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Morphological and molecular identification of plant parasitic nematodes in wheat fields of Eastern Anatolian Region (Türkiye)

Doğu Anadolu Bölgesi (Türkiye) buğday alanlarında bitki paraziti nematodların morfolojik ve moleküler teşhisi

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ABSTRACT

The aim of this study is to identify plant-parasitic nematodes molecularly and morphologically in wheat (*Triticum* spp.) fields in the Eastern Anatolia Region (Türkiye) between 2017-2019. For this purpose, a total of 258 soil samples were collected from 7 provinces (Erzincan, Elazığ, Erzurum, Iğdır, Kars, Malatya, and Sivas) in the Eastern Anatolia Region. Nematodes were morphologically identified using a light microscope at the genus (some of them species) level. DNA extraction was performed and PCR products were used to DNA sequencing and nucleotide analysis for 28S ribosomal DNA region by comparing the results with the database. According to the obtained data, a total of 20 genera and 7 species belonging to 2 orders and 9 families were identified: *H. digonicus* Perry, 1959; *H. canadensis* Waseem, 1961; *H. vulgaris* Yuen, 1964 (Nematoda: Hoplolaimidae); *Ditylenchus myceliophagus* Goodey, 1958 (Nematoda: Anguinidae); *Amplimerlinius macrurus* (Goodey, 1932) (Nematoda: Dolichodoridae); *Scutylenchus quadrifer* (Andrassy, 1954); (Nematoda: Dolichodoridae), and *Pratylenchoides alkani* Yüksel, 1977 (Nematoda: Pratylenchidae). *H. vulgaris* was identified at the species level using molecular techniques. The rates of presence of economically important plant parasitic nematodes were determined as 73%, 43%, 36%, 33% and 28% for *Ditylenchus* spp., *Pratylenchus* spp., *Aphelenchus* spp., *Xiphinema* spp. and *Helicotylenchus* spp. respectively. It is thought that the results obtained will help to plan nematode control methods in the region.

INTRODUCTION

Türkiye's wheat, *Triticum* L. (Poales: Poaceae) production in 2021 was 17 million tons. (TUIK 2021). Approximately 10% of the wheat cultivated areas that are great importance in Türkiye's agriculture are located in the Eastern Anatolian Region. It is reported that plant parasitic nematodes cause a 10% loss in wheat production worldwide (Bongers and

Ferris 1999, Gaugler and Bilgrami 2004, Nicol et al. 2011, Sasser and Freckman 1987). There are 25.043 species identified in Nematoda phylum (Zhi-Qiang 2013) and 4305 plant parasitic nematodes species were identified (Maggenti 1991). In 48 distinct regions of Türkiye, 240 plant parasitic nematodes have been identified on 66 plant host species (Kepenekçi 2014). Plant parasitic nematodes can live in diverse habitats. The majority of nematodes cause damage to the root system of a plant and fewer nematodes cause damage to above-ground parts such as leaves and flowers (Nicol 2002). Nematodes create symptoms that resemble nutrient deficiencies in various parts of the plant. The feeding of plant parasitic nematodes results in a significant reduction in a plants root density and as a result, the plant turns yellow and becomes dwarf. Therefore, the areas with very serious nematode infection in the field are observed in distinct or visible patches (Kort 1972, Lung 1992).

Nematode species that cause economic losses in wheat cultivated areas worldwide. Although there are many plant parasitic nematode species that cause yield losses in wheat, the main species are cereal cyst nematodes (CCN), root lesion nematodes (RLN), root-knot nematodes (RKN), wheat gal nematode, and the stem-bulb nematode (Nicol et al. 2002). Although many studies have been carried out on plant parasitic nematodes damaging different hosts in different regions of Türkiye, studies in Eastern Anatolia are very limited. In the Eastern Mediterranean Region, *Geocenamus brevidens* and *Pratylenchus thornei* were identified as the most common of the 9 nematode species in the Eastern Mediterranean Region (Elekcioğlu 1996). In another study, *Heterodera ciceri*, *Pratylenchoides erzurumensis*, *Pratylenchoides leiocauda* and *Pratylenchus mediterraneus*, *P. penetrans*, *P. thornei* species were reported in chickpea and lentils in Türkiye (Di Vito et al. 1994). In a survey of plant parasitic nematodes associated with chickpea conducted in Türkiye, *Ditylenchus dipsaci*, *Pratylenchus neglectus*, *P. penetrans* and *P. thornei,* were found to be the most common plant parasitic nematodes (Behmand et al. 2019). In a study carried out by İmren (2007), nematodes belonging to 8 families, 10 subfamilies, 12 genera and 23 species were reported in vegetable and vineyard areas of Diyarbakır province. In another study, among the 39 nematode species determined in the wheat, barley, vegetable and fruit production areas of the Southeastern Anatolia Region, 6 species including *Ditylenchus longicauda*, *Filenchus hamatus*, *Helicotylenchus crassatus*, *H. goodi*, *H. oleae* and *Rotylenchus echelimae* were newly recorded in Türkiye (Uludamar Kasapoğlu et al. 2018). It was determined that the vineyards of Malatya, Şanlıurfa and Mardin provinces were infected with Dagger nematode, *Xiphinema* spp. (Öztüzün 1970). In the Eastern Anatolia Region, *Pratylenchus thornei*, *P. neglectus*, *P. penetrans* and *P. crenatus* were identified (Yüksel 1974). It has been reported that the highest infection rate in root lesion nematodes is in Erzurum region with a rate of 42.50% and in Sivas region with a low rate of 17.14% (Toktay et al. 2015). In the study by Toktay et al. (2021) *Heterodera cruciferae* populations were identified and characterized using molecular techniques for the first time in Türkiye. It was revealed that there were no polymorphisms in H. cruciferae populations in Niğde according to ribosomal DNA region (rDNA-ITS) and cytochrome oxidase subunit 1 (mtDNA-COI) gene regions.

Although there are local studies conducted in Eastern Anatolia wheat cultivation areas, there is no comprehensive study covering the whole region. Within the scope of this study, sampling was carried out in seven different provinces covering the whole region and the plant parasitic nematode fauna in wheat cultivated areas was determined.

MATERIALS AND METHODS

Material collection

Soil samples from 258 different wheat fields from Elazığ, Erzincan, Erzurum, Iğdır, Kars, Malatya and Sivas provinces in the Eastern Anatolia Region of Türkiye between 2015 and 2016 within the scope of TUBITAK project (112O565) were used in the study (Table 1, Figure 1). Each soil sample was taken according to a zigzag pattern in each field with a soil auger at a depth of 30 cm (Southey 1986) and final weight per sample was 1.5–2 kg soil. For each sampled field, GPS coordinates of sampling sites were recorded. Soil samples were stored in a cold storage at 4 °C for further morphological and molecular evaluations.

Table 1. The location of soil samples collected from the Eastern Anatolia Region of Türkiye

Provinces	Number of soil samples
Elazığ	29
Erzincan	29
Erzurum	29
Iğdır	28
Kars	34
Malatya	35
Sivas	74
Total	258

Figure 1. Provinces where the samples are taken in Türkiye

Morphological identification

Each soil sample was thoroughly mixed and a 100 g of sub-sample was processed by a Petri Sieving Method, which is a modification of Baermann Funnel Method, to extract migratory nematodes from the samples (Barker 1985, Southey 1986). Permanent slides were prepared to identify the plant parasitic nematodes at the species level. For this purpose, nematodes extracted from the soil were fixed in TAF solution [7 ml of formalin (40% formaldehyde) at 65 ºC, 2 ml of triethanolamin and 91 ml of pure water] (Hooper 1986). After the fixation process, the nematodes were incubated in solution 1 (1 part of glycerol and 79 parts of pure water) at 35-40 ºC for 10-12 h. Then, they were transferred to solution 2 (5 parts of glycerin and 95 parts of 96% ethanol) and incubated at 40 ºC for 3 h. Fixed nematodes were put in a desiccator for the required period of time for all remaining water to evaporate (Seinhorst 1959). The nematodes divided into groups according to their genus under a dissecting light microscope (Leica DM 5500 B, Germany). Then, each genus was permanently fixed on glass slides using the wax-ring method (Hooper 1986), and the specimens were examined under a compound light microscope (Leica, Germany). Measurements of L, a, b, c, c', V (%), stylet length and tail length, and taxonomic identification were done according to the formula and keys cited by Siddiqi (1986) from the second stage juveniles, females or cysts. L value measurement was taken as 'mm' and other measurements as 'µm' (Siddiqi 2000). Finally, taxonomic classification of the nematode species of Nematoda and Dorylaimida (Longidoridae family) order was done according to De Ley and Blaxter (2002).

Molecular identification

DNA isolation was performed for molecular identification of morphologically undetectable species. The DNA was isolated from a single individual from the populations of wheat nematodes collected from wheat fields after extraction freshly, according to the protocol of Holterman et al. (2006). Each individual was transferred into an Eppendorf tube containing 25 µl of double distilled water (ddH_2O) and was kept at -80 ºC for a 10 min. Then, 950 µl of Worm Lysis Buffer (WLB (-)), 10 µl of beta-mercaptoethanol and 40 µl of proteinase K (20 mg/ml) were added to each tube. Tubes were incubated at 65 ºC for 2 h, after that successively incubated at 95 ºC for 10 min using a thermal cycler. After incubation, tubes were centrifuged for 1 min at 10.000 rpm, then stored at -20 ºC until the samples were used.

For molecular identification of the nematodes, the LSU-rDNA region (1050 bp) was amplified by using LSU primers (11F and 21R) in a PCR reaction (Holterman et al. 2006). Two µl of DNA was added to the PCR reaction mixture containing 21 µl of ddH2O, 25 µl of 2× DreamTaq PCR Master Mix (Thermo Scientific, Belgium) and 1 µM of each forward (11F: 5'GTCGTGATTACCCGCTGAACTTA3') and reverse primers (21R: 5'TCGGAAGGAACCAGCTACTA3'). For the PCR amplification, the thermal cycler program was set up to 1 cycle for 5 min at 94 ºC followed by 35 cycles of incubation at 94 ºC for 30 s, then at 54 ºC for 30 s and finally at 72 ºC for 110 s for elongation. For final elongation, reaction was incubated at 72 ºC for 5 s. Following the PCR amplification, 5 µl of each PCR product was mixed with 1 µl of 6x loading buffer (Thermo Scientific, Belgium) and loaded on a 1.5% standard agarose gel in 1x TAE buffer. After the electrophoresis (at 120 V for 40 min), the gel was stained with ethidium bromide (0.1 µg/ml) for 15-20 min, photographed and visualized under UV-light. The remaining PCR products were stored at -20 ºC. The products obtained from the PCR amplification using LSU primers were sent to a company (Macrogen Inc., Ankara) to obtain the DNA sequences in a bidirectional Sanger sequencing. After sequencing, the LSU sequences were blasted in GeneBank for identification. Phylogenetic trees are constructed from sequences *H. vulgaris* from a range of countries available in GeneBank. The sequences of *Helicotylenchus multicinctus* (MT321731, Colombia), *H. caudatus* (MN 764335, South Korea), *H. pseudorobustus* (MG653533, Poland), *H. microlobus* (MN 764322, South Korea), *Heterodera schachtii* (MH790255, USA), *H. vulgaris* (MK825777, Iran and MG770483, Greece), *Globodera rostochiensis* (MG994942, UK), *G. pallida* (JN712219, UK), *Rotylenchus goodeyi* (MW960041, Poland), *Pratylenchus thornei* (MZ 956971, Türkiye), *P. penetrans* (MW720692, Netherlands), were included for analyses of LSU locus.

The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model

(Tamura and Nei 1993). The tree with the highest log likelihood (-4277,83) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura-Nei model, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites [5 categories (+G, parameter = 0,4228)]. This analysis involved 15 nucleotide sequences. There were a total of 1164 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar et al. 2018).

RESULTS AND DISCUSSION

Plant parasitic nematodes negatively impact the plant growth and yields. It is essential to identify the nematode species in the field before deciding the best strategy to control. Identification of nematodes in the wheat fields from the Eastern Anatolia Region, which is one of the important winter wheat production centers of Türkiye, is limited. Therefore, it was followed a systematic sampling method to collect the soils from monoculture wheat cultivated areas in 7 provinces of the Eastern Anatolia Region of Türkiye (Bora and Karaca 1970). As a result of study 20 genera (Table 2) and 7 species (Table 3) were identified in 9 families in the orders of Rhabditida and Dorylaimida in the Nematoda phylum. In previous studies, 12 plant parasitic nematodes *Anguina tritici*, *Meloidogyne incognita*, *Xiphinema* spp., Heterodera avenae, Pratylenchus thornei, P. neglectus, P. penetrans, P. crenatus, Pratylenchoides alkani, P. erzurumensis, Heterodera filipjevi and H. latipons were detected in Eastern Anatolian Region. Xiphinema spp. was observed in vineyards in Malatya, Şanlıurfa, Mardin while Anguina tritici Steinbuch, 1799 was observed in wheat fields in Şanlıurfa, Mardin, Van and Bitlis. Meloidogyne incognita Kofoid and White, 1919 was observed in Malatya and Elazığ (Öztüzün 1970).When the distribution of nematode genera was evaluated in the soil samples, the highest soil infestation was observed in the genus of Ditylenchus (73.25%) followed by Paratylenchus, (43.02%), Aphelenchus (36.82%), Xiphinema (33.33%) and Helicotylenchus (28.29%). In this study, the lowest rate of contamination was found in the genera of Bitylenchus (0.77%), Zygotylenchus (1.16%), Amplimerlinius (1.93%), Telotylenchus (1.93%) and Tylenchorhynchus (2.71%) (Table 2). In a previous survey performed in wheat fields in Adıyaman province, a total of 17 species, 7 families and 9 subfamilies were identified. The most common nematodes

Table 1. Distribution of plant parasitic nematodes in the soil samples collected from seven provinces of the Eastern Anatolia

Nematode Genera	Provinces							Total	Soil infestation (%)
	Sivas*	Erzurum	Erzincan	Iğdır	Kars	Elazığ	Malatya		
Ditylenchus spp.	65	14	23	9	27	23	28	189	73.25
Pratylenchus spp.	29	12	13	21	25	4	7	111	43.02
Aphelenchus spp.	30	6	15	9	3	9	23	95	36.82
Xiphinema spp.	27	13	13	7	6	10	10	86	33.33
Helicotylenchus spp.	22	11	7	9	8	8	8	73	28.29
Merlinius spp.	29	10	5	6	11	12	8	71	27.51
Scutylenchus spp.	14	7	7	6	6	2	9	51	19.76
Tylenchus spp.	23	4	8	2	7	4	6	49	18.99
Trophurus spp.	9	5	8	0	5	5	3	35	13.56
Aphelenchoides spp.	9	3	5	1	3	6	5	32	12.41
Filenchus spp.	12	2	1	6	2	2	5	30	11.62
Pratylenchoides spp.	13	0	5	1	4	2	5	30	11.62
Paratylenchus spp.	10	2	2	0	4	4	7	29	11.24
Rotylenchus spp.	2	0	0	1	4	1	1	9	3.48
Geocenamus spp.	5	0	2	0	0	1	0	8	3.10
Tylenchorhynchus spp.	3	1	1	1	0	1	0	7	2.71
Telotylenchus spp.	2	0	1	0	1	0	1	5	1.93
Amplimerlinius spp.	2	0	2	0	0	1	0	5	1.93
Zygotylenchus spp.	0	0	0	3	0	0	0	3	1.16
Bitylenchus spp.	0	0	0	1	0	0	1	2	0.77

* Number of soil samples including nematodes

were Aphelenchus avenae, H.latipons, Merlinius brevidens, P. thornei and Scutylenchus quadrifer (Öcal and Elekcioğlu 2015). Many genera known to cause significant economic losses have been identified as a result of our survey study. Because the provinces where the samples were taken in the Eastern Anatolia region are close to each other in terms of geographical height and climatic characteristics, similar genera were identified in many of the provinces. Therefore, our results show soil infestation of different nematode genera than the ones identified in Adıyaman province.

Considering the distribution of plant parasitic nematodes in each province, the two most abundant genera in Sivas province were Ditylenchus spp. and Aphelenchus spp. with 87.8% and 39.2%, respectively, while Zygotylenchus spp. or Bitylenchus spp. were not detected (Table 2). Ditylenchus spp. and Xiphinema spp. were the top two contaminating nematode genera in soil samples collected from Erzurum province. In this province, the soil infestation of Ditylenchus spp. and Xiphinema spp. were calculated as 48.3, 44.8%, respectively. In soil samples collected from Erzincan province, Ditylenchus spp. and Aphelenchus spp. were the top two contaminating nematode genera with soil infestation of 79.3 and 51.7%, respectively. Interestingly, Pratylenchus spp. was the top contaminating nematode genera in the soil samples collected from Iğdır province with a soil infestation of 75.0%. Finally, Ditylenchus spp. was the nematode genera with the highest soil infestation in the soil samples collected from Kars, Elazığ and Malatya provinces, where the rates reached to 79.4, 79.3 and 80.0%, respectively, in each province. Pratylenchus thornei, P. neglectus, P. penetrans and P. crenatus were found in wheat fields of Eastern Anatolian Region in a previous survey study (Yüksel 1974). P. alkani and P. erzurumensis were reported for the first time in Eastern Anatolian Region by Yüksel (1977). These results are parallel with our observations. The highest infection rate of root lesion nematodes occurred in Erzurum region with the rate of 42.50% and the lowest rate was reported in Sivas region with 17.14% by Toktay et al. (2015). Moreover, Heterodera filipjevi and H. latipons were also identified in wheat fields of Eastern Anatolian Region in the same study. These previous studies combined with our observations suggest that these nematode species are widely distributed in the region. As opposed to our findings, Heterodera, Pratylenchus, Pratylenchoides, Paratylenchus, Merlinius, Helicotylenchus and Tylenchorhynchus genus were detected in soil samples collected from Bolu in North West Black Sea Region (İmren et al. 2015). In this study, the most harmful plant parasitic nematodes were determined as Heterodera (82.6%) and Pratylenchus (73.3%). Finally, the root-lesion nematodes (Pratylenchus neglectus and P. thornei) and cereal cyst nematodes (Heterodera avenae, H. filipjevi and H. latipons) were found to be economically important in wheat fields in the Eastern Mediterranean and Central Anatolian Regions (Kasapoğlu et al. 2015). In a study conducted in wheat fields in the Eastern Mediterranean Region, nine nematode species were identified, and it was reported that Geocenamus brevidens and P. thornei were wide-spread and could be of economic importance (Elekçioğlu 1996).

Table 3. Plant parasitic nematode species identified in this study

Genus	Species	L	a	b	c	c'	V (%)	stylet length	tail
Ditylenchus Filipjev, 1936	*Ditylenchus myceliophagus* Goodey, 1968	0,750 ± 0,16	40,00 ± 4,06	5,59 ± 0,30	15,00 ± 1,13	4,10 ± 0,63	81,12 ± 1,50	7,00 ± 1,05	50,00 ± 12,06
Helicotylenchus steiner, 1945	*Helicotylenchus canadensis* Waseem 1961	0,953 ± 0,46	26,00 ± 6,26	5,12 ± 1,03	52,94 ± 5,16	not detected	66,11 ± 3,40	29,18 ± 1,05	18,00 ± 4,06
Helicotylenchus steiner, 1945	*Helicotylenchus vulgaris* Yuen, 1964	0,807 ± 0,91	26,03 ± 2,03	5,02 ± 2,88	62,78 ± 8,02	0,65 ± 0,67	61,02 ± 1,21	30,12 ± 2,04	13,69 ± 0,15
Pratylenchoides winslow, 1958	*Pratylenchoides alkani* Yüksel, 1977	0,872 ± 0,06	30,00 ± 1,02	5,40 ± 0,16	16,50 ± 1,09	3,20 ± 0,03	56,70 ± 3,90	24,60 ± 0,05	47,12 ± 6,16
Amplimerlinius Siddiqi, 1976	*Amplimerlinius macrurus* Goodey, 1932 Siddiqi, 1976	0,852 ± 0,17	27,12 ± 5,01	5,20 ± 0,93	17,00 ± 1,09	2,40 ± 0,96	56,37 ± 1,80	26,60 ± 7,00	55,12 ± 1,09
Scutylenchus Jairajpuri, 1971	*Scutylenchus quadrifer* Andrassy, 1954: Siddiqi,1979	0,772 ± 0,97	27,00 ± 2,56	5,23 ± 1,93	13,50 ± 4,03	2,34 ± 0,01	48,37 ± 3,50	21,60 ± 0,95	50,12 ± 3,06

Morphological identification of seven species isolated from sample collection sites was performed. Accordingly, Ditylenchus myceliophagus was isolated from wheat fields in Erzincan in our survey (Figure 2, Table 4). Previously it was reported by Goodey (1958) in mushrooms in England. It was first reported by Ağdacı et al. (1990) in mushrooms in Türkiye.

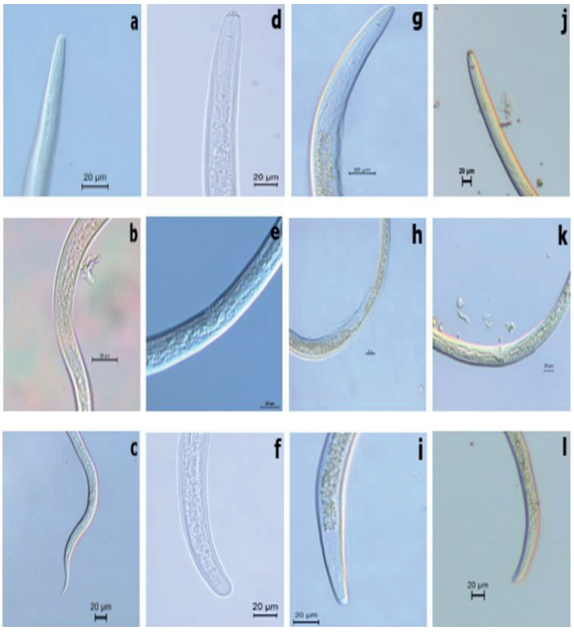

Figure 2. *Ditylenchus myceliophagus*, a. anterior end of the female of *D. myceliophagus*, b. vulva of the female of *D. myceliophagus*, c. tail of the female of *D. myceliophagus*. *Pratylenchoides alkani*, d. anterior end of the female of *P. alkani* e. vulva of the female of *P. alkani* f. tail of the female of *P. alkani*. *Scutylenchus quadrifer*, g. anterior end of the female of *S. quadrifer*, h. vulva of the female of *S. quadrifer*, i. tail of the female of *S. quadrifer*, *Amplimerlinius macrurus*, j. anterior end of the female of *A. macrurus*, k. anterior end of the female of *A. macrurus*, l. tail of the female of *A. macruruse*

Pratylenchoides alkani was first reported by Yüksel (1977) in beans in Türkiye (Erzurum). In this study, it was reported in wheat fields of Sivas (Center and Gemerek) (Figure 2, Table 4). Scutylenchus quadrifer was found in wheat areas of Elazığ-Center of Türkiye (Figure 2, Table 4). Ercan (1976) found this species in ornamental plants in Istanbul in Türkiye. Amplimerlinius macrurus was identified in wheat areas of Sivas-Center (Figure 2, Table 4). It was described for the first time by Saltukoğlu (1973) in watermelons in Istanbul of Türkiye. Helicotylenchus canadensis was identified in wheat cultivated areas in Erzurum (Karaçoban-Duman), Kars (Çıldır-Çanaksu) and Sivas (Center -Yıldızeli and Yavu-Ekecik) (Figure 3, Table 4). This species was previously reported by Waseem (1961) in vineyards in Canada. Previously reported by Kepenekçi (1999) in lentil in Türkiye (Nevşehir and Yozgat). Helicotylenchus digonicus was identified in wheat areas of Sivas (Kayseri Road) and Kars (Susuz-Arpaçay road-Akçalar) (Figure 3, Table 4). This species was first described by Yuen (1964) in grass in England and was detected by Saltukoğlu (1974) in grass and garlic in Istanbul. Finally, Helicotylenchus vulgaris was detected in samples collected from Kars (Çıldır road-Çanaksu) and Malatya (Arguvan-Bozburun) in wheat fields (Figure 3, Table 4). This species was first described by Yuen (1964) in grass in England and was found by Ertürk et al. (1973) in potato cultivated areas of Türkiye (Çanakkale and İzmir). The morphological and morphometric measurements of the species identified in the study were found to be compatible with the reference values (Table 3).

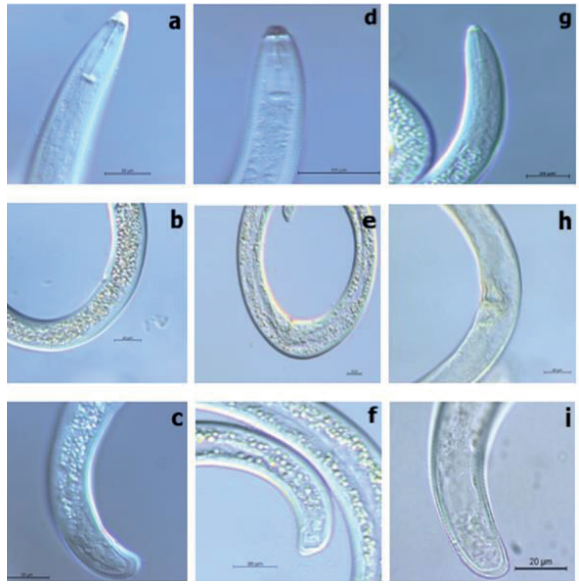

Figure 3. *Helicotylenchus canadensis* a. anterior region, b. vulval region, c. tail region, *Helicotylenchus digonicus* d. anterior region, e. vulval region, f. tail region, *Helicotylenchus vulgaris*, g. anterior region, h. vulval region, i. tail region

For the first time in Eastern Anatolia Region (Türkiye), nematode species have been identified by molecular methods in this study. Molecular diagnostic of H. vulgaris is not been reported in the literature in Türkiye. Nematode species were determined by molecular methods for the first time in the wheat fields in the provinces of Erzincan (Kemaliye), Sivas (Center) and Sivas (Gemerek) (Figure 4). After the females were identified at the genus and species levels under light microscope, some species were identified molecularly by using LSU primers to determine the species. After DNA isolation, bands of 1050 bp in length

Table 4. Locations of nematodes identified by morphological or molecular methods at the species level

Species	*PPNs/F	Moleculer	Morphology	Cities/District	Location
Ditylenchus myceliophagus Goodey, 1968	F		X	Erzincan/Center	N 39°47'91.4." E38°58'81.3"
Helicotylenchus canadensis Waseem 1961	PPNs		X	Erzurum / Karaçoban-Duman Kars / Çıldır Sivas/ Yıldızeli Sivas/ Yavu- Ekecik	N 39°30'71.8." E 41°93'14.6" N 41°03'96.8." E 43°30'51.8" N 39°86'97.4." E 36°61'93.1" N 39°80'76.4." E 36°14'33.2"
Helicotylenchus vulgaris Yuen, 1964	PPNs	X	X	Kars/ Çıldır-Çanaksu Malatya/Arguvan-Bozburun Sivas/ Center Sivas/ Kayseri-Road Erzincan/ Kemaliye-İliç	N 40°99'94.7." E 43°30'27.9" N 38°66'00.1." E 38°33'03.3" N 39°69'66.6." E 37°00'72.0" N 39°57'27.8." E 37°00'89.2" N 39°44'58.8." E 38°47'22.0"
Helicotylenchus digonicus, Perry, Darlind and Thorne, 1959	PPNs		X	Sivas/ Kayseri-Road Kars/ Susuz-Arpaçay	N 39°51'23.3." E 36°84'93.4" N 40°75'15.3." E 43°24'64.9"
Pratylenchoides alkani Yüksel, 1977	PPNs		X	Sivas/ Center Sivas/ Gemerek	N 39°55'68.6." E 37°02'72.2" N 39°25'72.9." E 36°12'11.1"
Amplimerlinius macrurus Goodey, 1932 Siddiqi, 1976	PPNs		X	Sivas/ Center	N 39°24'51.5." E 37°40'58.7"
Scutylenchus quadrifer Andrassy, 1954:Siddiqi, 1979	PPNs		X	Elazığ/ Center	N 38°67'83.3." E 39°15'38.6"

**PPNs (Plant parasitic nematode, F (Fungivore nematode)*

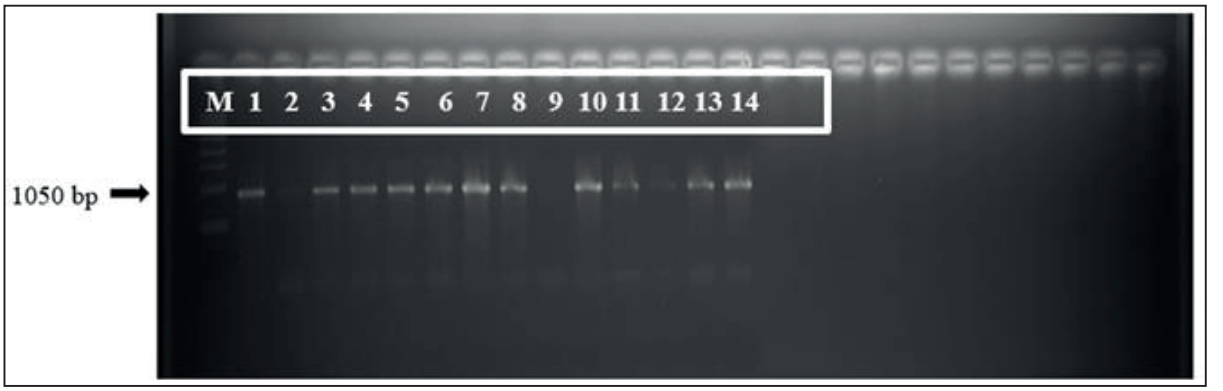

Figure 4. Band image obtained by PCR using LSU primers. M: 100bp DNA ladder (Thermo Scientific).3. *Helicotylenchus vulgaris* (N 39°57'27.8." E 37°00'89.2"), (Sivas, Gemerek): 4. *H. vulgaris* (N 39°69'66.6." E 37°00'72.0"), (Sivas, merkez): 10. *H. vulgaris* (N 39°44'58.8." E 38°47'22.0"), (Erzincan, Kemaliye)

were obtained from the samples by using LSU (11F and 21R) primers. PCR products obtained from PCR using LSU primers and were sent for sequence analysis. Then, the species of these genera were determined with BLAST (Wageningen University- Netherlands Database) analysis. H. vulgaris was determined in 3 locations after sequencing. Among the samples sent for sequencing, 4 populations did not a match with any species in the database.

Based on phylogenetic analysis, three H. vulgaris populations obtained from this study separately clustered into a group which indicates nucleotide differences of them. The sequences obtained from Erzincan and Sivas (Gemerek) provinces are highly similar than the sequences of Sivas (Merkez) (Figure 5). The LSU gene region sequences of the two Turkish population were similar to the Greece population, an example is similar to the Iranian population.

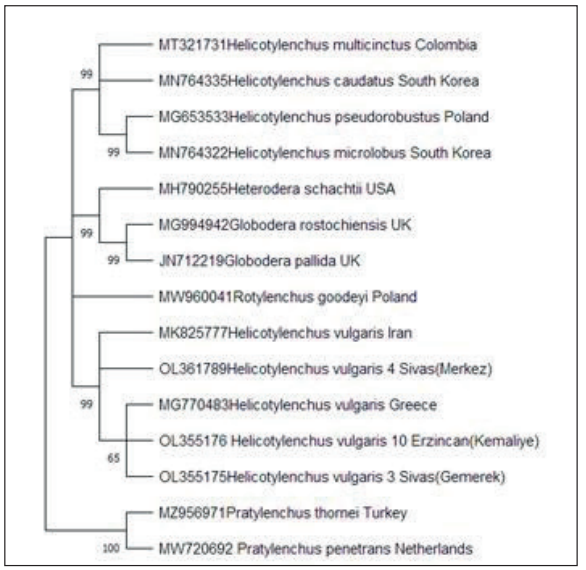

Figure 5. Molecular phylogenetic status of *Helicotylenchus vulgaris* LSU sequences

Considering the species that were not determined in the database search, further molecular characterization studies are suggested from this region. Overall, H. digonicus (from the samples of Sivas and Kars), S. quadrifer (from the samples of Elazığ), D. myseliophagus (from the samples of Erzincan), A. macrurus (from the samples of Sivas), H. canadensis (from the samples of Erzurum, Kars and Sivas), H. vulgaris (from the samples of Kars, Erzincan, Sivas and Malatya), and P. alkani (from the samples of Sivas) were reported for the first time in Eastern Anatolia Region according to the molecular identification studies.

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Author's Contributions

Authors declare the contribution of the authors is equal.

Statement of Conflict of Interest

The authors have declared no conflict of interest.

ÖZET

Bu çalışmanın amacı, Doğu Anadolu Bölgesi'nde (Türkiye) 2017-2019 yılları arasında buğday (*Triticum* spp.) alanlarında bulunan bitki paraziti nematod türlerinin moleküler ve morfolojik olarak belirlenmesidir. Bu amaçla, Doğu Anadolu Bölgesi'ne ait toplam 7 ilden (Erzincan, Elazığ, Erzurum, Iğdır, Kars, Malatya ve Sivas) toplam 258 toprak örneği alınmıştır. Elde edilen nematodlar cins veya tür düzeyinde ışık mikroskobu kullanılarak morfolojik olarak tanımlanmıştır. Tanımlanamayan nematod türlerinden bazılarının 28S ribosomal DNA bölgesi kullanılarak dizi analizi oluşturulmuş ve veribankasında karşılaştırmaları yapılmıştır. Elde edilen verilere göre; toplam 2 takım 9 familyaya ait 20 cins ve 7 tür, *Helicotylenchus digonicus* Perry, 1959, *Helicotylenchus canadensis* Waseem, 1961, *Helicotylenchus vulgaris* Yuen, 1964 (Nematoda: Hoplolaimidae), *Ditylenchus myceliophagus* Goodey, 1958 (Nematoda: Anguinidae), *Amplimerlinius macrurus* (Goodey, 1932) (Nematoda: Dolichodoridae), *Scutylenchus quadrifer* (Andrassy, 1954) (Nematoda: Dolichodoridae) ve *Pratylenchoides alkani* Yüksel, 1977 (Nematoda: Pratylenchidae) morfolojik olarak teşhis edilmiştir. *Helicotylenchus vulgaris* moleküler tekniklerle tür düzeyinde belirlenmiştir. Ekonomik açıdan önemli bitki paraziti nematodların bulunuş oranları *Ditylenchus* spp., *Pratylenchus* spp., *Aphelenchus* spp., *Xiphinema* spp. ve *Helicotylenchus* spp. için sırasıyla %73, %43, %36, %33 ve %28 olarak belirlenmiştir. Elde edilen sonuçların bölgedeki nematod mücadele yöntemlerinin planlanmasına yardımcı olacağı düşünülmektedir.

Anahtar kelimeler: ektoparazit nematodlar, teşhis, nematod, *Triticum* spp., Türkiye

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