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Original article (Orijinal araştırma)

Occurrence and abundance of cereal nematodes in Konya and Karaman Provinces in Turkey[1]

Konya ve Karaman (Türkiye) illerinde tahıl nematodlarının dağılımı ve popülasyon yoğunluğu

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Abstract

Distribution and populations of cereal nematodes in Konya and Karaman Provinces were investigated in 2016 and 2017. Root lesion nematodes, *Pratylenchus thornei* Sher & Allen, 1953, *Pratylenchus neglectus* Rensch, 1924 and *Pratylenchus vulnus* Allen & Jensen, 1951 (Tylenchida, Pratylenchidae), were found at 69, 7 and 17 locations, respectively. Cereal cyst nematode, *Heterodera filipjevi* (Madzhidov, 1981) Stelter, 1984 (Tylenchida, Heteroderidae), was found at 33 locations. Stem and bulb nematode, *Ditylenchus dipsaci* (Kuhn, 1857) Filipjev, 1936 (Tylenchida, Anguinidae), was not found in any samples. *Pratylenchus* spp. were found in 40.5% (6±1 nematodes 100 g dry soil^{-1}) and *Heterodera* spp. in 29% (3±1cysts 250 g dry soil^{-1}) of the soil samples. *Ditylenchus* spp. (32.6%, 18±3 nematodes 100 g dry soil^{-1}) and *Tylenchus* spp. (21.4%; 12±3 nematodes 100 g dry soil^{-1}) were widely distributed. *Paratylenchus* spp. (0.9%; 1±1 nematodes 100 g dry soil^{-1}) and *Pratylenchoides* spp. (0.5%; 1±1 nematodes 100 g dry soil^{-1}) were found in few locations and in low abundance. *Aphelenchus* spp. (39.1%; 33±5 nematodes 100 g dry soil^{-1}) and *Aphelenchoides* spp. (51.2%; 52±7 nematodes 100 g dry soil^{-1}) were identified as the fungal-feeding nematodes. *Acrobeloides* (65.1%; 109±13 nematodes 100 g dry soil^{-1}) and *Cephalobus* (19.1%; 7±1 nematodes 100 g dry soil^{-1}) were the most abundant bacterial-feeding nematode genera.

Keywords: Free living nematodes, *Heterodera* spp., morphometrics, *Pratylenchus* spp., species-specific PCR

Öz

Konya ve Karaman illerinde tahıl alanlarında nematodlarının dağılımı ve popülasyonları 2016 ve 2017 yıllarında araştırılmıştır. Kök yara nematodları; *Pratylenchus thornei* Sher & Allen, 1953, *Pratylenchus neglectus* Rensch, 1924 ve *Pratylenchus vulnus* Allen & Jensen, 1951 (Tylenchida, Pratylenchidae) sırasıyla 69, 7 ve 17 lokasyonda tespit edildi. Tahıl kist nematodu *Heterodera filipjevi* (Madzhidov, 1981) Stelter, 1984 (Tylenchida, Heteroderidae) 33 lokasyonda bulunmuştur. Soğan sak nematodu, *Ditylenchus dipsaci* (Kuhn, 1857) Filipjev, 1936 (Tylenchida, Anguinidae) incelenen örneklerde bulunmamıştır. *Pratylenchus* spp. toprak örneklerinde %40.5 (6±1 nematod 100 g kuru toprak^{-1}) ve *Heterodera* spp. %29 (3±1 kist 250 g kuru toprak^{-1}) oranında tespit edilmiştir. *Ditylenchus* spp. (%32.6; 18±3 nematod 100 g kuru toprak^{-1}) ve*Tylenchus* spp. (%21.4, 12±3 nematod 100 g kuru toprak^{-1})'nin yaygın olarak dağıldığı belirlenmiştir. *Paratylenchus* spp. (%0.9, 1±1 nematod 100 g kuru toprak^{-1}) ve *Pratylenchoides* spp. (%0.5; 1±1 nematod 100 g kuru toprak^{-1}) birkaç lokasyonda ve düşük yoğunlukta bulunmuştur. *Aphelenchus* spp. (%39.1; 33±5 nematod 100 g kuru toprak^{-1}) ve *Aphelenchoides* spp. (%51.2; 52±7 nematodes 100 g kuru toprak^{-1}) fungal beslenen türler olarak belirlenmiştir. *Acrobeloides* (%65.1; 109±13 nematod 100 g kuru toprak^{-1}) ve *Cephalobus* (%19.1; 7±1 nematod 100 g kuru toprak^{-1}) en yoğun bulunan bakteriyel beslenen nematod cinsleridir.

Anahtar sözcükler: Serbest yaşayan nematodlar, *Heterodera* spp., morfometrik ölçümler, *Pratylenchus* spp., türe özgü PCR

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Introduction

Cereals have an important place in the human diet and in animal feed throughout the world. Konya Province has the highest cereal production on the Central Anatolian Plateau of Turkey. Excluding maize, cereals are produced over 1.2 Mha, which is the 11.7% of Turkey production area (about 10 Mha) in Konya and Karaman Provinces. Cereal production in the provinces is about 3.5 Mt, 12.5% of the total production in Turkey (28 Mt) (TÜİK, 2019).

Nematodes are round worms that include parasitic and free living species found in the rhizosphere region of the soil. Nematodes are grouped according to their feeding characteristics (Yeates et al., 1993). Around the world, the most damaging nematode species in cereals are root lesion and cereal cyst nematodes, and likewise in Turkey (Nicol et al., 2003; Imren et al., 2012; Abd-Elgawad & Askary, 2015; Toktay et al., 2015). The yield losses in wheat caused by cereal cyst and root lesion nematodes were reported up to 50% on the Central Anatolian Plateau of Turkey (Nicol & Ortiz-Monastterio, 2004). Cereal cyst nematodes of *Heterodera filipjevi* (Madzhidov, 1981) Stelter, 1984, *Heterodera latipons* Franklin, 1969 and *Heterodera mani* Mathews, 1971 (Tylenchida, Heteroderidae) were found in cereals on the Central Anatolian Plateau (Enneli et al., 1994; Rumpenhorst et al.,1996; Ozturk et al., 1998; Abidou et al., 2005; Yavuzaslanoglu et al., 2012). *Pratylenchus thornei* Sher & Allen, 1953 and *Pratylenchus neglectus* Rensch, 1924 (Tylenchida, Pratylenchidae) were prevalent species of root lesion nematodes on the Central Anatolian Plateau (Yavuzaslanoglu et al., 2012).

Morphology and morphometrics are the basic methods in nematode diagnosis. However, generally there is considerable variation between the specimens investigated witin a species; the studies require experience and are time consuming. In particular, *Pratylenchus* spp. has considerable intraspecific variation. Additionally, the low number of diagnostic features, depending on the reproductive strategy of the species necessitates molecular identification (Castillo &Vovlas, 2007). Molecular characterization is a practical and reliable method, and supports nematode diagnosis studies. To date, data on root lesion nematodes on the Central Anatolian Plateau of Turkey were based on morphological and molecular identification. The study aimed to investigate the root lesion nematodes using molecular methods in addition to morphology and morphometrics.

The long-term assessment of nematode communities in cereal growing areas provides important information on the damaging potential of parasitic nematodes and also effect of free living nematodes on soil fertility and sustainability. Hence, monitoring nematode communities in regulated terms is required; especially nematode communities on the production areas of strategic plant species. For this purpose, the occurrence and abundance of nematodes in cereal production areas in Konya and Karaman Provinces which represent the largest cereal production area of Turkey were investigated in 2016-2017. The most damaging plant-feeding nematode species were identified using the species-specific PCR technique.

Materials and Methods

Sampling locations

Wheat and barley planted fields in Konya and Karaman Provinces on the Central Anatolian Plateau were sampled for the prevalence and population estimation of the nematodes in the years of 2016 and 2017. A total of 215 plant and soil samples were collected representing the districts systematically stopping every 3-4 km. Sixty-one samples were collected from Karaman Province and 154 samples were collected from Konya Province. The sampling was performed in April 2016 and 2017 for migratory nematodes. Cereal cyst nematode (CCN) cysts were sampled from the same locations with migratory nematodes in July-August 2017. The samples were taken every 15-20 paces in a zigzag pattern from 15-20 points constituting two kg of a bulk sample in a field. A 2.5 cm diameter soil corer was used for soil sampling to a depth of 30 cm. The plant samples were collected from the same points as the soil cores. The sampled cereal fields were mapped using a GPS.

Nematode extraction and population estimation

The nematodes were extracted from the plant and soil samples using a modified Baermann funnel technique (Hooper, 1986a). Three plants were used for the nematode extraction which was performed separately for each plant. The nematode counts from the plant samples were presented as average number per plant. These plant samples were incubated for 1 d and the soil samples for 2 d for the nematode extraction. The percentage of the moisture of each soil samples was calculated drying 10 g of fresh soil in an oven at 90ºC for 2 d. The nematode counts from the soil samples were converted to the number of nematodes 100 g of dry soil^{-1}. The nematodes obtained from the soil samples were divided into trophic groups according to Yeates et al. (1993).

The cysts of the CCN were extracted using a flotation technique (Kort, 1960) from a 250 g soil sample. Number of cysts in each soil sample was counted under a stereo microscope. The plant roots were washed gently with water and evaluated for the cyst formation under the stereo microscope.

Species identification of key plant-feeding nematodes

The nematodes obtained from the plant and soil samples were counted at genus level and divided into trophic groups according to Yeates et al. (1993). The permanent slides of the plant-feeding nematodes for the morphological species identification were prepared using all the available specimens (Hooper; 1986b). Measurements and morphometric calculations were compared with the literature.

Five species of *Pratylenchus* spp. [*P. thornei, P. neglectus, Pratylenchus vulnus* Allen & Jensen, 1951*, Pratylenchus scribneri* Steiner, 1943 and *Pratylenchus penetrans* (Cobb, 1917) Filipjev & Schuurmans-Stekhoven, 1941(Tylenchida, Pratylenchidae)], three species of *Heterodera* spp. [*H. filipjevi, H. latipons* and *Heterodera avenae* Wollenweber, 1924 (Tylenchida, Heteroderidae)] and *Ditylenchus dipsaci* (Kuhn, 1857) Filipjev, 1936 (Tylenchida, Anguinidae) were investigated using the species-specific PCR in addition to morphological diagnosis. This molecular method was applied as described by Karaca (2018) and Yavuzaslanoglu et al. (2018). Species-specific primers used for identification of root lesion nematodes multiplying D3 expansion regions of 26S-rDNA were presented on Table 1. Nematodes were identified obtaining the specific bands at 290, 278, 286, 288 and 287 bp for *P. neglectus*, *P. penetrans*, *P. scribneri*, *P. thornei* and *P. vulnus,* respectively (Al-Banna et al., 2004).

Table 1. Species of root lesion nematodes (*Pratylenchus* spp.) identified using 26S-rDNA D3 elongation regions, short name of used primers, 5'-3' forward sequences, annealing temperature, PCR product size and references obtained

Nematode species	Short name of primer	5'-3' Primer sequence	Annealing temperature (°C)	PCR product size (bp)	Reference
P. neglectus	PNEG	ATGAAAGTGAACATGTCCTC	63	290	Al-Banna et al., 2004
P. penetrans	PPEN	TAAAGAATCCGCAAGGATAC	62	278	Al-Banna et al., 2004
P scribneri	PSCR	AAAGTGAACGTTTCCATTTC	63	286	Al-Banna et al., 2004
P. thornei	PTHO	GAAAGTGAAGGTATCCCTCG	68	288	Al-Banna et al., 2004
P. vulnus	PVUL	GAAAGTGAACGCATCCGCAA	68	287	Al-Banna et al., 2004

Primers specific to rDNA-ITS regions of cereal cyst nematodes are shown in Table 2. *Heterodera filipjevi*, *H. avenae* and *H. latipons* were identified with obtained specific bands at 170, 242 and 204 bp, respectively (Toumi et al., 2013; Yan et al., 2013).

Molecular identification of *D. dipsaci* were performed using nine species-specific primers suggested by Vrain et al. (1992); Marek et al. (2005); Zouhar et al. (2007); Marek et al. (2010) and Vovlas et al. (2011) (Table 3). Specific bands for identification of *D. dipsaci* were investigated at 327, 396, 517, 263, 333, 967, 256, 325, 245 bp, for PF1-PR1, PF2-PR2, DdpS1-rDNA2, DitNF1-rDNA2, DipU F-DipU R, 18S-26S, DipU F-Dip1 R, DIT2 F-DIT2 R, DIT5 F-DIT5 R primers, respectively.

Table 2. Species of identified cereal cyst nematodes (*Heterodera* spp.) using rDNA-ITS regions, short name of used primers, 5'-3'sequences, annealing temperature, PCR product size and references obtained

Nematode species	Short name of primer	5'-3' Primer sequence	Annealing temperature (°C)	PCR product size (bp)	Reference
H. filipjevi	HfITS-F1	F: CCCGTCTGCTGTTGAGA	58	170	Yan et al., 2013
	HfITS-R1	R: ACCTCAGGCTTTTATTATCAC			
H. avenae	HaITS-F	F: ATGCCCCCGTCTGCTGA	64	242	Yan et al., 2013
	HaITS-R	R: GAGCGTGCTCGTCCAAC			
H. latipons	Hlat-actF	F: ATGCCATCATTATTCCTT	50	204	Toumi et al., 2013
	Hlat-actR	R: ACAGAGAGTCAAATTGTG			

Table 3. The primers and sequences, PCR product size, target regions on nematode genome and references used for species-specific detection of *Ditylenchus dipsaci*

Primer	5'-3' Primer sequence	PCR product size (bp)	Target region	Reference
PF1	5′-AACGGCTCTGTTGGCTTCTAT-3	327	Flanking ITS regions	Marek et al., 2005
PR1	5′-ATTTACGACCCTGAGCCAGAT-3′			
PF2	5′-TCGCGAGAATCAATGAGTACC-3′	396	Flanking ITS regions	Marek et al., 2005
PR2	5′-AATAGCCAGTCGATTCCGTCT-3′			
DdpS1	5′-TGGCTGCGTTGAAGAGAACT-3′	517	5.8S rDNA	Vrain et al., 1992
rDNA2	5′-TTTCACTCGCCGTTACTAAGG-3′			
DitNF1	5′-TTATGACAAATTCATGGCGG-3′	263	18S rDNA - ITS1	Vrain et al., 1992
rDNA2	5′-TTTCACTCGCCGTTCTAAGG-3′			
DipU F	5' -CCCATTTTTGAACTTTTTTACAAG-3'	333	Flanking ITS regions	Vovlas et al., 2011
DipU R	5' -CTAGATTAGCAAAGACGTATATC-3'			
18S	5' -TTGATTAGGTCCCTGCCCTTT-3'	967	ITS1-5.8S-ITS2	Marek et al., 2010
26S	5' -TTTCACTCGCCGTTACTAAGG-3'			
DipU F	5' -CCCATTTTTGAACTTTTTTACAAG-3'	256	ITS1-ITS2	Marek et al., 2010
Dip1 R	5' -GAAAAGCACCCAACCAGTACC-3'			
DIT2 F	5′-GCAATGCACAGGTGGATAAAG-3′	325	Flanking ITS regions	Zouhar et al., 2007
DIT2 R	5′-CTGTCTGTGATTTCACGGTAGAC-3′			
DIT5 F	5′-GAAAACCAAAGAGGCCGTAAC-3′	245	Flanking ITS regions	Zouhar et al., 2007
DIT5 R	5′-ACCTGATTCTGTACGGTGCAA-3′			

Statistical analysis

Statistically significant differences of the population densities of the nematode genera among districts were investigated using analysis of variance and Student's t-test. Statistical analyses were performed using the JMP5.01.a program (JMP, 2009).

Results and Discussion

The occurrence and abundance of the nematodes on wheat and barley fields in Konya and Karaman Provinces, which is the largest cereal production area of Turkey was investigated in detail in all the districts represented with 215 samples. Seventy-six percent of the samples contained plant-feeding nematodes.

The occurrence of the economically important nematode species of *Pratylenchus* spp. was 40.5%, supporting previous reports (Yavuzaslanoglu et al., 2012) (Table 4). The maximum *Pratylenchus* spp. population was recorded in Karapınar District of Konya Province with a mean of 14±14 nematodes 100 g dry soil^{-1}. The plant samples contained a higher number of nematodes in Kadınhanı, Selçuklu and Cihanbeyli Districts of Konya Province; a mean of 13±8, 16±16 and 9±4 nematodes 100 g dry soil^{-1}, respectively (Table 5). However, the population density was relatively lower in comparison to previous reports. It was a maximum 111 nematodes 100 g dry soil^{-1} in the current study, while it had been reported to be up to 274 nematodes in 100 g dry soil in 2003 (Yavuzaslanoglu et al., 2012).

Table 4. Prevalence of individual nematode genera and feeding groups identified in samples

Province	District	Number of samples collected	*Pratylenchus* spp.	*Heterodera* spp.	*Pratylenchus* spp. + *Heterodera* spp.	*Ditylenchus* spp.	*Tylenchus* spp.	*Paratylenchus* spp.	*Pratylenchoides* spp.	Plant Feeding Nematodes	*Aphelenchus* spp.	*Aphelenchoides* spp.	Fungal Feeding Nematodes	*Cephalobus* spp.	*Eucephalobus* spp.	*Acrobeles* spp.	*Acrobeloides* spp.	*Rhabditis* spp.	Bacterial Feeding Nematodes	*Dorylaimid*aorder
Karaman	Ayrancı	9	2	2	0	4	1	0	0	5	5	3	7	1	1	0	6	0	8	1
	Başyayla	2	2	0	0	1	0	0	0	2	2	2	2	0	0	0	2	0	2	0
	Ermenek	4	2	0	0	2	0	0	0	3	3	2	4	0	1	0	3	0	3	0
	Central	44	17	8	3	18	8	0	0	32	24	28	33	13	0	1	34	1	37	4
	Sarıveliler	2	0	0	0	2	1	0	0	2	2	2	2	1	0	0	2	0	2	0
Konya	Ahırlı	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
	Akşehir	4	2	0	0	0	1	0	0	3	1	3	4	2	0	1	1	0	3	0
	Altınekin	3	3	2	2	2	1	0	0	3	0	2	2	0	0	0	3	0	3	0
	Beyşehir	5	2	1	1	4	2	0	0	5	3	4	4	1	0	0	5	0	5	2
	Bozkır	4	2	1	1	2	0	1	0	4	1	1	1	1	0	0	3	0	3	1
	Central	2	1	1	0	0	0	0	0	2	1	0	1	0	0	0	1	0	1	0
	Çeltik	3	0	0	0	0	0	0	0	0	1	2	2	2	0	0	0	0	2	1
	Cihanbeyli	26	12	11	6	2	5	0	1	18	5	3	7	7	0	2	14	0	17	11
	Çumra	11	4	4	1	5	1	0	0	9	9	8	10	1	0	2	10	0	10	0
	Doğanhisar	3	1	0	0	1	2	0	0	3	3	2	3	1	1	0	3	0	3	0
	Ereğli	1	0	1	0	0	0	0	0	1	1	0	1	0	0	0	1	0	1	0
	Güneysınır	5	1	1	1	3	0	0	0	4	3	3	3	0	0	0	3	0	3	0
	Hüyük	2	1	0	0	2	1	0	0	2	1	1	1	0	0	0	2	0	2	0
	Ilgın	7	1	4	0	2	2	0	0	6	2	6	6	0	0	0	6	0	6	1
	Kadınhanı	8	4	4	3	6	4	0	0	8	1	5	5	0	0	0	8	0	8	0
	Karapınar	8	3	1	0	1	1	0	0	5	1	1	1	0	0	0	1	0	1	0
	Karatay	15	8	5	2	4	5	0	0	13	5	9	9	1	0	0	9	0	10	1
	Kulu	12	6	4	2	0	2	0	0	9	0	4	4	4	1	2	3	0	7	7
	Sarayönü	6	5	4	3	4	3	0	0	6	2	6	6	0	0	0	6	0	6	0
	Selçuklu	5	1	2	0	2	2	0	0	4	1	5	5	0	0	0	4	0	4	0
	Seydişehir	6	1	2	1	0	2	1	0	4	3	3	4	1	0	0	4	0	5	1
	Tuzlukçu	6	3	1	1	0	1	0	0	3	1	3	3	2	0	2	1	0	3	4
	Yalıhöyük	4	2	1	1	3	1	0	0	3	1	1	1	0	0	0	2	0	2	0
	Yunak	7	1	2	0	0	0	0	0	3	2	1	3	3	0	1	3	0	4	0
Number of total samples		215	87	62	28	70	46	2	1	163	84	110	134	41	4	11	140	1	161	34
Frequency %		100	40	29	13	32	21	0,9	0,5	76	39	51	62	19	1	5	65	0,5	75	15

Table 5. Mean±standard error of mean and (range) population densities of nematode genera and order Dorylaimida in the samples collected from Konya and Karaman Provinces in Turkey. All values are (100 g dry soil)$^{-1}$, except where indicated

Province	District	*Pratylenchus* spp.	*Pratylenchus* spp. plant^{-1}	*Heterodera* spp. (250 g dry soil)$^{-1}$	*Heterodera* spp. plant^{-1}	*Ditylenchus* spp.	*Tylenchus* spp.	*Paratylenchus* spp.	*Pratylenchoides* spp.	*Aphelenchus* spp.	*Aphelenchoides* spp.	*Cephalobus* spp.	*Eucephalobus* spp.	*Acrobeles* spp.	*Acrobeloides* spp.	*Rhabditis* spp.	Dorylaimida order
Karaman	Ayrancı	5±3 (0-25)	0	1±1 (0-8)	0	10±4 (0-25)	3±3 (0-24)	0	0	43±24 (0-204)	31±16 (0-127)	2±2 (0-21)	5±5 (0-41)	0	129±60 (0-527)	0	3±3 (0-25)
	Başyayla	11±11 (0-22)	10±10 (0-20)	0	0	22±22 (0-44)	0	0	0	54±10 (44-63)	88±67 (21-154)	0	0	0	625±456 (169-1081)*	0	0
	Ermenek	11±6 (0-22)	10±6 (0-20)	0	0	11±6 (0-22)	0	0	0	95±67 (0-293)	22±13 (0-45)	0	6±6 (0-23)	0	84±63 (0-270)	0	0
	Central	4±2 (0-44)	5±1 (0-40)	2±1 (0-36)	1±1 (0-2)	28±10 (0-410)	8±3 (0-91)	0	0	44±11 (0-290)	53±12 (0-342)	8±2 (0-46)	0	1±1 (0-22)	93±22 (0-758)	1±1 (0-21)	2±1 (0-22)
	Sarıveliler	0	0	0	0	57±12 (45-69)	11±11 (0-23)	0	0	215±146 (69-360)	171±14 (158-185)	12±12 (0-23)	0	0	567±244 (323-811)	0	0
Konya	Ahırlı	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Akşehir	11±6 (0-22)	0	0	0	0	5±5 (0-21)	0	0	5±5 (0-21)	16±5 (0-22)	11±6 (0-22)	0	5±5 (0-21)	28±28 (0-111)	0	0
	Altınekin	7±7 (0-21)	13±6 (0-20)	15±11 (0-36)	0	83±48 (0-166)	28±28 (0-83)	0	0	0	97±50 (0-166)	0	0	0	166±78 (22-291)	0	0
	Beyşehir	9±9 (0-46)	4±4 (0-20)	1±1 (0-7)	0	35±11 (0-60)	10±6 (0-26)	0	0	119±65 (0-348)	172±77 (0-371)	5±5 (0-24)	0	0	181±57 (20-348)	0	19±14 (0-70)
	Bozkır	17±17 (0-68)	5±5 (0-20)	1±1 (0-1)	0	17±11 (0-44)	0	6±6 (0-23)	0	11±11 (0-44)	44±44 (0-176)	6±6 (0-22)	0	0	23±10 (0-47)	0	6±6 (0-23)
	Çeltik	0	0	0	0	0	0	0	0	7±7 (0-22)	15±8 (0-23)	30±20 (0-69)	0	0	0	0	15±15 (0-44)
	Central	0	10±1 (0-20)	2±1 (0-3)	0	0	0	0	0	11±11 (0-22)	0	0	0	0	22±22 (0-43)	0	0
	Cihanbeyli	5±2 (0-22)	9±4 (0-100)	5±2 (0-37)	1±1 (0-4)	3±2 (0-45)	6±3 (0-66)	0	5±5 (0-133)	12±8 (0-194)	8±5 (0-112)	14±7 (0-154)	0	3±3 (0-66)	76±40 (0-939)	0	16±5 (0-89)
	Çumra	6±3 (0-22)	2±2 (0-20)	2±1 (0-14)	0	20±9 (0-88)	2±2 (0-22)	0	0	88±28 (0-263)	49±12 (0-108)	2±2 (0-22)	0	4±3 (0-23)	149±35 (0-389)	0	0
	Doğanhisar	14±14 (0-42)	0	0	0	7±7 (0-21)	50±39 (0-127)	0	0	43±1 (42-44)	78±58 (0-190)	15±15 (0-44)	30±30 (0-89)*	0	299±203 (0-698)	0	0
	Ereğli	0	0	1	0	0	0	0	0	45	0	0	0	0	23	0	0
	Guneysınır	0	4±4 (0-20)	1±1 (0-1)	0	45±24 (0-136)	0	0	0	106±45 (0-217)	121±95 (0-497)	0	0	0	111±65 (0-339)	0	0
	Hüyük	11±11 (0-21)	0	0	0	44±20 (24-63)	180±180 (0-359)*	0	0	32±32 (0-63)	127±127 (0-254)	0	0	0	604±369 (236-973)	0	0
	Ilgın	0	3±3 (0-20)	3±1 (0-7)	0	11±7 (0-47)	7±5 (0-27)	0	0	21±14 (0-93)	135±63 (0-434)	0	0	0	129±65 (0-488)	0	3±3 (0-21)
	Kadınhanı	13±6	13±8	2±1	1±1	40±15	59±50	0	0	3±3	67±23	0	0	0	190±58	0	0
	Karapınar	14±14	8±3	0	0	3±3	3±3	0	0	3±4	48±48	0	0	0	4±4	0	0
	Karatay	6±3	5±2	6±3	0	10±6	17±9	0	0	34±21	93±56	1±1	0	0	187±73	0	2±2
	Kulu	4±3	10±3	3±2	0	0	5±4	0	0	0	7±3	22±10	2±2	4±3	10±5	0	17±6
	Sarayönü	7±4	13±4	14±9	1±1	46±20	22±11	0	0	10±7	85±20	0	0	0	123±23	0	0
	Selçuklu	4±4	16±16 (0-	1±1	0	33±28	25±17	0	0	4±4	58±23	0	0	0	151±51	0	0
	Seydişehir	3±3	0	2±2	1±1	0	8±5	7±7	0	29±21	36±19	4±4	0	0	59±33	0	4±4
	Tuzlukçu	7±5	7±4	1±1	0	0	11±11	0	0	7±7	33±18	19±15	0	7±4	4±4	0	26±14
	Yalıhöyük	11±6	5±5	2±2	0	52±32	6±6	0	0	6±6	22±22	0	0	0	28±21	0	0
	Yunak	3±3	6±6	7±7	1±1	0	0	0	0	21±15	13±13	12±6	0	3±3	19±12	0	0

*highest population density.

Three species of root lesion nematodes were found in the cereal fields surveyed. *Pratylenchus thornei* was the most widely distributed root lesion nematode, identified from 69 sampling locations in Çumra, Karatay, Karapınar, Central, Guneysınır, Bozkır, Yalıhöyük, Seydişehir, Beyşehir, Hüyük, Doğanhisar, Ilgın, Akşehir, Tuzlukçu, Yunak, Kulu, Cihanbeyli, Altınekin, Selçuklu, Sarayönü and Kadınhanı Districts in Konya and Central, Ayrancı, Başyayla, Ermenek Districts in Karaman Province using D3b-R/Ptho-F primer set (Figure 1). The species-specific bands were obtained at 288 bp as suggested by

Al-Banna et al. (2004) (Figures 2 and 3). The morphology and morphometricss for *P. thornei* agreed with the previous literature (Sher & Allen, 1953; Elekcioglu, 1992; Kepenekçi, 1999; Osmanoglu, 2006; Imren & Elekcioglu, 2008) (Table 6).

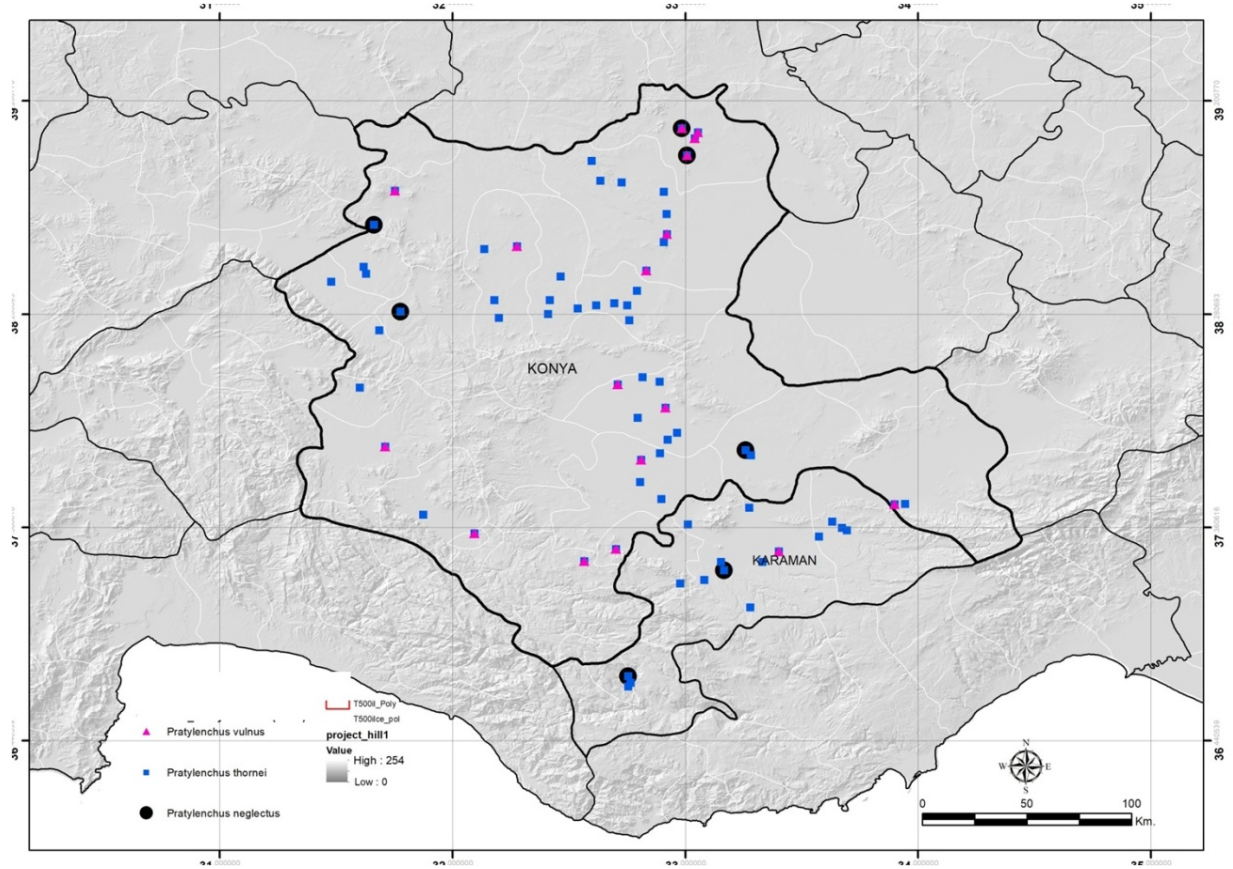

Figure 1. Root lesion nematodes identified in the sampling locations.

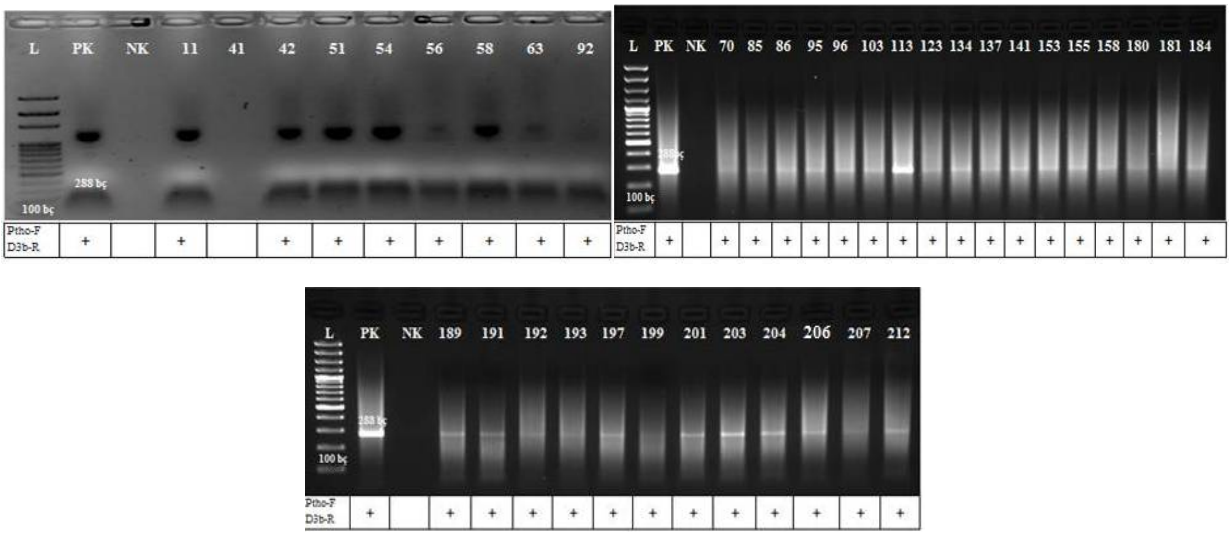

Figure 2. Identification of *P. thornei* in plant samples using PTHO primer in Konya and Karaman provinces. L, 100-bp ladder; PK: positive control; NK, negative control; numbers are survey samples numbers.

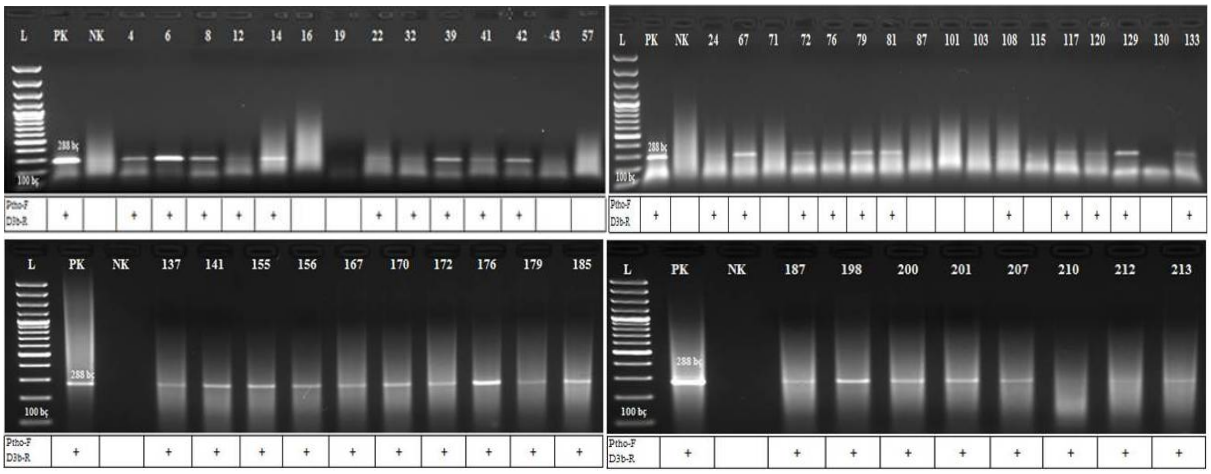

Figure 3. Identification of *P. thornei* in soil samples using PTHO primer in Konya and Karaman provinces. L, 100-bp ladder; PK, positive control; NK, negative control; numbers are survey samples numbers.

Table 6. Morphometrics of *Pratylenchus thornei* identified in this study and in references

Measurements*	This study	Sher & Allen, 1953	Kepenekci, 1999	Osmanoglu, 2006	Imren & Elekcioglu, 2008
n	5		20	8	14
L (mm)	0,54	0,45-0,77	0,48-0,63	0,50-0,68	0,48-0,60
a	29,64	26-39,6	26,9-34,4	33,28-38,83	29,9-36,6
B	6,13	5,5-8,0	4,7-5,9	4,26-6,18	4,8-6,2
b'	5,36		4,2-5,1	6,16-8,26	
c	18,8	18-22	16,6-21,0	16,64-25,5	16,0-26,6
c'	2,5		2,3-2,9	2,07-3,36	2,01-2,72
MB (%)	49,37		51,7-57,6	40,51-51,30	
Stylet (µm)	14,75	17-19	16-18	15,68-16,90	16,2-18,5
Tail (µm)	28,78		25-36	21,56-36,26	22,5-30,0
V (%)	77,54	73-80	73,8-79,2	73-77	71,6-79,0

* L (mm), total body length; a, body length/the largest width part of body; b, body length/distance from esophagus intestine overlapping part to anterior end of body; c, body length/tail length; c', tail length/body width at anus; MB (%), distance from anterior to median bulb/esophagus length × 100; V (%), distance from anterior end of body to vulva/body length × 100.

Pratylenchus neglectus species was found in seven locations in Karapınar, Ilgın, Tuzlukçu and Kulu Districts in Konya and Başyayla and Central Districts in Karaman Province using D3b-R/Pneg-F primer set at 290 bp as suggested by Al-Banna et al. (2004) (Figures 1, 4 and 5). One specimen of *P. neglectus*investigated by morphology and morphometricss agreed with the literature (Sher & Allen, 1953; Akgül & Okten, 1997; Kepenekci, 1999; Imren & Elekcioglu, 2008) (Table 7).

Pratylenchus vulnus was found in 17 locations in Çumra, Güneysınır, Bozkır, Yalıhöyük, Beyşehir, Yunak, Kulu, Cihanbeyli, Karatay and Kadınhanı Districts in Konya and Central and Ayrancı Districts in Karaman Province using D3b-R/Pvul-F primer set at 287 bp (Figures 1, 6 and 7).

Pratylenchus vulnus was recorded for the first time in cereals in Turkey in addition to the reported root lesion nematode species of *P. thornei* and *P. neglectus* (Yavuzaslanoglu et al., 2012). It was second most common species of root lesion nematodes in survey area after *P. thornei*. The prevalence of *P. neglectus* was lower than expected on Central Anatolian Plateau based on earlier reports (Yavuzaslanoglu et al., 2012).

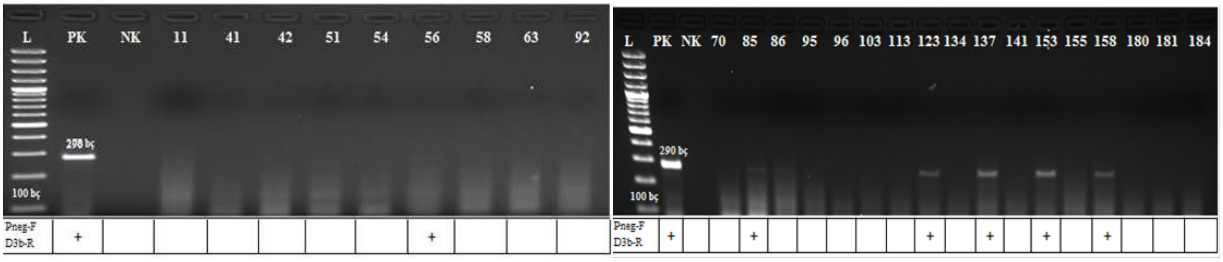

Figure 4. Identification of *P. neglectus*in plant samples using PNEG primer in Konya and Karaman provinces. L, 100-bp ladder; PK, positive control; NK, negative Control; numbers are survey samples numbers.

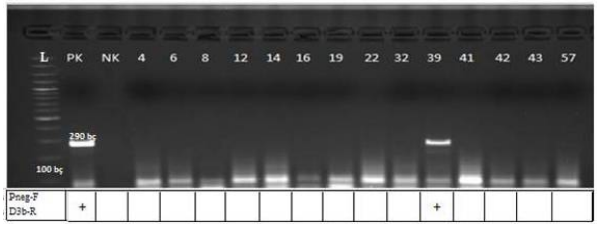

Figure 5. Identification of *P. neglectus*in soil samples using PNEG primer in Konya and Karaman provinces. L, 100-bp ladder; PK, positive control; NK, negative control; numbers are survey samples numbers.

Table 7. Morphometrics of *Pratylenchus neglectus* identified in this study and in references

Measurements*	This study	Sher & Allen (1953)	Akgül & Okten (1997)	Kepenekçi (1999)	Imren & Elekcioglu (2008)
n	1		8	20	6
L (mm)	0,51	0,31-0,55	0,34-0,46	0,38-0,51	0,398-0,460
a	25,75	18-25	18,9-32,2	21,7-27,8	18,2-26,1
B	5,02	4,0-6,3	3,75-6,52	4,1-6,0	4,1-5,6
b'	4,58		3,53-5,92	3,9-4,8	
C	17,68	16-22	16,7-31,7	14,9-23,3	14,01-23,00
c'	2,26		1,8-2,3	1,5-3,1	1,9-2,6
MB (%)	46,86		41,0-59,4	43,0-63,1	
Stylet (µm)	15,38	16-18	11,7-16,2	16-20	14,2-19
Tail (µm)	29,04		13,5-20,7	20-31	20,0-28,4
V (%)	79,45	80-88	74,8-81,3	79,7-84,8	77,0-86,4

* L (mm), total body length; a, body length/the largest width part of body; b, body length/distance from esophagus intestine overlapping part to anterior end of body; c, body length/tail length; c', tail length/body width at anus; MB (%), distance from anterior to median bulb/esophagus length × 100; V (%), distance from anterior end of body to vulva/body length × 100.

The study showed the value of molecular studies which are useful for differentiation of morphologically similar and difficult to distinguish species. Morphologically *P. vulnus* is quite similar to *P. thornei* and is difficult to differentiate. Also, *P. vulnus* has high intraspecific morphometric variation and low number of diagnostic features so requires molecular identification (Castillo & Vovlas, 2007).

Pratylenchus penetrans and *Pratylenchus scribneri* were not found in any of the samples using the species-specific primers (D3b-R/PPEN-F and D3b-R/PSCR-F).

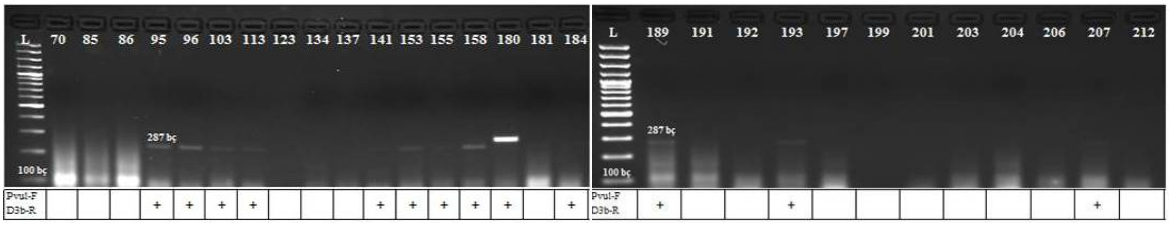

Figure 6. Identification of *P. vulnus* in plant samples using PVUL primer in Konya and Karaman provinces. L, 100-bp ladder; numbers are survey samples numbers.

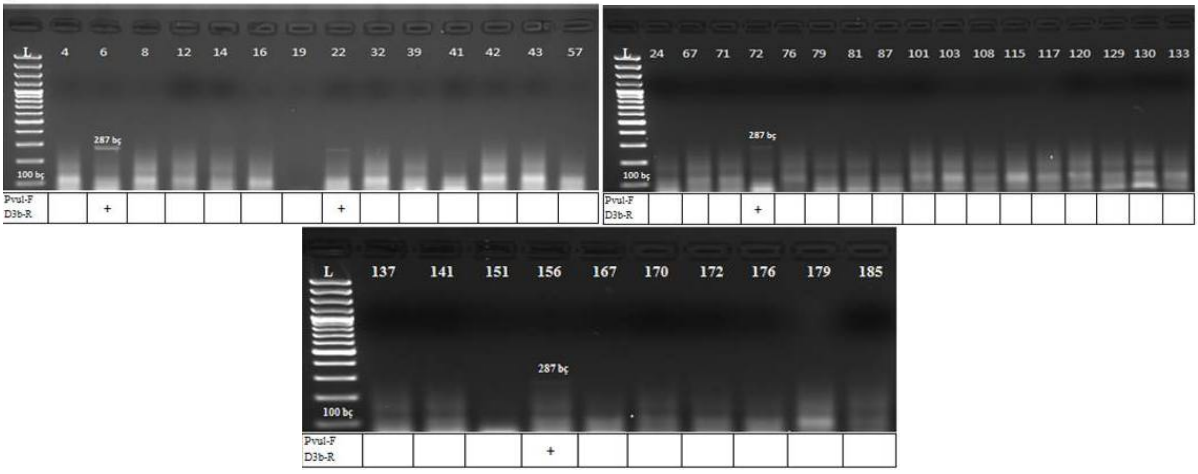

Figure 7. Identification of *P. vulnus* in soil samples using PVUL primer in Konya and Karaman provinces. L, 100-bp ladder; numbers are survey samples numbers.

The prevalence of cereal cyst nematodes was about 29%.Cereal cyst nematode cyst numbers were the highest in Altınekin and Sarayönü Districts of Konya Province; a mean of 15±11 and 14±9 cysts 250 g dry soil^{-1}, respectively. The number of cysts from the plant samples was between 0-4 cysts (Table 5). Population density of *Heterodera* spp. was found higher in comparison to the previous report in 2003 (Yavuzaslanoglu et al., 2012).

Heterodera filipjevi was found in 33 locations in Çumra, Karatay, Central, Beyşehir, Hüyük, Ilgın, Tuzlukçu, Yunak, Cihanbeyli, Altınekin, Sarayönü and Kadınhanı Districts in Konya Province and in Central and Ayrancı Districts in Karaman Province using the HfITS-R/HfITS-F primer set at 170 bp as suggested by Yan et al. (2013) (Figures 8 and 9). *Heterodera filipjevi* was identified by morphology and morphometrics of the vulval region and these measurements agreed with previous reports (Subbotin, 1999; Abidou, 2005; Handoo, 2002; Imren et al., 2012) (Table 8).The study showed that *H. filipjevi* was the main cereal cyst nematode in this part of the Central Anatolian Plateau (Enneli et al., 1994; Rumpenhorst et al.,1996; Ozturk et al*.,* 1998; Abidou et al*.,* 2005; Yavuzaslanoglu et al., 2012).

Heterodera latipons and *H. avenae* were not found in the samples using the species-specific primers (HaITS-R, HaITS-F, Hlat-actF and Hlat-actR). In previous research, it was established that the distribution of the cereal cyst nematodes in Turkey was closely related to climatic conditions prevalent throughout Anatolia, with *H. filipjevi* is found on the Central Anatolian Plateau and *H. avenae* in temperate zone of East Anatolia (Toktay et al., 2015). *Heterodera latipons* was reported to be predominant in Southeast Anatolia with a mediterranean climate (Imren et al., 2012). Molecular and morphological identification studies supported the previous observations on the distribution of cereal cyst nematodes in Turkey.

The prevalence of root lesion and cereal cyst nematodes together was about 13% in survey area which was lower than in earlier studies (Rumpenhorst, 1996; Yavuzaslanoglu et al., 2012).

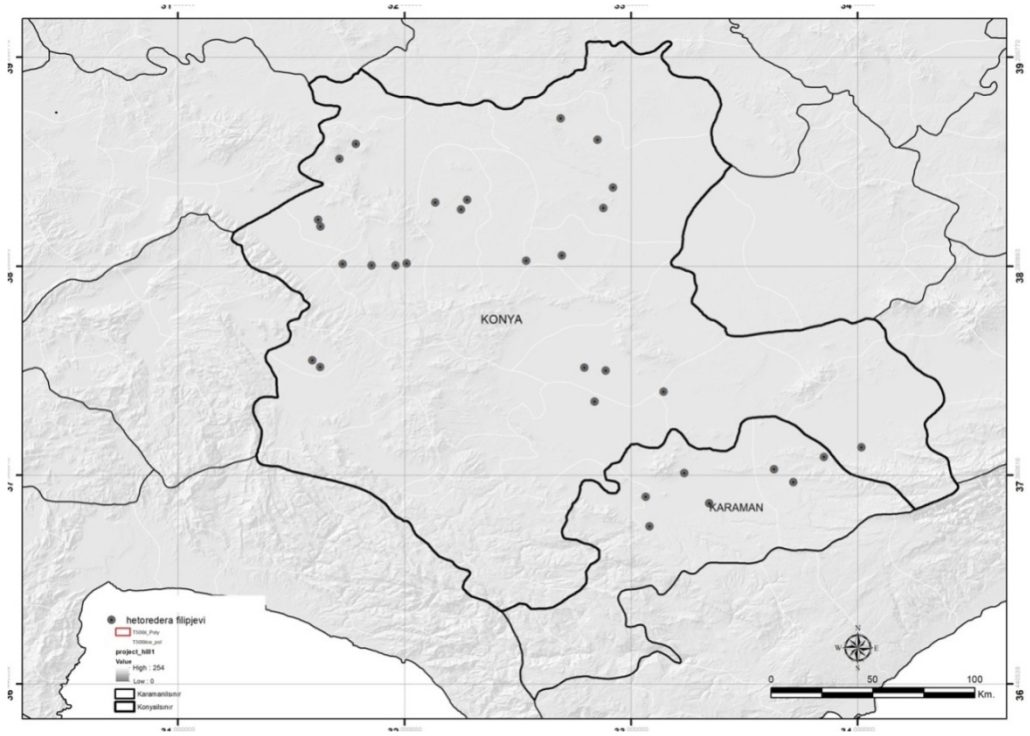

Figure 8. *Heterodera filipjevi* identified in the sampled locations.

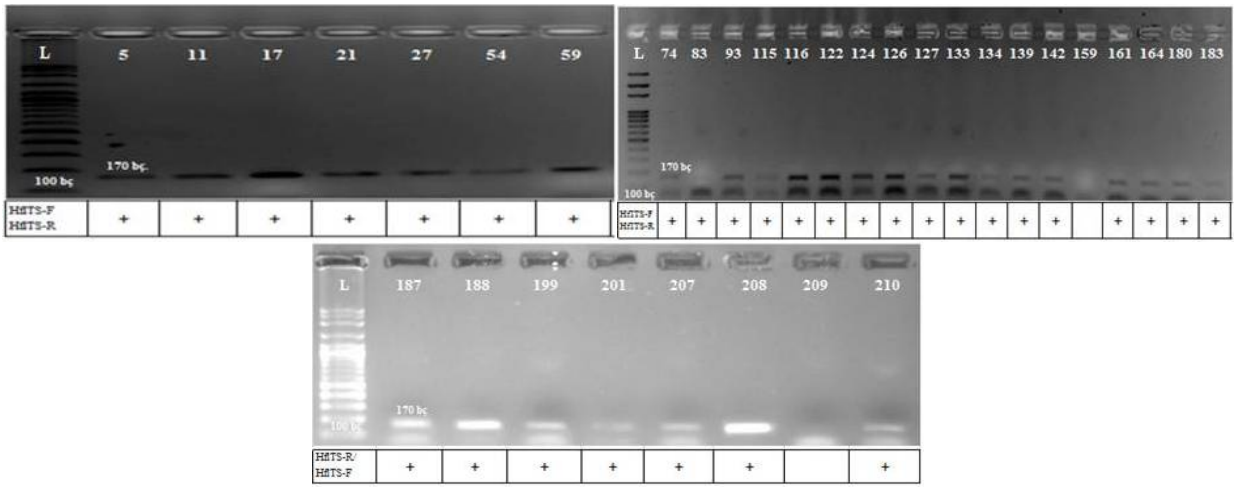

Figure 9. Identification of *H. filipjevi* in soil samples using HfITS-F1 HfITS-R1 primer in Konya and Karaman Provinces. L, 100-bp ladder; numbers are survey samples numbers.

Table 8. Morphometrics of *Heteroderafilipjevi* identified in this study and in references

Measurements (µm)	This study	Subbotin (1999)	Abidou (2005)	Handoo (2002)	Imren et al. (2012)
Fenestra length	64,13	50,00	50.41	52,00	66,24
Semifenestra length		27,00	25.30		23,04
Vulval bridge width	15,60	11.80	7.27	8 (6-9)	19,52
Vulval slit length	29,60	11.00	6.95	7(6-8)	24,64
Fenestra width	13,00			28 (21-33)	

Ditylenchus (32.6%)*, Tylenchus* (21.4%)*, Paratylenchus* (0.9%) and *Pratylenchoides* (0.5%) were the other plant-feeding nematodes found in the wheat and barley fields surveyed.

The maximum population density of the *Ditylenchus* spp. was 410 nematodes 100 g dry soil^{-1} in Karaman Province Central District (mean: 28±10 nematodes 100 g dry soil^{-1}). The *Ditylenchus* spp. populations were higher in Altınekin (mean: 83±48 nematodes 100 g dry soil^{-1}), Sarıveliler (mean: 57±12 nematodes 100 g dry soil^{-}), Yalıhüyük (mean: 52±32 nematodes 100 g dry soil^{-1}), Sarayönü (mean: 46±20 nematodes 100 g dry soil^{-1}) and Guneysınır (mean: 45±24 nematodes 100 g dry soil^{-1}) Districts (Table 5). No species-specific bands for *D. dipsaci* in any of the samples tested were with the nine species-specific primer sets used.

Tylenchus spp. populations were significantly different between districts (P<0.05). The highest population was recorded in Hüyük District in Konya Province, with a mean of 180±180 nematodes 100 g dry soil^{-1} (Table 5).

Paratylenchus spp. was recorded in Bozkır (mean: 6±6 nematodes 100 g dry soil^{-1}) and Seydişehir (mean: 7±7 nematodes 100 g dry soil^{-1}) Districts in Konya Province (Table 5).

Pratylenchoides spp. was only found in Cihanbeyli District in Konya Province, with a mean of 5±5 nematodes 100 g dry soil^{-1} (Table 5).

Fungal-feeding nematodes were in 62% of the soil samples. *Aphelenchoides* spp. (51.2%) and *Aphelenchus* spp. (39.1%) were observed (Table 4). The population density of the fungal-feeding nematodes was generally high in all districts. The maximum number of *Aphelenchus* spp. was recorded in Sarıveliler District in Karaman Province (mean: 215±146 nematodes 100 g dry soil^{-1}). *Aphelenchoides* spp. was the highest in Beyşehir District in Konya Province (mean: 172±77 nematodes 100 g dry soil^{-1}) (Table 5).

Bacterial-feeding nematodes were found in 75% of samples. *Acrobeloides* was the most prevalent bacterial-feeding nematode genera in 65.1% of samples, followed by *Cephalobus*in 19.1% of samples. *Eucephalobus* (1.9%), *Acrobeles* (5.1%) and *Rhabditis* (0.5%) prevalence was relatively low. The most abundant bacterial-feeding nematode genus was *Acrobeloides.* The maximum population density was in Başyayla District in Karaman Province at 625±456 nematodes 100 g dry soil^{-1} (P<0.05) (Table 5). *Eucephalobus* spp. populations were significantly different between districts (P<0.05). The maximum population density was 30±30 nematodes 100 g dry soil^{-1} in Doğanhisar District in Konya Province. *Eucephalobus* spp. was recorded in Ayrancı (mean: 5±5 nematodes 100 g dry soil^{-1}) and Ermenek Districts (mean: 6±6 nematodes 100 g dry soil^{-1}) in Karaman Province and in Kulu District (mean: 2±2 nematodes 100 g dry soil^{-1}) in Konya Province. It was not found in the other districts (Table 5). The population densities of *Cephalobus* spp. were the highest in Çeltik District in Konya Province (30±20 nematodes 100 g dry soil^{-1}) (Table 5). *Acrobeles* spp. was recorded in seven districts. The maximum population density was in Tuzlukçu District in Konya Province (mean: 7±4 nematodes 100 g dry soil^{-1}) (Table 5). *Rhabditis* spp. was only found in the Central District in Karaman Province (mean: 1±1 nematodes 100 g dry soil^{-1}) (Table 5).

Nematodes in the Dorylaimida were found in 15.8% of the samples (Table 4). The population densities of the nematodes in the Dorylaimida were significantly different between districts (P<0.05). The maximum population density was 26±14 nematodes 100 g dry soil^{-1} in Tuzlukçu District in Konya Province (Table 5).

The soil environment and host plant are the main factors affecting nematode survival and population growth (Wallace et al., 1993). In addition, climate change and global warming affect spatial distribution and damage potential of pathogens and pests (Iglesias et al., 2001; Ghini et al., 2008; Morgan & Wall, 2009). Changes in prevalence and population density of the main damaging plant parasitic nematode species are probably due to the changes in soil conditions such as temperature and moisture with application of different crop rotation over years.

The study provides useful information on the prevalence and population densities of *Heterodera* spp. and *Pratylenchus* spp. which are the major pests of the economically important cereal crops for making risk analysis effectively and taking appropriate control actions.

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