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Original article (Orijinal araştırma)

Identification of root-knot nematode species (*Meloidogyne* spp.) (Nemata: Meloidogynidae) in the potato fields of Central Anatolia (Turkey) using molecular and morphological methods [1]

Orta Anadolu Bölgesi (Türkiye) patates ekiliş alanlarındaki kök-ur nematodu türlerinin (*Meloidogyne* spp.) (Nemata: Meloidogynidae) moleküler ve morfolojik yöntemlerle belirlenmesi

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Summary

Root-knot nematodes are considered to be the world's most damaging plant parasitic nematodes. *Meloidogyne chitwoodi* and *Meloidogyne fallax* are quarantined organisms in Europe and Turkey, and special regulations exist for planting and transporting seed potatoes in order to control these organisms. *M. chitwoodi,* was firstly identified from infected potato tubers collected from Niğde and after that it was determined in Nevşehir, İzmir, Balıkesir, Kütahya, Manisa and Bitlis provinces in Turkey. This study was conducted in 2009 with the aim of identifying *Meloidogyne* spp. species in Central Anatolia, which is the most important potato production region of Turkey. 73 samples in total were collected from Niğde (49 samples), Nevşehir (18), Aksaray (2), Isparta (2), Kayseri (1), and Konya (1) provinces of Turkey. All populations were identified using molecular methods, also fifteen selected populations were identified based on their morphological and morphometric features. Morphological characters and morphometric measurements were taken from the perineal pattern of mature females, second-stage juveniles, and eggs. Two different PCR protocols were used: (1) JMV1, JMV2 and JMV hapla multiplex PCR primers and (2) Fc and Rc species specific primers. Also sequence analysis was used for molecular identification. Results obtained both from morphological and molecular methods showed that all populations were positive for *M. chitwoodi* and there is no evidence was found regarding the existence of *M. fallax* or the other *Meloidogyne* species.

Key words: *Meloidogyne chitwoodi*, potato, identification, molecular, PCR

Özet

Köklerde oluşturduğu urlarla tanınan *Meloidogyne* cinsine dahil olan türler dünyada en çok zarar yapan nematod grubu olarak kabul edilmektedir. *Meloidogyne chitwoodi* ve *Meloidogyne fallax*, Avrupa ve Türkiye'de karantina zararlısı olup bu etmenler açısından tohumluk patateslerin taşınması ve dikilmesi için özel şartlar bulunmaktadır. Türkiye'de ilk olarak Niğde ilinden toplanan bulaşık patates yumrularında tespit edilen *M. chitwoodi* daha sonra Nevşehir, İzmir, Balıkesir, Kütahya, Manisa ve Bitlis'te tespit edilmiştir. 2009 yılında gerçekleştirilen bu çalışmanın amacı Türkiye'nin en önemli patates üretim alanı olan Orta Anadolu bölgesindeki *Meloidogyne* türlerinin belirlenmesidir. Niğde (49), Nevşehir (18), Aksaray (2), Isparta (2), Kayseri (1) ve Konya (1) illerinden toplam 73 örnek toplanmıştır. Bütün popülasyonlar moleküler yöntemlerle ayrıca seçilen 15 popülasyon morfolojik ve morfometrik karakterlerine göre teşhis edilmiştir. Morfolojik karakterler ve morfometrik ölçümler ergin dişilerin perennial alanlarından, 2. dönem larvalardan ve yumurtalardan yapılmıştır. Moleküler teşhislerde iki farklı PCR protokolü (1) JMV1, JMV2 ve JMVhapla multiplex PCR primerleri (2) türe spesifik Fc ve Rc primerleri ile sekans analizi kullanılmıştır. Moleküler ve morfolojik teşhis metotları kullanılarak yapılan teşhislerin her ikisinde de sadece *M. chitwoodi* tespit edilmiş olup Orta Anadolu patates üretim alanlarında *M. fallax* ve diğer kök-ur nematode türlerinin varlığına dair herhangi bir sonuç elde edilememiştir.

Anahtar sözcükler: *Meloidogyne chitwoodi*, patates, teşhis, moleküler, PCR

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Introduction

Root-knot nematodes are an economically important polyphagous group of highly adapted obligate plant parasites. They are distributed worldwide and parasitize nearly every species of higher plant (Karssen & Moens, 2006). Up to 2009, 98 different species of *Meloidogyne* genus had been discovered (Jones et al., 2013). In Europe, 23 root-knot nematode species have been identified up to today, and 14 of those were first records (Wesemael et al., 2011). Among all the discovered root-knot species, *M. incognita*, *M. javanica*, *M. arenaria*, *M. chitwoodi*, *M. fallax,* and *M. hapla* species are the most ones, and they account for more than 95% of the occurrences of this genus (Adam et al., 2007). *Meloidogyne arenaria*, *M. incognita* and *M. javanica* are the species most in hot regions. In colder regions, *M. hapla* and *M. naasi* are important species in field-grown crops (Wesemael et al., 2011). Also since 1990's, *M. chitwoodi* and *M. fallax* species became species effecting potato and vegetable production in colder regions (Wesemael et al., 2011). In Turkey, 9 different species has been found in the studies conducted on various culture plants in various regions up to today and these are *M. arenaria*, *M. artiellia, M. chitwoodi*, *M. ethiopica, M. exigua*, *M. hapla*, *M. incognita, M. javanica*, and *M. thamesi* (Yüksel, 1966 ve 1967; Elekçioğlu, 1992; Kepenekci et al., 2002; Özarslandan et al., 2009; Aydınlı et al., 2013; İmren et al., 2014).

Root-knot nematodes on potatoes were first recorded by Borazancı et al. (1985) in Manisa province, but these authors did not give any information on the species they found. Later studies showed that existence of *M. hapla* in Niğde, Nevşehir, and Aksaray provinces and *M. incognita* in Edirne province (Kepenekci et al., 2006; Erdoğuş et al., 2011). *M. chitwoodi* was first found by Özarslandan et al. (2009) in 2006 on infected potato tubers collected from the fields in Niğde province. *M. chitwoodi* species, also known as Columbian root-knot nematode, was first discovered in 1980 at the Northwest Pacific region of A.B.D. (Santo et al., 1980). *M. chitwoodi* is found in Holland, Belgium, France, Germany, Portugal, South Africa, America, Mexico, Argentina and Turkey. It is found in Turkey are Niğde, Nevşehir, Bitlis, İzmir, Manisa, Kütahya, and Balıkesir provinces (Özarslandan et al., 2009; Devran et al., 2009; Yıldız et al., 2009; Özarslandan & Elekçioğlu, 2010; Ulutaş, 2010; Özarslandan et al., 2013).

Meloidogyne chitwoodi is included in the quarantine lists by European and Mediterranean Plant Protection Organization (EPPO) and North American Plant Protection Organization (NAPPO). It also exists in the Turkish quarantine list as *Meloidogyne spp*. As a result of feeding and growth of nematodes, females cause brownish spots that can be seen when peeled and also external galling on tubers. Because of quality problems caused by these necrosis and galling, products could be rejected either at market or at the field (Ingham et al., 2007). Even though necrosis consists only 5% of the tuber, it is considered as commercially not suitable and also total yield is reduced because of the damage caused by nematodes (Anonymous, 2014). Infected tubers which use as a seed play an important role for spreading and cause problems in seed potato trade.

This study aims to identify the root-knot nematode species in potato fields and to determine the infection pattern of *M. chitwoodi*. To this end, 73 samples collected from potato fields in Central Anatolia was used as an ecologic region were analyzed using molecular and morphological methods.

Material and Methods

The material of this study consists of 73 potato tuber samples collected from potato areas infested by *Meloidogyne* spp. between 2009 and 2012 (Table 1).

Root-knot nematode populations

In order to obtain pure cultures, tomato seedlings were inoculated with potato tubers collected from fields. For every population, tubers were first washed and peeled 0.5 to 1 cm deep using sterile scalpel. These samples were dissected to pieces in the size of 1.5-2 cm, added to 100 ml of water and mixed in Waring blender for 30-60 seconds. Resulting mixture was agitated, poured into pots in which there had tomato seedlings in the 2-4 true leaf stage and sterile sand-soil mixture [85% silver sand, 15% soil], and covered with silver sand-soil mixture. All experiments were conducted in a temperature controlled

glasshouse at 20-30°C and pots were watered and fertilized as required. Tomatoes were harvested approximately 3 months later and every population was inoculated to new tomato seedlings as single egg mass for 5 times. Approximately 3 months after the inoculation, tomatoes were harvested, roots were checked for egg packages and best performing ones were selected.

Table 1. Isolates and sources of *Meloidogyne* spp. populations used in this study

Population Code	Location	Accession numbers	Population Code	Location	Accession numbers
NIG-1	Niğde/Merkez/Tırhan	KF557796	NIG-41	Niğde/Merkez/Edikli	KF557825
NIG-2	Niğde/Merkez/Tırhan	KF557804	NIG-42	Niğde/Merkez/Edikli	KF557826
NIG-3	Niğde/Merkez/Tırhan	KF557816	NIG-43	Niğde/Merkez/Edikli	KF557827
NIG-5	Niğde/Merkez/Hasaköy	KF557834	NIG-44	Niğde/Merkez/Kiledere	KF557828
NIG-6	Niğde/Merkez/Ağcaşar	KF557838	NIG-45	Niğde/Merkez/Konaklı	KF557829
NIG-7	Niğde/Merkez/Yeşilgölcük	KF557839	NIG-46	Niğde/Merkez/Konaklı	KF557830
NIG-8	Niğde/Merkez/Alay	KF557840	NIG-47	Niğde/Merkez/Konaklı	KF557831
NIG-9	Niğde/Merkez/Tırhan	KF557841	NIG-48	Niğde/Merkez/Hasaköy	KF557832
NIG-10	Niğde/Merkez/Tırhan	KF557793	NIG-49	Niğde/Merkez/Orhanlı	KF557833
NIG-11	Niğde/Merkez/Hasaköy	KF557794	NIG-50	Niğde/Merkez/Orhanlı	KF557835
NIG-12	Niğde/Merkez/Hasaköy	KF557795	NIG-51	Niğde/Merkez/Orhanlı	KF557836
NIG-13	Niğde/Merkez/Hasaköy	KF557797	NIG-52	Niğde/Merkez/Orhanlı	KF557837
NIG-14	Niğde/Merkez/Bağlama	KF557798	NEV-1	Nevşehir /Acı Göl/Karapınar	KF557775
NIG-15	Niğde/Merkez/Bağlama	KF557799	NEV-2	Nevşehir /Acı Göl/Karapınar	KF557784
NIG-16	Niğde/Merkez/Bağlama	KF557800	NEV-3	Nevşehir/ Derinkuyu	KF557786
NIG-17	Niğde/Merkez/Alay	KF557801	NEV-4	Nevşehir/ Derinkuyu	KF557787
NIG-18	NiğdeMerkez/Bağlama	KF557802	NEV-5	Nevşehir/ Derinkuyu	KF557788
NIG-19	Niğde/Merkez/Kiledere	KF557803	NEV-6	Nevşehir /Derinkuyu	KF557789
NIG-20	Niğde/Merkez/Kiledere	KF557805	NEV-7	Nevşehir /Derinkuyu	KF557790
NIG-21	Niğde/Merkez/Kiledere	KF557806	NEV-8	Nevşehir /Derinkuyu	KF557791
NIG-22	Niğde/Merkez/Kiledere	KF557807	NEV-9	Nevşehir /Derinkuyu/Suvermez	KF557792
NIG-23	Niğde/Merkez/Kiledere	KF557808	NEV-10	Nevşehir /Derinkuyu/Suvermez	KF557776
NIG-24	Niğde/Merkez/Kiledere	KF557809	NEV-11	Nevşehir /Derinkuyu/Yazıhöyük	KF557777
NIG-25	Niğde/Merkez/Kiledere	KF557810	NEV-14	Nevşehir / Ürgüp/Mazı	KF557778
NIG-26	Niğde/Merkez/Kiledere	KF557811	NEV-15	Nevşehir/Ürgüp/Mazı	KF557779
NIG-27	Niğde/Merkez/Ağcaşar	KF557812	NEV-16	Nevşehir/Merkez/Güvercinlik	KF557780
NIG-29	Niğde/Merkez/Kiledere	KF557813	NEV-17	Nevşehir/Acı Göl/Tepeköy	KF557781
NIG-30	Niğde/Merkez/Kiledere	KF557814	NEV-18	Nevşehir/Acı Göl/Karapınar	KF557782
NIG-31	Niğde/Merkez/Kiledere	KF557815	NEV-19	Nevşehir/Acı Göl/Tepeköy	KF557783
NIG-33	Niğde/Merkez/Bağlama	KF557817	NEV-20	Nevşehir/Derinkuyu/-	KF557785
NIG-34	Niğde/Merkez/Alay	KF557818	ISP-1	Isparta/Merkez/Gelincik	KF557771
NIG-35	Niğde/Merkez/Konaklı	KF557819	ISP-2	Isparta/ Merkez/Gelincik	KF557772
NIG-36	Niğde/Merkez/Konaklı	KF557820	AKS-1	Aksaray/Gülağaç	KF557769
NIG-37	Niğde/Merkez/Konaklı	KF557821	AKS-2	Aksaray/Gülağaç	KF557770
NIG-38	Niğde/Merkez/Konaklı	KF557822	KYS-1	Kayseri/Kocasinan /Erkilet	KF557773
NIG-39	Niğde/Merkez/Edikli	KF557823	KON-1	Konya/Sarayönü/Kuyulusebil	KF557774
NIG-40	Niğde/Merkez/Edikli	KF557824			

Molecular identification

DNA extraction

Meloidogyne spp. egg mass to be used in DNA extraction were collected from the infected roots of pure cultures cultivated in greenhouse. DNA extraction was performed according to the protocol stated in the DNA easy Tissue and Blood extraction kit (Qiagen, Hilden, Germany). Before the PCR process, Nanodrop (The Thermo Scientific Nano Drop 2000) was used in order to confirm the DNA extracts and to determine the DNA amount.

PCR

Primer sequences used in PCR studies are given in Table 2 and used as stated in the literature (Wishart et al., 2002, Zijlstra, 2000). In every PCR process both the positive (The Netherlands population) and negative controls were used.

Table 2. Primer sequences used for the identification of *Meloidogyne spp*. populations

Name of Primer	Primer Sequences (5-3)	Species	Fragment (bp)	References
JMV1	GGATGGCGTGCTTTCAAC	*M. chitwoodi*	540 bp	
JMV2	TTTCCCCTTATGATGTTTACCC	*M. fallax*	670 bp	Wishart vd. 2002
JMVhapla	AAAAATCCCCTCGAAAAATCCACC	*M. hapla*	440 bp	
Fc	TGGAGAGCAGCAGGAGAAAGA	*M.chitwoodi*	800 bp	Zijlstra 2000
Rc	GGTCTGAGTGAGGACAAGAGTA			

Multiplex PCR reactions were performed with JMV1/JMV2/JMVhapla primers in a 25 µl volume that consist of 10x PCR Buffer (2.5 µL), dNTP mix (5mM) (0.5 µL), JMV1 JMV2 and JMV hapla primers (0.35 µL), Taq DNA polymerase (5U) (0.1 µL), dH2O and 20 ng DNA DNA (5 µL). DNA amplifications were performed with thermocycler (Techne, TC-5000) programmed with the following sequence: initial denaturation step at 94 °C for 3 min. followed by 35 cycles of 94 °C for 30 s., 55 °C for 30 s. and 72 °C for 90 s. and a final extension step at 72 °C for 10 min.

PCR reactions were performed with species specific Fc/Rc primers in a 25 µl volume that consist of 10x PCR Buffer (2.5 µL), dNTP mix (5mM) (0.5 µL), Fc and Rc primers (0.6 µL), Taq DNA polymerase (5U) (0.1 µL), dH_2O and 20 ng DNA (5 µL). DNA amplifications were performed with thermocycler (Techne, TC-5000) programmed with the following sequence: initial denaturation step at 94 °C for 2 min. followed by 42 cycles of 94 °C for 30 s., 60 °C for 30 s. and 72 °C for 60 s. and a final extension step at 72 °C for 10 min. PCR products were separated by electrophoresis in Tris-EDTA (TAE) buffer with 1.7% agarose gel stained with Pronosafe (Condalab, Spain) at 80 V for 1 h and then visualized with image acquisition system (Vilber QUANTUM ST4 1000).

Sequence analysis

PCR products obtained using primers reported by Wishart et al. (2002) were confirmed in electrophoresis and sequence process was performed by sending the PCR product (≈15 µL) to GENOKS Molecular Biology and Biotechnology Laboratory. Sequencing data were analyzed using Chromas Pro 1.7.6 (Technelysium, Australia).

Morphological identification

For morphological identification, 15 populations were selected among 63 populations, of which pure cultures are obtained, based on location difference criteria. Eggs were extracted from infected tomato roots according to Hussey & Barker (1973). Second stage larvae were fixed using the method developed by De Grisse (1969). Permanent slides were prepared using wax-ring method (Hooper, 1986). Standard deviations of measurements were calculated with 95% confidence interval as stated by Fortuner (1984). Perineal pattern slides were prepared using “Preparation Method of Perineal Samples” given by Taylor & Netscher (1974) and developed by Hartman & Sasser (1985). Morphological features of populations were examined by light microscope (Leica DM4000 B LED) and the LAS (Leica Application Suite) program was used for measurement. Species level identifications were performed according to Jepson (1987) and Karssen (2002).

Results and Discussion

Molecular identification

Multiplex PCR with JMV1/JMV2/JMVhapla primers produced 540 bp DNA fragment, which specific to *M. chitwoodi* in all populations. There were no amplification products for *M. fallax* (670 bp) and *M. hapla* (440 bp) (Figure 1). Other studies used the same primer set also found similar results (Devran et al., 2009; Özarslandan et al., 2013). PCR performed with species specific Fc/Rc primers produced about 940 bp fragment. This fragment was seen both in all 73 populations and in positive controls (Figure 2). We reached to the opinion that the 800 bp value stated in the original description may be wrong, because Fc/Rc primers are species specific to *M. chitwoodi* and all fragments was found in the same level both in all population and in positive control. Other factors supporting this opinion are that the DNA markers used by Zijlstra (2000) were covering a wide spectrum and no indicating the 800 bp level. And also digital gel imaging systems weren’t widespread and marker variety was very limited in that time. Sequences of 73 populations were compared to other sequences in NCBI Genbank using BLAST, all were identified as *M. chitwoodi*. Sequences were submitted to GenBank with accession numbers KF557769-KF557841 (Table 2).

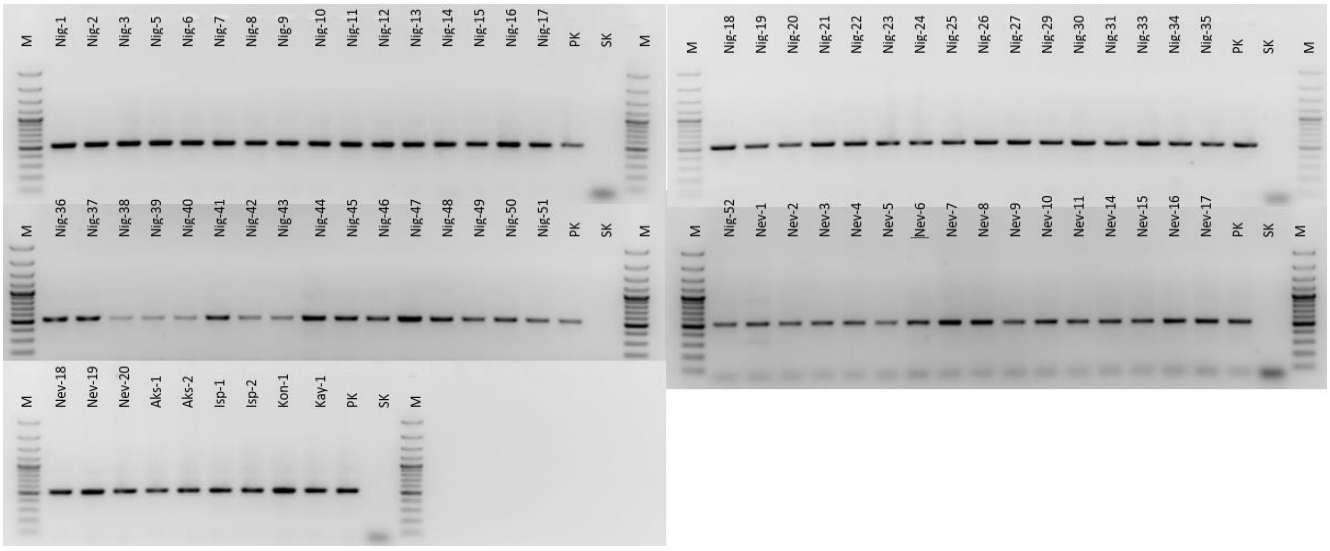

Figure 1. 540 bp PCR product obtained from 73 root-knot nematode samples using JMV1, JMV2 and JMV hapla (M: 100 bp DNA Ladder (ThermoFisher, SM0321), PK: Positive control, SK: water control).

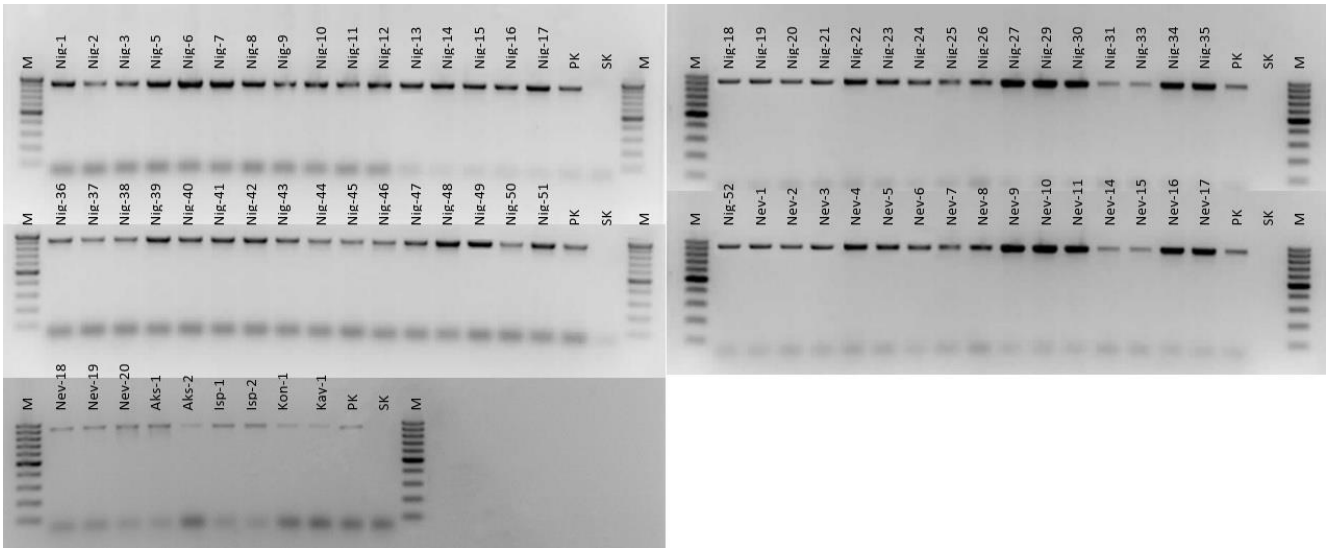

Figure 2. 940 bp PCR product obtained from 73 root-knot nematode samples using Fc and Rc primers (M: 100 bp DNA Ladder (ThermoFisher, SM0241), PK: Positive control, SK: water control.

Morphological identification

In general, perineal pattern of the populations of *M. chitwoodi* under this study showed generally consistent with previous descriptions (Golden et al., 1980; Karssen, 2002). The overall shape of the pattern rounded to oval. Dorsal arch ranging from low and rounded to relatively high and angular, stria near perineal area broken, curved, twisted. Vulva was sunken in an area variable in shape and devoid of striae. Above and below the anus and continuing laterally and ventrally a clear area was bounded by wavy striae (Figure 3).

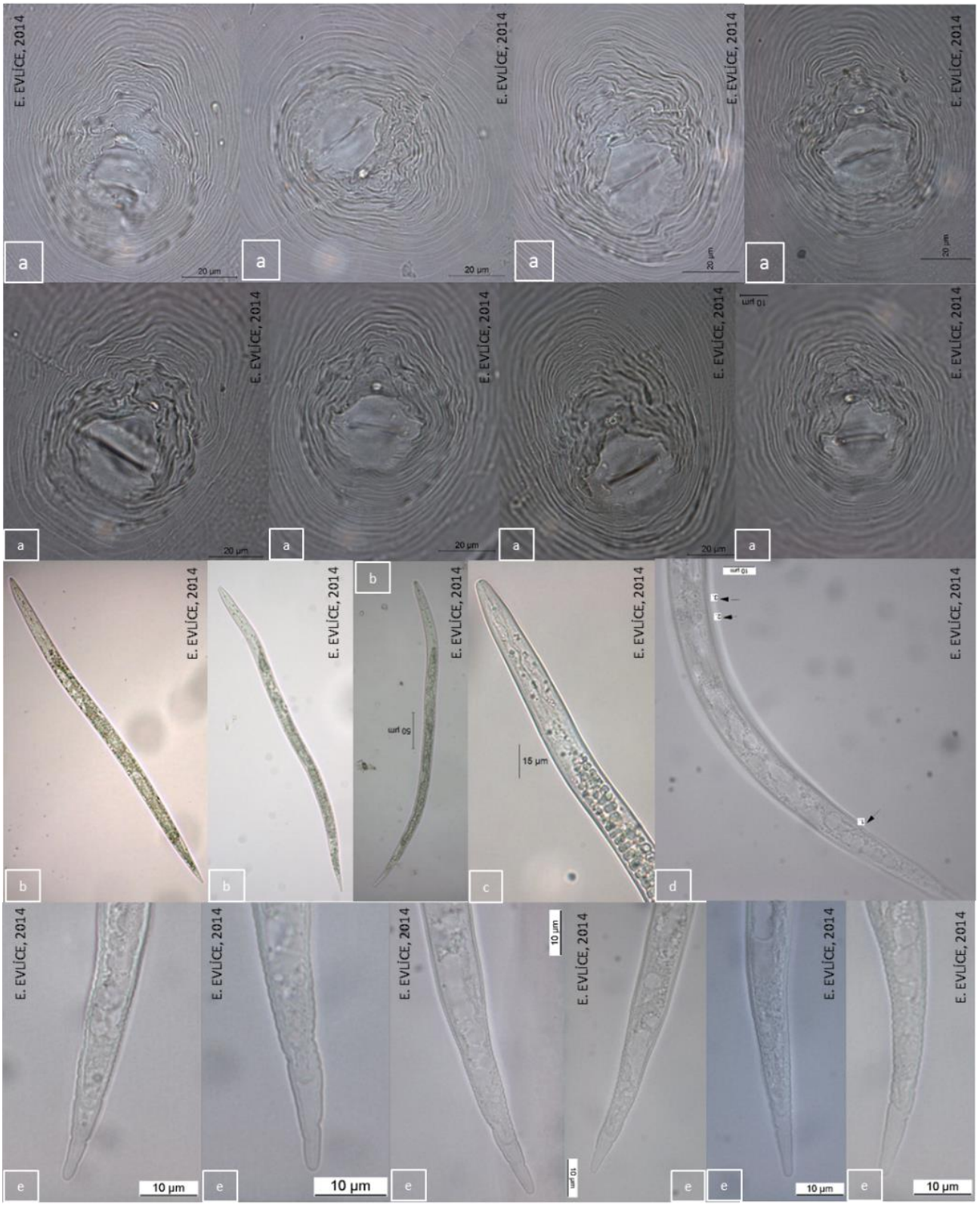

Figure 3. *Meloidogyne chitwoodi* (a) Perineal pattern, (b) general view of second stage larvae, (c) anterior view of second stage larvae, (d) genital primordium view, and (e) tail view of second stage larvae.

Table 3. Second stage larvae measurements of *Meloidogyne chitwoodi* populations (n= 20)

	Niğde Populations	Nevşehir Populations	Aksaray Populations	Golden vd. 1980
N	180	100	20	10
L (μm)	366.8±11.3 (352.3-390)	379.8±12.1 (363.4-398.6)	373.6±9.3 (361.9-395.1)	380.0±11.5 (362-394)
Greatest body diameter (μm)	13.1±0.4 (12.5-13.9)	13,2±0.3 (12.6-14.0)	13.2±0.4 (12.6-13.6)	13.1±0.5 (12.6-13.9)
Body diam. at stylet knobs (μm)	8.8±0.3 (8.2-9.4)	9,1±0.3 (8.3-10.1)	8.6±0.2 (8.3-8.8)	-
Body diam. at S-E pore (μm)	12.4±0.4 (11.5-13.4)	12,3±0,3 (11.3-13.3)	11.9±0.3 (11.4-12.2)	11.8±0.3 (11.4-12.0)
Body diam. at anüs (μm)	9.6±0.5 (8.8-10.8)	9,4±0.4 (8.6-10.2)	9.5±0.3 (9.0-10.0)	9.4±0.4 (8.9-10.1)
Stylet length (μm)	10.0±0.4 (9.3-10.8)	10,4±0.4 (9.6-11.4)	10.3±0.7 (9.5-11.3)	9.7±0.3 (9.5-10.1)
DGO (μm)	3.0±0.2 (2.6-3.3)	3.4±0.3 (2.6-3.8)	3.6±0.3 (3.1-3.8)	3.4±0.4 (2.5-3.8)
Tail length (μm)	43±2.1 (40.2-47.9)	43.2±2.2 (36.9-47.1)	43.8±1.4 (40.7-45.7)	43.2±1.6 (39.8-44.8)
Tail terminus length (μm)	10.4±0.9 (8.8-12.2)	11,2±0.7 (9.0-12.4)	11.5±0.6 (10.2-12.5)	10.9±0.8 (8.9-12.0)
Anus primordium (μm)	89.2±6.3 (80.4-97.8)	96.5±6.9 (83.0-110.6)	91.7±5.7 (82.9-102.8)	93.0±8.5 (82-109)
a	28.1±0.8 (26-29.1)	28.9±1.2 (26.3-31.9)	28.2±1.0 (26.6-30.0)	29.1±1.3 (26.0-31.0)
b’	7.6±0.4 (7.0-8.3)	7.5±0.6 (6.4-8.6)	7.5±0.3 (7.0-8.0)	7.6±0.9 (5.7-8.8)
c	8.5±0.4 (7.4-9.0)	8.8±0.3 (7.9-9.4)	8.5±0.2 (8.3-8.9)	8.8±0.4 (8.1-9.5)
c’	4.5±0.2 (4.1-4.7)	4.5±0.2 (4.0-5.2)	4.6±0.2 (4.4-5.0)	4.6±0.2 (4.2-5.0)
(S-E pore/L)x100	18.3±0.6 (17.5-19.3)	18.5±0.6 (17.2-20.1)	18.4±0.6 (17.5-19.3)	18.5±0.8 (18.0-19.1)

Morphologic and morphometric features of the second stage larvae of *M. chitwoodi* population were compatible with the original description. Body moderately long, tail conical, hyaline tail part relatively short, anterior region clearly deliminated, tail tip blunty rounded. But some morphometrical features such as L, VG, stylet, and hyalin length were different than the original description in some populations (Golden et al., 1980; Karssen, 2002) (Table 3). These differences we believed to be caused by intraspecies variations are also observed among other populations. In a study, which evaluated the morphological features of male and the second stage larvae belonging to four different American isolate of *M. chitwoodi,* very high morphological variation was reported (Humphreys-Pereira & Elling, 2014). Karssen (2002) reported that rectum inflated, whereas Golden et al. (1980) reported rectum not inflated. In our study, we failed to observe rectum on some larvae.

Egg measurement showed eggs diameter shorter (41.51 µl, 42 µl) and eggs length longer (92.99 µl, 85 µl) than the original, thus we found a wider L-W ratio (2.25 µl, 2 µl) (Table 4). These results indicated that morphologic and morphometrical features of the 15 root-knot populations matched those of *M. chitwoodi.*

Table 4. Egg measurements of *Meloidogyne chitwoodi* populations

	Niğde populations	Nevşehir populations	Aksaray populations	Golden vd. 1980
N	450	250	50	50
Length (μm)	93.2±4.9 (73.7-112.5)	93.1±5.0 (80.2-114.2)	90.4±4.9 (77.5-109.0)	85±3.6 (79-92)
Width (μm)	41.1±1.7 (36.3-50.5)	42.3±2.6 (38.0-52.0)	41.0±1.8 (37.3-44.9)	42±1.8 (40-46)
L-W ratio	2.3±0.1 (1.8-2.9)	2.2±0.2 (1.7-2.7)	2.2±0.2 (1.8-2.5)	2±0.1 (1.8-2.3)

Our findings inticated that *M. chitwoodi* is to be only all samples analysed after performing identification using morphological and morphometrical features also molecular methods. Similar studies conducted on potato fields in Niğde and Nevşehir provinces also identified only *M. chitwoodi* species (Devran et al., 2009; Özarslandan et al., 2009; Özarslandan & Elekcioglu, 2010). These findings are compatible with the finding of our study. This study showed that the area of infection has reached to 11 provinces after eight years since its first detection in Isparta, Aksaray, Konya and Kayseri provinces. This picture proves the seriousness of the situation as these provinces contains 48.6% of the potato fields in Turkey and produces 56.8% of the total potato production of Turkey (Anonymous, 2013). Other important hosts of *M. chitwoodi* such as tomato, corn, and wheat are also widely cultivated in Turkey. This may increase the damaging effects of the infection. In order to prevent this possibility, tubers that do not have the seed qualifications should not be used in uninfected fields and management strategies must be created including region specific rotation schemes with uninfected plants and prevention methods.

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