TCGGCGATAGAGGTAAATGAC	420	Zijlstra et al., 2000
	Rar	TCGGCGATAGACACTACAACT		
M. javanica	Fjav	GGTGCGCGATTGAACTGAGC	670	Zijlstra et al., 2000
	Rjav	CAGGCCCTTCAGTGGAACTATAC		
M. incognita	INCK14R	CCCGCTACACCCTCAACTTC	399	Randig et al., 2002
	INCK14F	GGGATGTGTAAATGCTCCTG		
M. hapla	JMV1	GGATGGCGTGCTTTCAAC	440	Wishart et al., 2002
	JMV2	TTTCCCCTTATGATGTTTACCC		
	JMV hapla	AAAAATCCCCTCGAAAAATCCACC		

Table 3. Root-knot nematode primers and their PCR cycles

INCK14F/INCK14R and Fjav/Rjav	Far/Rar	JMV1/JMV2/JMV hapla	
94°C 3 min	94°C 3 min	94°C 3 min	
94°C 30 s 60°C 30 s 72°C 60 s	94°C 30 s 56°C 30 s 72°C 60 s	94°C 30 s 48°C 30 s 72°C 2 min	35 cycles
72°C 7 min	72°C 7 min	72°C 7 min	

Results and Discussion

Morphological identification

Root-knot nematodes were detected in 83 soil and root samples and 68 populations were cultured and multiplied. Fifteen populations did not multiply on the tomatoes. Of the 68 root-knot nematode populations, 25 were identified as *M. incognita*, 18 as *M. javanica*, 22 as *M. hapla* and one as *M. arenaria* by perineal patterns. However, the two pure cultures could not be identified by their perineal patterns.

Tail length, hyaline terminus length, stylet length, distance of DGO from the stylet base of J2 are the most important morphometric characters for identification of *Meloidogyne* spp. (Whitehead, 1968; Eisenback et al., 1981; Jepson, 1987; Karssen, 2002). However, Kaur & Attri (2013) showed that body length, stylet length, head to median bulb length, tail length, c and c' ratios of J2 for *M. incognita* were highly variable from different host plants and district in India. Table 4 shows means of morphometric measurements of J2 for three root-knot nematode species in the Lakes region. J2 of *M. arenaria* could not measure because no population of this species was cultured.

Meloidogyne incognita: Although there were small differences between some isolates, all perineal patterns of the 25 populations showed typical *M. incognita* features. The perineal region generally had an angularly oval structure with a high dorsal arch in a typical pyriform and typically inverted-V shape formed by striae in the dorsal to the tail. Striae were in distinct waves which bent towards the lateral lines and were not interrupted. Lateral fields were not distinct. Striae were straighter with an oval appearance in ventral region (Figure 1). All perineal pattern features for *M. incognita* isolates were similar to those described as Jepson (1987).

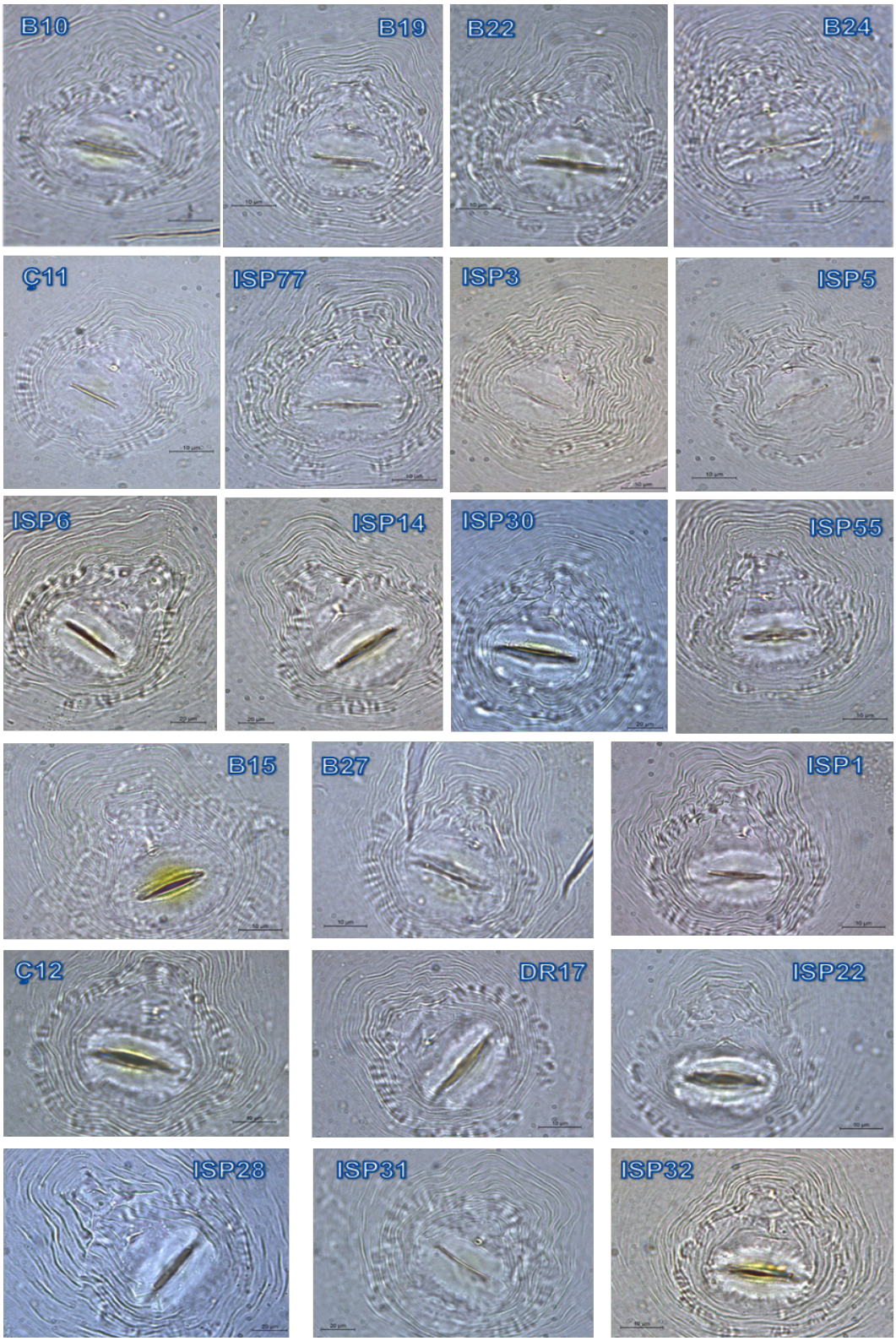

Figure 1. Perineal patterns of *Meloidogyne incognita* isolates collected from Lakes Region of Turkey. Bar: 10 µm.

Table 4 give some morphometric measurements of J2 of *M. incognita* in the study. J2 had the longest tail length (57.6 µm) compared to *M. javanica* and *M. hapla* (Table 4). This value was longer than in reported by Jepson (1987), Özarslandan (2009) and Kaur & Attri (2013). Hyaline terminus length was the smallest among three root-knot nematode species (Table 4) and similar to measurements of Jepson (1987) and Özarslandan (2009). The stylet length (13.3 µm) and DGO to stylet knob distance (3.4 µm) of the J2 were longer than in described by Whitehead (1968) and Özarslandan (2009), but, stylet length was shorter than Indian populations in described by Kaur & Attri (2013).

Meloidogyne javanica: Distinct lateral fields formed by double incisures are typically clear in the perineal patterns of *M. javanica* (Eisenback et al., 1981; Jepson, 1987). Similarly, perineal patterns of all *M. javanica* populations in our study show clear double lateral lines separating dorsal and ventral regions (Figure 2). Also, *M. javanica* had a general oval or oval to pyriform with a medium height and occasionally compressed dorsal arch in perineal regions. There was no transverse striae between vulva and anus in our patterns of populations (Figure 2).

Hyaline terminus length of J2 (14.40 µm) was markedly longer than the other two species and tail length was moderately long (55.2 µm) (Table 4). These lengths were in agreement with Jepson (1987), but longer than reported by Özarslandan (2009). In our study, stylet length was markedly longer than in measured by Özarslandan (2009), whereas DGO to stylet knob distance (3.4 µm) was similar to that reported by Özarslandan (2009) (Table 4).

Meloidogyne hapla: The concentration of punctuations between anus and tail terminus is the most characteristic feature of the *M. hapla* perineal pattern (Eisenback et al., 1981; Jepson, 1987). Likewise, in our study, there were punctuations with a stippled area between the anus and tail terminus in the patterns of *M. hapla* isolates (Figure 3). Some specimens (DR21 and ISP78) in Figure 3 had punctuations entirely in tail terminal area, which might have been an artifact of the fixation or preparation process. All cross-sectioned perineal patterns were roughly oval, regularly spaced with smooth and softly waved striae and with low dorsal arch. Lateral lines clearly appeared in softly irregular lined structure leading to outward from the punctuations as stated by Jepson (1987). The striae of the ventral and lateral regions intersected on one or both sides to become elongated and have wing shaped structure (Figure 3). Additionally, in our study, the physical appearance and position of galls on roots can helped in the diagnosis for *M. hapla*. Similarly, the relatively small and irregular galls of *M. hapla* often had lateral roots as described by Hunt & Handoo (2009).

The body length of the J2 (380.5 µm) was shorter than for *M. incognita* and *M. javanica* J2 (Table 4). In our study, tail length and hyaline terminus length of the J2 were relatively short compared to those reported by Chitwood (1949) and Jepson (1987). The DGO to stylet knob distance of *M. hapla* J2 (4.8 µm) was extremely long compare to the J2 of *M. incognita* and *M. javanica* and the measurements reported by Chitwood (1949) (Table 4).

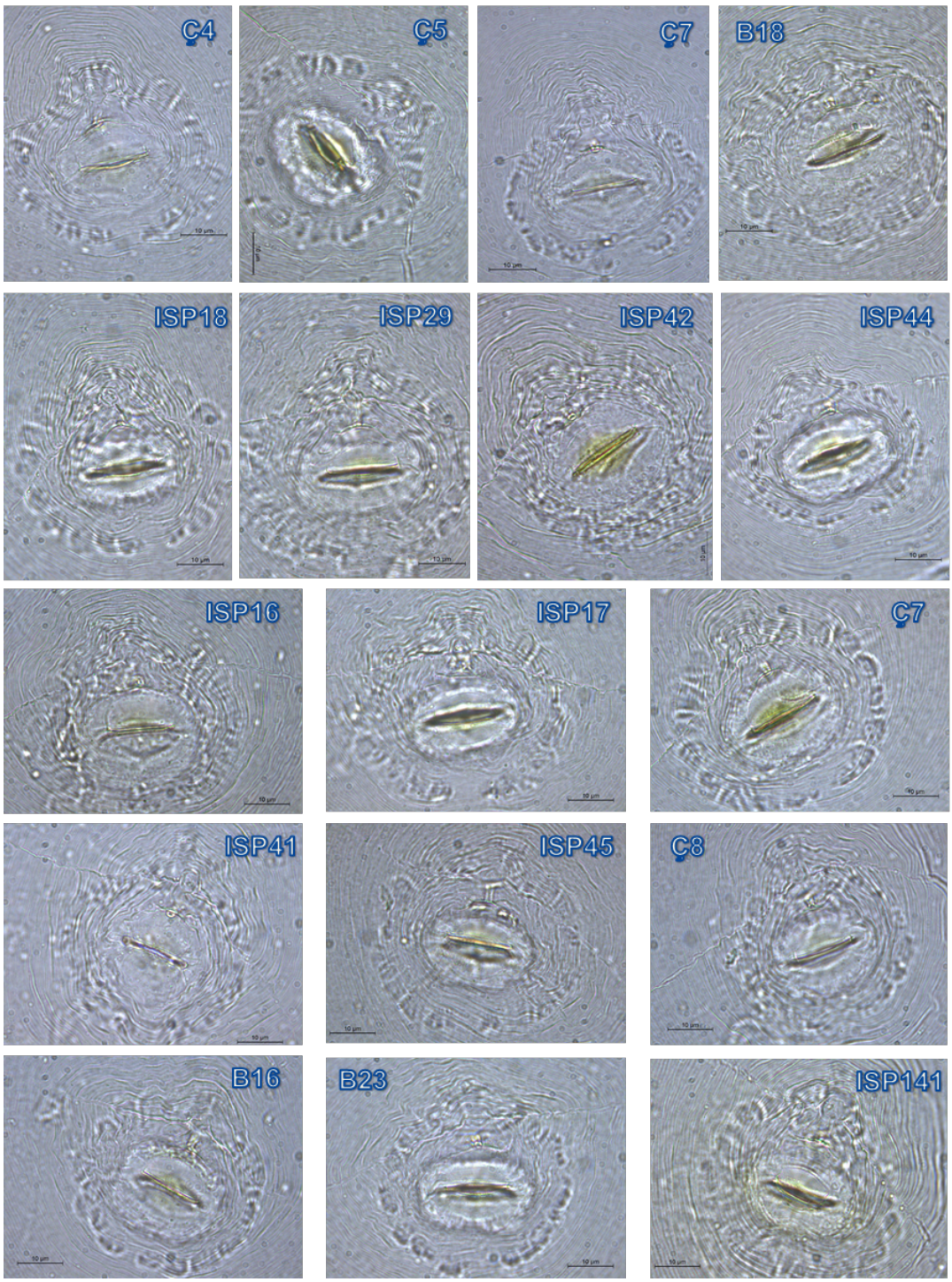

Figure 2. Perineal patterns of *Meloidogyne javanica* isolates collected from Lakes Region of Turkey. Bar: 10 µm.

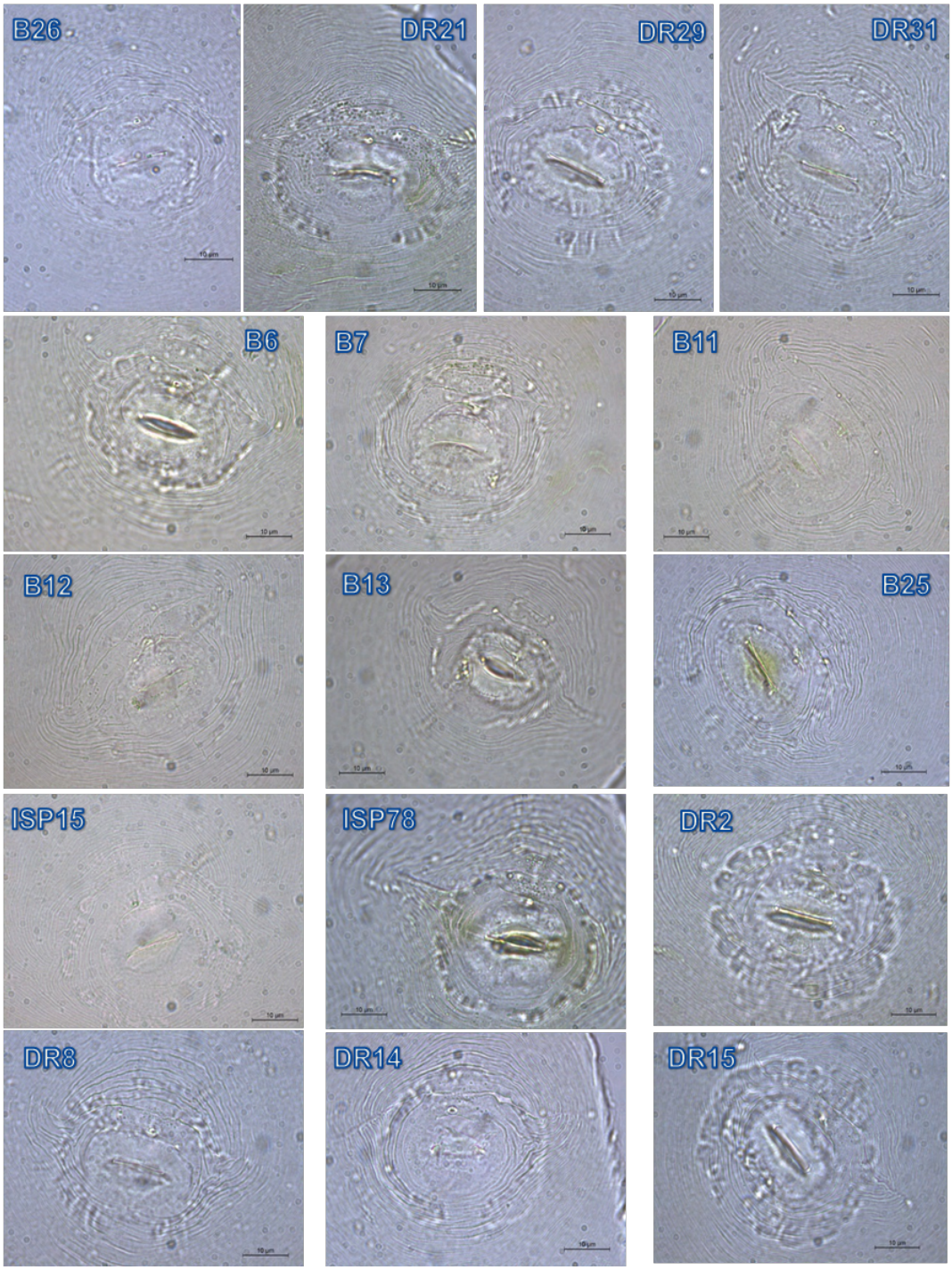

Figure 3. Perineal patterns of *Meloidogyne hapla* isolates collected from Lakes Region of Turkey. Bar: 10 µm.

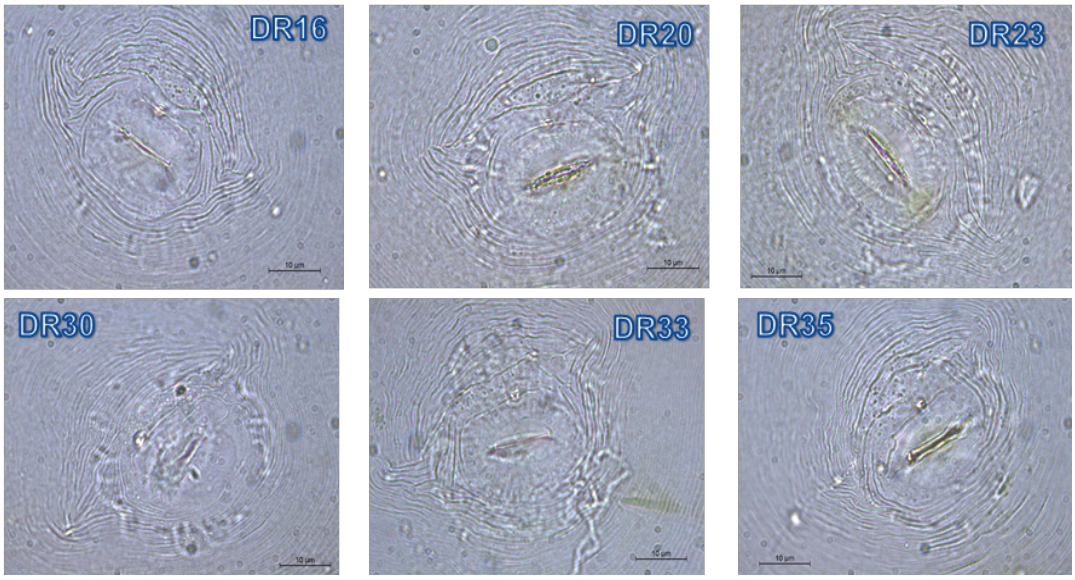

Figure 3. (continued).

Only one population was identified as *M. arenaria*. The lateral field was forked and the broken striae within lateral line region were curving with a striational winged form as described by Chitwood (1949) and Jepson (1987). Striae were distinctly separated and smoother in the ventral region (Figure 4). The dorsal arch was low and prevulval region was free of striae.

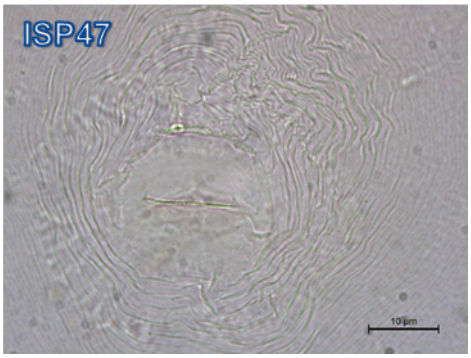

Figure 4. Perineal patterns of *Meloidogyne arenaria* isolates collected from Lakes Region of Turkey. Bar: 10 µm.

Table 4. Morphometric data for second stage juveniles of root-knot nematode species collected from Lakes Region of Turkey

Morphometric characters	J2 measurements (µm)		
Meloidogyne incognita	This study (n = 15)	Özarslandan (2009)	Whitehead (1968)
Body length (L)	409.7[x] (360-441.6)[y] [22.4][z]	407.60 [x]± 4.7[w] (387.8-428.8)[y]	360-393[y]
Tail length	57.6 (50.4-68.8)[5.5]	47.0±1.5 (38.4-52.8)	
Hyaline terminus length	11.6 (6.4-16)[2.2]	10.10± 0.4(8.0-11.2)	
DGO – Stylet knob distance	3.4 (3.2-4.8)[0.5]	2.56 ±0.1(2.4-2.9)	2-2.25
Stylet length	13.3 (12-14.4)[0.7]	11.40±0.4(9.6-12.8)	10
a	29.5	33.17	29-33
c	7.1	8.77	8-9.4
Meloidogyne javanica	This study (n:15)	Özarslandan (2009)	Whitehead (1968)
Body length (L)	448 (427.2-465.6) [16.3]	426.56 ±4.4 (408.0-454.4)	387-459
Tail length	55.2 (52.8-60.8)[3.81]	51.44 ±1.1(46.40-59.20)	36-56
Hyaline terminus length	14.40 (12.8-17.6)[2.3]	12.96 ±0.4(11.20-15.20)	
DGO – Stylet knob distance	3.4 (3.2-4)[0.4]	3.36 ±0.1(3.2-4.0)	4
Stylet length	14.0 (13.6-14.4)[0.5]	13.36 ±0.4(11.20-14.40)	9.4-11.4
a	32	30.33	27.1-35.9
c	8.1	8.31	7.3-11.1
Meloidogyne hapla	This study (n:20)	-	Chitwood (1949)
Body length (L)	380.5 (328-412.8) [23.7]		357-467
Tail length	49.5 (44.8-56)[2.9]		46-58
Hyaline terminus length	13.1 (11.2-17.6)[2.1]		12-19
DGO – Stylet knob distance	4.8 (3.2-6.4)[1.7]		3-4
Stylet length	12.4 (11.2-13.6)[0.8]		10-12
a	29.2		
c	7.7		

[x] mean, [y] max-min value; [z] standard deviation; [w] standard error.

Molecular identification

For 25 *M. incognita* populations, a PCR was conducted using primer set INCK14F/INCK14R (Randig et al., 2002) and a DNA band of 399 bp was obtained for all *M. incognita* populations. This result was in agreement with the results of Tesarova et al. (2003) and Devran & Söğüt (2009). For *M. arenaria* and *M. javanica*, specific SCAR primers (Zijlstra et al., 2000) produced 420 bp and 670 bp DNA bands, respectively, and this result was in agreement with previous studies in Turkey (Devran & Söğüt, 2009; Özarslandan & Elekçioğlu, 2010; Akyazı et al., 2012; Aydınlı & Mennan, 2016). All *M. hapla* populations were identified using primer set JMV1/JMV2/JMV hapla and a 440 bp DNA bands was obtained as described by Wishart et al. (2002) and this result was in agreement with previous a study in Turkey (Akyazı et al., 2012) (Figure 5).

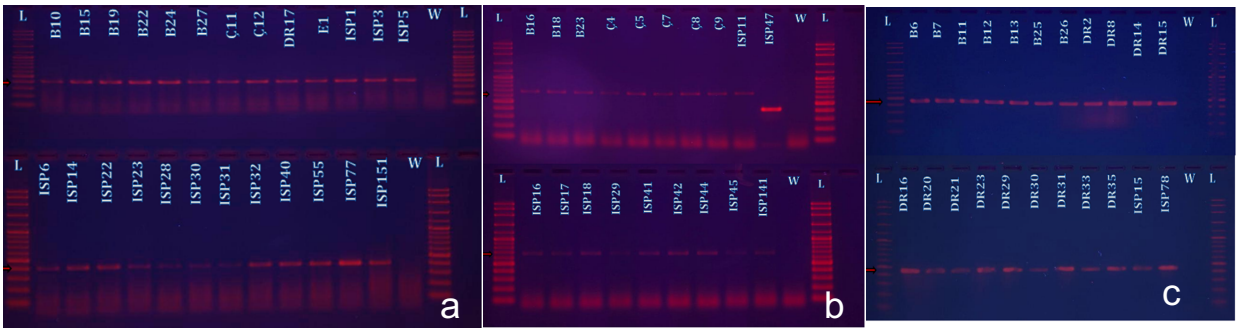

Figure 5. Amplification products with the *Meloidogyne* spp. collected from Lakes Region of Turkey a) *M. incognita* populations (B10-ISP151); b) *M. javanica* populations (B16-ISP141) and *M. arenaria* (ISP47); c) *M. hapla* populations (B6-ISP78). L: 100 bp DNA ladder, W: Water.

Root-knot nematode races

Meloidogyne incognita races 2, 4 and 6 and *M. javanica* races 1 and 3 were determined. *Meloidogyne arenaria* races could not be determined because the species was not mass cultured. Table 5 shows *M. incognita* and *M. javanica* races and their reaction on differential host plants and the altitude at which they were collected.

Seventeen *M. incognita* populations reproduced well on tomato, pepper and tobacco cultivars, but did not on cotton and peanut, so these populations were identified as race 2. Six populations developed well on tobacco, however there were no galls or egg masses on the other differential host cultivars. Therefore, these were identified as race 6. The other two populations reproduced on tomato, cotton, pepper and tobacco, but not on peanut and were identified as race 4. *Meloidogyne incognita* race 2 was found to be the most common (68% of samples) in this study. Similarly, race 2 was found to be as widespread in the Mediterranean and Black Sea Regions (Söğüt & Elekçioğlu, 2000; Mennan & Ecevit, 2001; Devran & Söğüt, 2011). *Meloidogyne incognita* race 6 was first reported in western coastal areas of the Mediterranean Region of Turkey by Devran & Söğüt (2011). In the current study, *M. incognita* race 6 was more common (24% of samples) than in the western coastal areas of the Mediterranean Region (3% of samples). In addition, Kaçar (2011) reported the occurrence of *M. incognita* races 5 and 6 in Turkey and Robertson et al. (2009) reported *M. incognita* races 6 and 5 in vegetable growing areas of Spain. *Meloidogyne incognita* race 4 was not common (8% of samples) in the Lakes region. Similarly, Söğüt & Elekçioğlu (2000) reported *M. incognita* race 4 in several vegetables growing areas in eastern areas of the Mediterranean Region of Turkey, whereas, Akyazı & Ecevit (2010) reported that *M. incognita* race 1 was more common in Tokat than race 2 in the Black Sea Region of Turkey.

Fifteen *M. javanica* populations developed well and formed egg masses on the roots of tobacco and tomato but not on cotton, pepper and peanut. Thus, these were identified as *M. javanica* race 1. Only one *M. javanica* population (B16) formed galls and egg masses on the roots of tobacco, tomato and peanut, but not on cotton and pepper. This *M. javanica* population was determined as race 3 and represents the first detection of this race in Turkey. The other two *M. javanica* populations (ISP41 and ISP45) were not tested with differential host because of they were not cultured. In a previous study, *M. javanica* race 1 was reported to be widespread in eastern and western areas of the Mediterranean Region of Turkey (Söğüt & Elekçioğlu, 2000; Devran & Söğüt, 2011). *Meloidogyne javanica* races 1 and 3 did not reproduce on pepper, however, the other races of *M. javanica* develop well in pepper and have been reported from different parts of the world. *Meloidogyne javanica* races 2 and 3 were reported by Rammah & Hirschmann (1990). Carneiro et al. (2004) identified *M. javanica* race 4 in Parana State, Brazil and Robertson et al. (2009) reported *M. javanica* race 1 and 5 from vegetable growing areas of Spain.

Table 5. Differential host test to classifying races of *Meloidogyne incognita* and *M. javanica* populations and the altitude at which they were collected from Lakes Region of Turkey

Meloidogyne incognita						*Meloidogyne javanica*					
Code	Race	Tobacco	Pepper	Cotton	Altitude (m)	Code	Race	Tobacco	Pepper	Peanut	Altitude (m)
B22	2	3.5±0.5*	2.7±0.5	0.0±0.0	276	B18	1	4.2±0.2	0.0±0.0	0.0±0.0	275
ISP14	2	4.2±0.5	2.7±0.2	0.0±0.0	303	B23	1	3.7±0.5	1.2±0.5	0.0±0.0	284
ISP31	2	2.2±1.3	4.2±0.2	1.2±0.2	353	Ç4	1	4.5±0.3	0.0±0.0	0.0±0.0	366
Ç11	2	3.5±0.3	2.2±0.8	0.0±0.0	338	Ç5	1	4.0±0.6	0.0±0.0	0.0±0.0	350
Ç12	2	4.0±0.4	4.0±0.6	0.0±0.0	339	Ç7	1	3.7±0.5	0.0±0.0	0.0±0.0	341
ISP151	2	3.5±0.6	2.7±0.2	0.0±0.0	357	Ç8	1	4.5±0.5	0.0±0.0	0.0±0.0	327
ISP40	2	3.7±0.5	2.7±0.2	0.0±0.0	451	Ç9	1	4.2±0.2	0.0±0.0	0.0±0.0	360
ISP55	2	3.5±0.3	3.2±0.2	0.0±0.0	596	ISP11	1	3.2±0.3	0.0±0.0	0.0±0.0	321
ISP5	2	3.0±0.4	4.2±0.5	0.0±0.0	595	ISP16	1	3.2±0.5	1.0±0.6	0.0±0.0	275
ISP6	2	3.0±0.4	5.0±0.0	0.5±0.5	595	ISP17	1	4.5±0.3	0.2±0.2	0.0±0.0	275
ISP1	2	3.7±0.5	3.5±0.3	0.0±0.0	602	ISP18	1	4.2±0.2	0.0±0.0	0.0±0.0	286
ISP3	2	4.0±0.4	2.7±0.2	0.0±0.0	607	ISP29	1	4.0±0.4	0.0±0.0	0.0±0.0	325
B10	2	4.2±0.2	4.0±0.0	0.5±0.5	925	ISP141	1	5.0±0.0	0.0±0.0	0.0±0.0	359
E1	2	3.7±0.3	3.0±0.4	0.0±0.0	930	ISP44	1	3.7±0.5	0.0±0.0	0.0±0.0	676
DR17	2	2.7±0.9	3.0±0.3	0.0±0.0	1075	ISP42	1	4.7±0.2	0.0±0.0	0.0±0.0	941
B24	2	3.7±0.2	3.5±0.3	0.2±0.2	1430	B16	3	4.2±0.2	0.0±0.0	3.2±0.2	276
B27	2	4.5±0.3	4.2±0.2	0.0±0.0	1436	ISP41**	nt				303
B27	4	3.2±1.1	4.5±0.3	3.2±0.2	350	ISP45**	nt				993
ISP23	4	3.7±0.5	4.2±0.2	2.7±0.5	1110						
B15	6	3.5±0.3	0.0±0.0	0.0±0.0	276						
B19	6	3.0±0.4	0.7±0.5	0.0±0.0	308						
ISP22	6	4.5±0.3	0.0±0.0	0.0±0.0	302						
ISP28	6	5.0±0.0	0.7±0.2	0.0±0.0	288						
ISP30	6	3.7±0.2	0.0±0.0	0.0±0.0	287						
ISP77	6	4.0±0.5	0.0±0.0	0.0±0.0	599						

* Mean ± standard errors of four replicates;
** nt: not tested.

Distribution of root-knot nematodes in Lakes Region of Turkey

Root-knot nematodes infested 52% of the samples taken from open fields and greenhouses in the Lakes region. Major root-knot nematode species, *M. incognita*, *M. javanica* and *M. hapla* were common in vegetable growing areas at different altitudes at 37, 27 and 32% of samples, respectively. Only one population was identified as *M. arenaria* collected from eggplant in Atabey District of Isparta Province at an altitude of about 1000 m. Two populations were not identified from their perineal patterns model and molecular assays. *Meloidogyne incognita* populations were found on tomato, eggplant, pepper, cucumber and bean. *Meloidogyne javanica* populations were detected on tomato, eggplant and cucumber, and *M. hapla* populations were predominantly detected on tomato and eggplant crops at higher altitudes. *Meloidogyne incognita* and *M. javanica,* as thermophilic species, were found to be more prevalent at lower altitudes. Six *M. incognita* isolates (ISP23, DR17, E1, B10, B24 and B27) were found at locations higher than 900 m. All *M. incognita* race 6 isolates were detected at lower altitudes. Similarly, 12 *M. incognita* race 2 isolates were found at lower altitudes. However, five race 2 isolates were found at higher altitudes. One of two *M. incognita* race 4 isolates was found at a lower altitude, whereas the other was found at over 1000 m. Also, 20% of *M. incognita* populations were detected in open fields and 80% in greenhouses. Only one *M. javanica* isolate (ISP42, race 1) was found on a high plateau area, while the others were found in vegetable growing areas across the lower plateau areas, which have warmer climatic conditions. *Meloidogyne javanica* race 3 was found in a cucumber greenhouse in Elsazı Village of Burdur Province at 276 m. Also, 17% of *M. javanica* populations were found in open fields and 83% in greenhouses. One *M. arenaria* population (ISP47) was found in an open field at 993 m. *Meloidogyne hapla* was observed in the cooler areas. About 91% of *M. hapla* populations were from high altitude sites with sandy soil. Greenhouses in Deregümü District of Isparta Province were commonly infested with *M. hapla* at altitudes over 900 m. The other 9% of populations were found at lower altitudes. Excluding two populations (DR20 and DR35), all *M. hapla* populations were collected from greenhouses.

Researchers have studied the identification and distribution of root-knot nematodes in different regions of Turkey. Özarslandan & Elekçioğlu (2010) reported that *M. incognita*, *M. arenaria*, *M. javanica* and *M. chitwoodi* were represented 28, 27 35 and 10%, respectively, of 79 root-knot nematode populations collected from all over Turkey. Devran & Söğüt (2009) showed that soils where the vegetables are grown along the western coastal areas of the Mediterranean Region were infested by *M. incognita* (63%), *M. javanica* (30%) and *M. arenaria* (7%). Elekcioğlu & Uygun (1994) were the first to to identify root-knot nematodes in the Mediterranean Region, with *M. incognita* and *M. javanica* the most commonly species detected. In the eastern Mediterranean Region, *M. javanica*, *M. incognita* and *M. hapla* were found in 55, 42 and 3%, respectively, of 38 samples collected from protected vegetable areas (Söğüt & Elekçioğlu, 2000). In the other regions of the Turkey, various studies have found root-knot nematode species. Yüksel (1974) reported that *M. incognita*, *M. javanica*, *M. arenaria* and *M. hapla* occurred in vegetable growing soils of the Marmara Region. Important vegetables growing areas of Aydın Province of the Aegen Region were found to have *M. incognita*, *M. javanica* and *M. hapla* when sampled in summer (Kaşkavalcı & Öncüer, 1999). Studies in the Black Sea Region have reported that Bafra and Çarşamba Plains are infested with *M. incognita*, Tokat vegetable areas with *M. incognita*, Ordu and Samsun Provinces with *M. arenaria* and *M. hapla* (Mennan & Ecevit, 2001; Akyazı & Ecevit, 2010; Akyazı et al., 2012). In recently studies, *M. arenaria* (42%), *M. ethiopica* (41%), *M. javanica* (12%) and *M. incognita* (4%) were reported in the central areas of the Black Sea Region (Aydınlı & Mennan, 2016) and *M. incognita* in Kahramanmaraş Province in the eastern Mediterranean Region (Çetintaş & Çakmak, 2016).

In conclusion, for vegetables grown during summer and early autumn in the Lakes region, fumigants, such as metam sodium or Dazomet, and soil solarization are not sufficient for control of root-knot nematodes. Thus, it is of great importance for root-knot nematodes species are identified so that appropriate available and alternative control methods can be chosen based on the species present and regional conditions. *Meloidogyne incognita* and *M. javanica* were the most common species on cultivated

plants in low altitude areas of the Lakes region, just as in the coastal areas of the Mediterranean Region. Importantly, three host races (2, 4 and 6) of *M. incognita* were detected. Race composition of these two-major species are important for plant resistance and breeding, and in crop rotation strategies, so this is a significant outcome of this study. In contrast, *M. hapla* was found to be the prevalent species in higher high altitude areas in the Lakes region, which is also an important finding as there are no suitable resistant cultivar available for vegetable crops grown in these areas. Given that *M. hapla* induced spherical galls on the proliferating or branching small roots of tomatoes and eggplants, and egg masses were clearly visible on the outside of the small branching roots, this symptomology could facilitate control by egg parasitizing fungi or bacteria, or nematicide treatments. *Meloidogyne hapla* has a wide host range, however, crop rotation with grasses and grain crops can significantly decrease its population density. Therefore, it is important to consider geographic and species distribution of root-knot nematode species when implementing control programs. This study has provided such data for the Lake region of Turkey, which should prove to be a valuable resource for future research and for developing effective control and management strategies for the region.

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