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Original article (Orijinal araştırma)

Detection of the root-knot nematode *Meloidogyne luci* Carneiro et al., 2014 (Tylenchida: Meloidogynidae) in vegetable fields of Samsun Province, Turkey

Samsun İli sebze alanlarında kök-ur nematodu *Meloidogyne luci* Carneiro et al., 2014 (Tylenchida: Meloidogynidae)'nin belirlenmesi

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Abstract

The root-knot nematode, *Meloidogyne luci* Carneiro et al., 2014 (Tylenchida: Meloidogynidae), is commonly found in greenhouses in Samsun Province in northern Turkey but has not been reported in open fields. However, the most recent study on the distribution of root-knot nematodes in open fields of this region was conducted more than 20 years ago and identification was based on perineal patterns. Therefore, the aim of the present study was to update the distribution of *Meloidogyne* spp. in the vegetable fields of Samsun Province. For that purpose, soil samples were collected from 50 vegetable fields during July 2017. Nematode isolates obtained from bioassay tests were identified based on their esterase enzyme phenotype and identification was confirmed with molecular techniques. Root-knot nematodes were detected in 20% of the fields sampled. *Meloidogyne luci* was the most prevalent species, as was reported from greenhouses in Samsun Province, followed by *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949 and *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949. *Meloidogyne luci* is reported for the first time from open fields in Turkey with this study.

Keywords: Distribution, esterase phenotype, *Meloidogyne*, mitochondrial DNA, Turkey

Öz

Türkiye'nin kuzeyindeki Samsun İli'ndeki seralarda yaygın bulunan kök-ur nematodu *Meloidogyne luci* Carneiro et al., 2014 (Tylenchida: Meloidogynidae), bu çalışmadan önce açık alanlarda tespit edilmemiştir. Ayrıca, bu bölgedeki açık alanlarda kök-ur nematodlarının dağılımı ile ilgili en son çalışma, 20 yıldan daha uzun süre önce yürütülmüş ve teşhis perineal desenlere göre yapılmıştır. Bu yüzden, mevcut çalışmanın amacı, Samsun ilindeki sebze tarlalarında *Meloidogyne* türlerinin dağılımına ait sonuçların güncellenmesidir. Bu amaçla, Temmuz 2017'de 50 sebze tarlasından toprak örnekleri alınmıştır. Test bitkilerinden elde edilen nematod izolatları, esteraz enzim fenotiplerine göre teşhis edilmiş ve teşhis moleküler teknikler ile teyit edilmiştir. Örnekleme yapılan sebze tarlalarının %20'sinde kök-ur nematodları tespit edilmiştir. Samsun ilinde daha önce seralarda belirlenen *M. luci,* en yaygın tür olarak tespit edilirken, bu türü *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949 ve *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 takip etmiştir. Türkiye'de açık alanlarda *M. luci*' nin varlığı ilk defa bu çalışma ile bildirilmiştir.

Anahtar sözcükler: Dağılım, esteraz fenotipi, *Meloidogyne,* mitokondrial DNA, Türkiye

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Introduction

Root-knot nematodes (*Meloidogyne* spp.) are among the most important threats to vegetable production. These parasitic nematodes infest the root system and typically cause abnormal swellings called galls which have adverse effects on the uptake of water and nutrients (Aydınlı & Mennan, 2016). This damage is seen above ground as the stunting and yellowing and results yield reduction. Wesemael et al. (2011) reported in a review that the degree of damage for important *Meloidogyne*-plant combinations in Europe ranges from 40% to 100%. Nearly 100 species have been described in this nematode genus and *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949, *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949*, Meloidogyne javanica* (Treub, 1885) Chitwood, 1949 and *Meloidogyne hapla* Chitwood, 1949 (Tylenchida: Meloidogynidae) are considered major pest species commonly detected in different vegetable production systems (Wesemael et al., 2011; Onkendi et al., 2014). Furthermore, *Meloidogyne chitwoodi* Golden et al., 1980 and *Meloidogyne fallax* Karssen, 1996, reported as emerging species by Moens et al. (2009), have become prevalent in vegetable production systems in European countries (Wesemael et al., 2011). Additionally, *Meloidogyne ethiopica* Whitehead, 1968 as a different species from those previously reported in Europe, was detected in tomatoes in Slovenia in 2003 (Sirca et al., 2004). After this report, *M. ethiopica* was recorded in Greece, Italy and Turkey (Conceição et al., 2012; Maleita et al., 2012; Aydınlı et al., 2013). In 2014, Carneiro et al. (2014) described *Meloidogyne luci* Carneiro et al., 2014 as a new root-knot nematode species from different crops in Brazil, Chile and Iran. This species has a very similar morphology to that of *M. ethiopica*. Moreover, a close relationship has been detected between *M. luci, M. ethiopica* and *Meloidogyne inornata* Lordello, 1956 based on phylogenetic analyses. Therefore, these three species are considered to be sister species (Carneiro et al., 2014; Gerič Stare et al., 2017). The esterase phenotypes of these species have three bands and are very similar but a slight difference in their band positions is sufficient to separate each of them (Carneiro et al., 2014), with only an esterase band position different between *M. ethiopica* and *M. luci*. However, before *M. luci* was described, populations in Europe were reported as *M. ethiopica*. Then, Janssen et al. (2016) reported that the Slovenian population accepted as *M. ethiopica* was actually *M. luci*. Later, Gerič Stare et al. (2017) reported that *M. ethiopica* populations in Europe, including Turkey, should be reclassified as *M. luci*. On the basis of that report, all *M. ethiopica* populations in Turkey were accepted as *M. luci*, including those found in vegetable greenhouses in Samsun Province (Aydınlı & Mennan, 2016).

Samsun Province, which is in the Middle Black Sea Region of northern Turkey, had total vegetable production of 1.16 Mt in 2017 (TUIK, 2017). The largest vegetable production areas are the Bafra and Çarşamba Plains, each with about 15,000 ha. Five *Meloidogyne* species, namely *M. arenaria, M. incognita, M. hapla, M. javanica* and *M. luci* (formerly *M. ethiopica*), have been reported from vegetable production areas of this province in different surveys (Bora, 1970; Yüksel, 1974; Mennan & Ecevit, 1996; Katı & Mennan, 2006; Aydınlı & Mennan, 2016). However, the most recent survey in open vegetable fields was conducted by Mennan & Ecevit (1996); they reported *M. incognita* as the most common root-knot nematode. Later surveys in the province were only conducted in greenhouses and the most recent showed that *M. luci* was the predominant species (Katı & Mennan, 2006; Aydınlı & Mennan, 2016). To date, there have been no reports of *M. luci* in open fields of Turkey. Hence, the aim of this study was to determine whether *M. luci* occurs in open vegetable fields. Additionally, the present study sought to update records of the distribution of *Meloidogyne* species in vegetable fields of Samsun Province.

Material and Methods

Surveys

Surveys were conducted in 2017 in 10 and 11 villages on the Bafra and Çarşamba Plains, respectively, in Samsun Province of northern Turkey (Figure 1). A total of 50 randomly selected vegetable fields in Bafra (25 fields; 8 pepper, 8 tomato, 6 melon and 3 watermelon) and Çarşamba (25 fields; 7 tomato, 7 bean, 6 pepper, 4 melon and 1 eggplant) were surveyed for the early presence of *Meloidogyne* infestation during July 2017. The roots of 20-30 plants from each field were checked for the presence of *Meloidogyne* galls and soil samples were collected from the rhizosphere of infected plants. The individual

soil samples collected in each field were combined into a composite sample, placed in a labeled, polyethylene bag and transported to the Nematology Laboratory of the Faculty of Agriculture at Ondokuz Mayıs University in Samsun for processing. Soil samples were used in bioassay tests within 2 days.

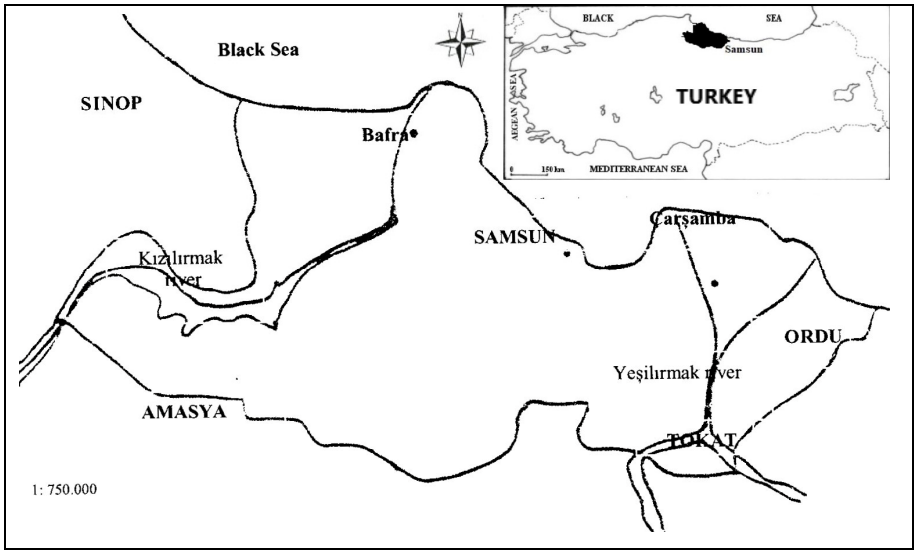

Figure 1. Locations of surveyed area in the map of Turkey.

Detection of *Meloidogyne* spp.

The presence of *Meloidogyne* spp. in soil samples was determined by bioassay test. For that purpose, 3-week-old seedlings of a nematode-susceptible tomato variety (*Solanum lycopersicum* L. cv. Falcon, May Seed, Turkey) were transplanted singly into pots containing 500 cm^3 of the homogenized soil sample from each field (Karuri et al., 2017). Plants were maintained at 25±2°C in the greenhouse, irrigated and fertilized as need. The plants were removed from the pots after 60 days and the roots were carefully washed with tap water. The roots were dissected under a stereomicroscope and females and egg masses were extracted from random positions of each root system. The females were immediately used and the egg masses were stored at -20ºC until analysis.

Identification of *Meloidogyne* spp. isolates

Meloidogyne spp. isolates were identified by the esterase phenotypes of females and identification was confirmed with molecular techniques based on DNA analysis. For the esterase studies, young females collected in 0.9% NaCl were transferred to an extraction buffer (20% sucrose and 1% Triton X-100) in a microhematocrit tube. Single or three females were crushed with a pestle. Samples were immediately stored at -20ºC. Twenty females from each plant root were analyzed. Females of *M. javanica* isolated from pot cultures in the laboratory were used as reference. The electrophoresis process was run at 6 mA per gel for the first 15 min and then at 20 mA per gel for 40-45 min in a Mini-Protean Tetra System (Bio-Rad Laboratories, Hercules, CA, USA). The gels were stained with the substrate α-naphthyl acetate for esterase activity (Esteves et al., 2015).

For the molecular studies, DNA was extracted from 10 egg masses for PCR with species-specific primers and from an individual male or second-stage juvenile for sequencing of the mitochondrial DNA (mtDNA) region. DNA was extracted with the DNeasy Tissue and Blood Kit (QIAGEN, Hilden, Germany), according to the manufacturer's instructions. PCR reactions were carried out with specific primers, namely Far/Rar (Zijlstra et al., 2000) for *M. arenaria*, Fjav/Rjav (Zijlstra et al., 2000) for *M. javanica*, inc-K14F/R (Randig et al., 2002) and SEC-1F/1R (Tesarova et al., 2003) for *M. incognita*, and JMV (Wishart

et al., 2002) for *M. hapla, M. chitwoodi* and *M. fallax*. Amplifications with specific primers were performed in a final volume of 25 µl that contained 2 µl of template DNA, 1 µl of 10 µM of each primer (0.4 µM of each primer) and 12.5 µl of BioMix Red (Bioline). PCR was conducted with a T-100 Thermal Cycler (Bio-Rad Laboratories) and amplification conditions were as follows: initial denaturation at 94ºC for 3 min; 35 cycles of 30 s at 94ºC, 30 s at 55ºC (SEC-1F/1R and JMV) or 60ºC (Far/ Rar, inc-K14F/R and Fjav/Rjav), and 1 min at 72ºC; and a final extension for 7 min at 72ºC. All samples were tested with all specific primer pairs in the study. The PCR products were analyzed by electrophoresis in 2% agarose gel stained with ethidium bromide and then visualized.

The mtDNA COI region was amplified with JB3/COI2R5 primers (Kiewnick et al., 2014; Maleita et al., 2018) for the sequencing of the three phenotype L3 isolates. The PCR reaction mixtures contained 5 µl of DNA, 5 µl of 10X standard Taq buffer, 4 µl of 25 mM MgCl2, 2 µl of 5 mM dNTPs, 2 µl of 10 µM of each primer, and 0.5 µl of 5 U/µl Taq DNA polymerase (New England Biolabs, Ipswich, MA, United States) and nuclease-free water made up to 50 µl. Thermal cycling was programmed as reported by Kiewnick et al. (2014). Amplified DNA was sequenced in both directions with the respective primers used for amplification. Sequences were checked and aligned with the BioEdit software package (Hall, 1999). The edited sequences were then compared with the sequences of *Meloidogyne* species available in the GenBank database.

Results

Meloidogyne species was detected in 10 of the 50 fields sampled in Samsun Province (Table 1). Infested soil samples were recovered from vegetable fields in seven of 11 villages on the Carşamba Plain but no infestations were detected on the Bafra Plain. These infested samples were from four bean, four pepper and two tomato fields. The occurrence of *Meloidogyne* spp. varied among vegetable species. Overall, 57% of the bean fields surveyed were infested, followed by 29% of pepper fields and 13% of tomato fields. However, no *Meloidogyne* species were detected in watermelon, melon and eggplant fields.

Root-knot nematode species were identified on the basis of their esterase phenotypes. The three different esterase phenotypes detected, namely L3, A2 and I2, were indicative of *M. luci, M. arenaria* and *M. incognita*, respectively (Figure 2). L3, the most common esterase phenotype in this study, was detected in seven isolates (Figure 3), while A2 was in three isolates and I2 in one isolate. In addition, a sample obtained from a tomato field contained both L3 and A2 phenotypes indicating the presence of *M. luci* and *M. arenaria* isolates.

Identification based on esterase phenotype was confirmed by molecular assays, namely PCR with species-specific primers and sequencing of the mtDNA region. In addition, primers specific for *M. javanica* and *M. hapla*, which had been detected in previous studies in this survey region, were used to check the DNA of isolates identified with esterase assays. The DNA of isolates was not amplified with these primers (data not shown). The PCR conducted with the *M. arenaria* species-specific primer, Far/Rar, produced amplification fragments of 420 bp from the three isolates of the A2 esterase phenotype (Figure 4a). The inc-K14F/R from primer pairs used for the identification of *M. incognita* gave a positive band of 400 bp for an isolate with I2 esterase phenotype (Figure 4b), whereas PCR with SEC-1F/1R primers produced a single band of 500 bp for both an isolate with I2 and seven isolates with L3 (Figure 4c). The PCR with primer pair used for sequencing of the mtDNA COI region yielded single bands of about 800 bp (data not shown). The sequences from the mtDNA COI region were 100% identical with the *M. luci* sequences in GenBank (accession numbers MF280973, MF280974, MF280975, MF280976 and KY563093). The sequences obtained were deposited in GenBank under the accession numbers MG969509, MG969510 and MG969511.

Table 1. *Meloidogyne* isolates obtained from vegetable fields in Samsun Province, Turkey

Isolate No	Field coordinate	Sample field	Esterase phenotype	Primer pairs*			Species
				Far/Rar	inc-F/R	SEC-1F/1R	
103	41°16'39" N 36°33'13" E	Pepper	L3	-	-	+	*M. luci*
108	41°16'20" N 36°35'24" E	Bean	A2	+	-	-	*M. arenaria*
109	41°13'13" N 36°37'17" E	Pepper	I2	-	+	+	*M. incognita*
110	41°15'35" N 36°36'46" E	Bean	L3	-	-	+	*M. luci*
111	41°15'17" N 36°37'28" E	Pepper	L3	-	-	+	*M. luci*
112	41°15'37" N 36°36'52" E	Tomato	L3	-	-	+	*M. luci*
113	41°14'42" N 36°38'14" E	Bean	L3	-	-	+	*M. luci*
114	41°13'58" N 36°38'40" E	Bean	A2	+	-	-	*M. arenaria*
121	41°14'19" N 36°40'03" E	Tomato	A2+L3	+	-	+	*M. arenaria* + *M. luci*
125	41°17'39" N 36°34'40" E	Pepper	L3	-	-	+	*M. luci*

* DNA of isolates was not amplified with Fjav/Rjav, specific for *M. javanica,* and JMV, specific for *M. hapla, M. chitwoodi and M. fallax*.

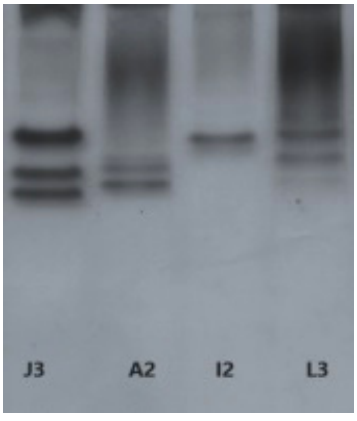

Figure 2. Esterase phenotypes detected from females of *Meloidogyne* isolates (J3, *M. javanica* as reference isolate; A2, *M. arenaria*; I2, *M. incognita*; L3, *M. luci*).

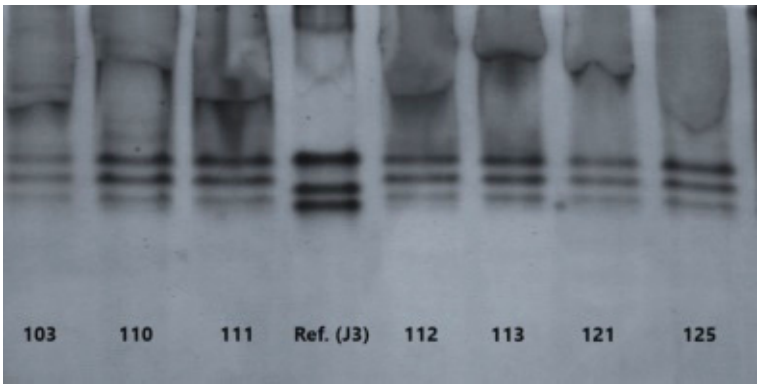

Figure 3. Esterase phenotypes (L3) of *Meloidogyne luci* isolates in this study (J3: reference isolate of *M. javanica*).

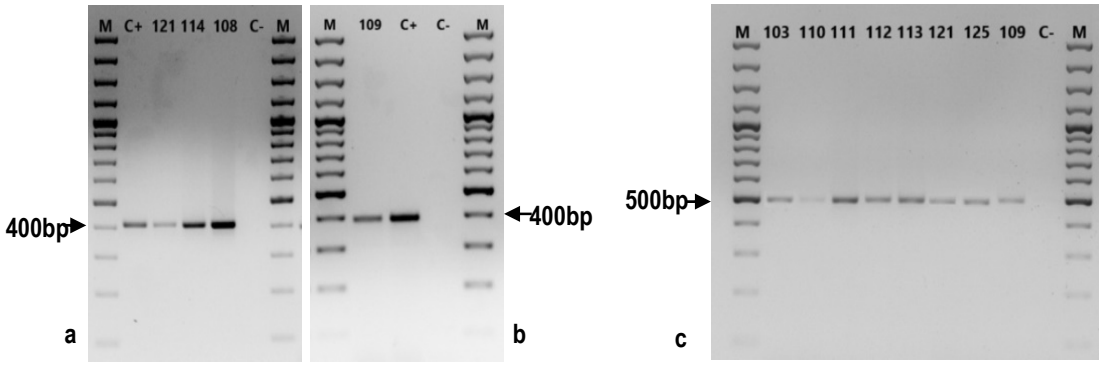

Figure 4. PCR products obtained with a) Far/Rar, primers specific for *Meloidogyne arenaria*; b) inc-K14F/R, primers specific for *Meloidogyne incognita*; and c) SEC-1F/1R, primers specific for *M. incognita* and *Meloidogyne luci* (Numbers indicated code of isolates in this study. C+: positive control DNA, C-: negative control (water), M: molecular marker with 100 bp.).

Discussion

This study reports the presence of *M. luci* (formerly *M. ethiopica*) in open fields in Turkey for the first time. The most recent open field survey for root-knot nematodes in Samsun was conducted over 20 years ago (Mennan & Ecevit, 1996). In that study, the identification of *Meloidogyne* species was based on the perineal patterns. Although the perineal patterns of females aid the identification of *Meloidogyne* species, this character alone is not sufficient anymore and can result in misidentification due to substantial similarity and overlap of perineal patterns between species (Brito et al., 2008; Garcia & Sanchez-Puerta, 2012). For example, the perineal patterns of *M. luci* are similar to those of *M. incognita* and are therefore not useful for identification purposes (Carneiro et al., 2014; Aydınlı & Mennan, 2016). In contrast, the present study reports the presence of *Meloidogyne* species in open fields through the use of reliable and sensitive methods, without relying on expertise in the interpretation of perineal morphology patterns.

Meloidogyne luci, the most prevalent root-knot nematode species in this survey, was collected from three pepper (isolates 103, 111 and 125), two bean (isolates 110 and 113) and two tomato fields (isolates 112 and 121), and *M. arenaria* was collected from two bean fields (isolates 108 and 114) and one tomato field (isolate 121). *Meloidogyne incognita* was detected in only one pepper field (isolate 109). In addition, a mixed population of *M. arenaria* and *M. luci* (isolate 121) was collected from a tomato field.

Meloidogyne luci was first detected in Turkey in 2009 in greenhouses in Samsun Province (Aydınlı et al., 2013). Later, an extensive survey of *Meloidogyne* species in greenhouses of the Middle Black Sea Region revealed that *M. luci* was the most common species, followed by *M. arenaria, M. javanica and*

M. incognita in Samsun (Aydınlı & Mennan, 2016). In the present survey, the occurrence of *Meloidogyne* species, except *M. javanica*, is consistent with the findings of Aydınlı & Mennan (2016). The absence of *M. javanica* in open fields in Samsun Province supports the results of previous studies (Bora, 1970; Yüksel, 1974; Mennan & Ecevit, 1996) and suggests that this species does not survive in open fields but does survive in the greenhouses in this region. However, *M. javanica* was detected in open fields in a different region of Turkey (Aydın, Aegean Region) that was more temperate than the Black Sea Region during the winter period (Kaşkavalcı & Öncüer, 1999). Van Gundy (1985) reviewed the environmental factors affecting survival of *Meloidogyne* spp. and noted that *M. javanica* had a lower resistance to cold stress than both *M. incognita* and *M. arenaria*. In the present study, *M. incognita* was collected in only one field, in contrast to the finding of Mennan & Ecevit (1996) who reported a wide distribution of this nematode in the same area. Differences between the results of the study of Mennan & Ecevit (1996) and the present study in terms of the distribution of this species were also reported in the greenhouse studies of Katı & Mennan (2006) and Aydınlı & Mennan (2016). A likely reason for this is the similarity of the perineal pattern of *M. incognita* and *M. luci*.

The *M. luci* specific esterase enzyme phenotype (L3) is the best tool for differentiating this species from other *Meloidogyne* species (Carneiro et al., 2014; Gerič Stare et al., 2017; Maleita et al., 2018). Additionally, esterase studies can be supported with data from mtDNA regions. The efficacy of mtDNA markers was previously reported by Gerič Stare et al. (2017) and Maleita et al. (2018). Janssen et al. (2016) also showed usefulness of mtDNA regions for tropical root-knot nematodes differentiation. Gerič Stare et al. (2017) reported that in phylogenetic analysis of the COII/IRNA region of mtDNA, *M. luci* formed a monophyletic clade and allowed a clear separation of this recently described species. In addition, Maleita et al. (2018) stated that the mtDNA COI was a useful region for the differentiation of *M. luci* from *M. ethiopica* but it could not be used to differentiate *M. arenaria, M. ethiopica, M. incognita, M. inornata* and *M. javanica*.

In conclusion, *M. luci* was detected for the first time in open vegetable fields of Turkey. This nematode was previously reported from open fields associated with kiwifruit and maize in Greece, and potatoes in Portugal in the EPPO region (Conceicão et al., 2012; Maleita et al., 2018), cucumber, lettuce, broccoli, okra, green bean, yakon, kiwifruit and lavender in Brazil, grapevine in Chili in South America (reviewed in Carneiro et al., 2014), rose, snapdragon and sedum in Iran in Asia (Carneiro et al., 2014). Moreover, Strajnar et al. (2011) reported that it survived in the winter in open fields in both sub-Mediterranean regions and also in the continental climates of regions in Slovenia, despite temperatures below zero. Therefore, *M. luci* appears to constitute a real threat to both open field and protected field crops in the world due to its wide host range and ability to persist in different climatic zones.

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