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Phytophagous nematodes in cereal fields in Niğde Province, Turkey

Niğde İli tahıl alanlarında bulunan fitofag nematodlar

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Abstract

This study evaluated the occurrence and incidence of phytophagous nematodes and identified the cereal cyst nematode species by morphological and molecular tools in the main cereal-growing areas in Niğde in 2018-2019. Phytophagous nematodes within twelve genera were detected in 95% of soil samples. The most common phytophagous nematodes in cereal soil were in the genera *Heterodera, Ditylenchus, Merlinius, Pratylenchus, Aphelenchus, Aphelenchoides, Tylenchus, Helicotylenchus, Trophurus, Pratylenchoides, Filenchus* and *Xiphinema* (in decreasing order of incidence). In particular, 75% of the soil samples from surveyed fields were infested with the cereal cyst nematodes (*Heterodera* spp.). Morphological characteristics of cysts and second-stage juveniles were calculated within the expected ranges for *Heterodera filipjevi* (Madzhidov, 1981) Stelter, 1984, however, two populations from Çamardı was determined as *Heterodera latipons* Franklin, 1969 (Tylenchida: Heteroderidae). Intraspecific variation was not observed within the populations of *H. filipjevi* which could be in the same genotypic group. In addition to the high incidence of these *Heterodera* spp., intensive cereal cropping systems with/without non-cereal rotations in wheat production areas of Niğde also resulted in high incidence of root lesion nematode, *Pratylenchus* species.

Keywords: Identification, *Heterodera* spp., phylogeny, phytophagous nematodes, wheat

Öz

Bu çalışma ile 2018-2019 yıllarında Niğde İli'ndeki başlıca tahıl yetiştirme alanlarında bulunan fitofag nematodların yoğunluk ve dağılımı belirlenmiş, tahıl kist nematodu türlerinin morfolojik ve moleküler yöntemlerle tanımı yapılmıştır. Fitofag nematodlar 12 cins olarak toprak örneklerinin %95'inde tespit edilmiştir. En yaygın fitofag nematodlar sırasıyla, *Heterodera*, *Ditylenchus*, *Merlinius*, *Pratylenchus*, *Aphelenchus*, *Aphelenchoides*, *Tylenchus*, *Helicotylenchus*, *Trophurus*, *Pratylenchoides*, *Filenchus* ve *Xiphinema* cinsleridir (bulunma yoğunluğuna göre sıralanmıştır). İncelenen toprak örneklerinin %75'inde tahıl kist nematodu (*Heterodera* spp.) bulunmuştur. Kistlerin ve 2. dönem larvaların ölçümleri ve morfolojik karakterlerinin değerlendirilmesiyle elde edilen sonuçlarda *Heterodera filipjevi* (Madzhidov, 1981) Stelter, 1984 olarak tespit edilmiş olup sadece Çamardı'dan iki popülasyon *Heterodera latipons* Franklin, 1969 (Tylenchida: Heteroderidae) olarak belirlenmiştir. *Heterodera filipjevi*'nin tür içi varyasyonlarına bakıldığında hepsinin aynı gurupta olabileceği değerlendirilmiştir. Buna ilave olarak tahıl kist nematodları yanında kök lezyon nematodlarının, *Pratylenchus* türlerinin de rotasyon yapılan/yapılmayan Niğde İli tarım alanlarında yüksek yoğunlukta bulunduğu sonucuna varılmıştır.

Anahtar sözcükler: Teşhis, *Heterodera* spp., filogenetik, bitki paraziti nematodlar, buğday

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Introduction

Wheat (*Triticum* spp.) is the third most important crop after rice and maize in terms of global production with an average annual production of almost 772 Mt in 2017 (FAO, 2018). As the most important food crops of many countries, bread wheat [*Triticum aestivum* L. (Poales: Poaceae)] and durum wheat [*Triticum durum* Desf. (Poales: Poaceae)] provides an important nutrition to the feeding both humans and livestock, and contribute nearly one-third of the total food grain production in the world. A complex of biotic (i.e., diseases and insects) and abiotic factors (i.e., soil and climate) affects directly wheat grain yield in the important wheat-growing areas of the world. Among the biotic factors, the plant parasitic nematodes (PPNs) are a major biotic constraint to wheat production systems worldwide, especially where the plants under stress by other biotic and abiotic factors, particularly drought. PPNs cause severe annual economic losses of up to 216 billion USD worldwide when simultaneously exposed to drought (Nyaku et al., 2017). Cereal cyst nematodes (CCNs) [*Heterodera avenae* Wollenweber, 1924 (Tylenchida: Heteroderidae) group], among the PPNs, are most commonly found in wheat cropping areas (Rivoal & Cook, 1993; Dababat & Fourie, 2018).

Heterodera avenae group occurs widely throughout the wheat-growing areas of the world (Dababat & Fourie, 2018). Cereal cyst nematodes are causing yield loss of about 3.4 million USD annually in the Pacific Northwest region of the USA (Smiley & Nicol, 2009). The main species of cyst nematode pests of cereals are *H. avenae*, *Heterodera filipjevi* (Madzhidov, 1981) Stelter, 1984 and *Heterodera latipons* Franklin, 1969 (Tylenchida: Heteroderidae) within *H. avenae* group, which contains 12 nematode species (Rivoal & Cook, 1993; Nicol, 2002). Accurate identification of the species causing the loss is critical for determining the most effective method to control cyst nematodes, and requires both morphological and molecular methods. The vulval region of cysts and second-stage juveniles (J2s) are the most common features used for identification of *Heterodera* spp. (Handoo, 2002; Subbotin et al., 2010). However, the use of these morphological characters of *Heterodera* spp. is difficult and needs experienced scientists. Currently, molecular identification techniques are used overcome the taxonomic difficulties with morphological species identification (Dababat et al., 2015; Imren et al., 2020). Determination of the genetic variation in cyst nematode populations also aids the development or improvement of resistant host plants.

Turkey, with an average annual production of about 20 Mt and a planted area of about 7.3 Mha, is one of the important wheat-growing countries of the world, but the average yield of around 2.7 t/ha is relatively low (FAO, 2018). The Central Anatolian Plateau (CAP) produces about 5 Mha wheat annually under rainfed conditions with only a limited area of irrigation. Located on the CAP, Niğde Province produces around 230 kt of wheat, barley and rye grain on over 125 kha (TurkStat, 2020). In Niğde, wheat crops are frequently exposed to drought stress due to insufficient annual rainfall (200-300 mm) and scarcity of supplementary irrigation. Several studies were conducted between 1974-2020 to detect the diversity in *H. avenae* group in the root zone of various crops in different areas of Turkey (Yüksel, 1974; Rumpenhorst et al., 1996; Abidou et al., 2005, Şahin et al., 2009; Dababat et al., 2015; Imren et al., 2018). In Turkey, cyst nematodes in the *H. avenae* group were first found in associated wheat in Erzurum in 1974 and since then *H. avenae* group nematodes have been increasingly found in the other wheat-growing areas of Turkey (Imren et al., 2012; Dababat et al., 2015). *Heterodera avenae* group populations from the CAP have mainly been identified based on their morphology and morphometrics; therefore, limited information is available on the variation in their morphometrics in relation to their genetic structure (Şahin et al., 2009).

This research was conducted to reveal the status of phytophagous nematodes including the *H. avenae* group in the cereal-growing areas of Niğde by completing an intensive survey in wheat fields in Altunhisar, Bor, Central, Çamardı and Ulukışla Districts. The objectives were to (1) determine the incidence of the important species of PPNs in Niğde Province, (2) determination and evaluate both cysts and J2s of CCN populations with morphology, morphometrics and molecular assays including sequencing of the internal transcribed spacer (ITS) of ribosomal DNA fragments, and (3) investigate the phylogenetic relationships with and between populations found.

Materials and Methods

Nematode populations

The survey was conducted in 2018 between physiological maturity and harvest stage of wheat from the cereal-growing areas of Altunhisar, Bor, Central, Çamardı and Ulukışla Districts in Niğde. In total 64 cereal fields (wheat and barley) were sampled (Figure 1 & Table 1) about 10-20 km apart with soil samples were taken arbitrarily along a zigzag transect. A minimum of 10 cores was taken per sample using 2.5 cm diameter soil corer, the soil mixed and a representative subsample of 2 kg was kept for nematode extraction. Details recorded included crop, district and geographic coordinates.

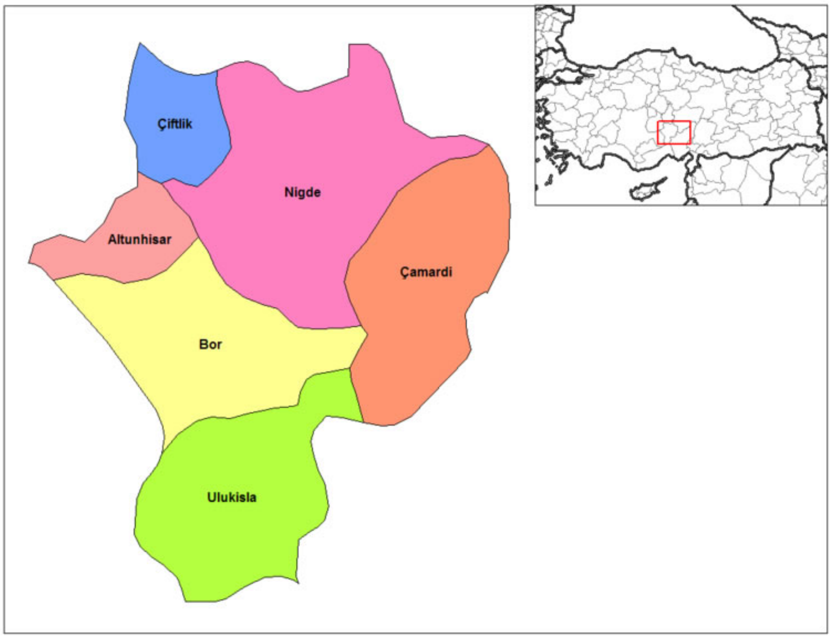

Figure 1. Districts of Niğde Province, Turkey surveyed for phytophagous nematodes (image source: www.istanbul-city-guide.com/map/turkey/nigde/map_of_nigde.jpg). The district labeled Niğde is Central District as used in the text. No fields were sampled in Çiftlik because cereal production is not a major land use in that district.

Migratory nematodes were extracted using a modified Baermann funnel from 100 ml of soil as described by Hooper (1986). After extraction, nematode suspensions were placed in measuring cylinders and allowed to settle for 8 h, the supernatant discarded, and the concentrated nematodes (plus debris) were transferred in 15-ml tubes. Nematode identification was made to genus under a light microscope at 100× magnification (Leica 5500, Wetzlar Germany). Cyst nematodes were extracted from 250 ml of soil using a modified decanting and sieving technique (Dababat et al., 2014). At least 20 full cysts were selected from each population, and stored at 4ºC for later morphological and molecular analysis. Cysts were morphologically identified to genus under a stereomicroscope at 20× magnification. The incidence of the nematodes was determined for each field as the quantity of the samples with nematodes as a proportion of the total number of samples.

Morphological identification of *Heterodera* specimens

Each *Heterodera* population was identified based on the vulval cones structure and J2 dimensions as well as their morphological features. Vulval cone slides were used to identified cyst nematode specimens according to Hooper (1986). Vulval slit length, vulval bridge width, fenestra width and length, and underbridge width and length were measured. The presence of underbridge and bullae of the perineal area were also determined (Handoo, 2002).

Table 1. Districts, locations, number of wheat and barley fields sampled (infested only) and their geographic coordinates in Niğde Province, Turkey sampled for phytophagous nematode assessment in 2018

District	Location	Infested fields	Geographic coordinates (N and E)
Altunhisar	Akmanlar	1	38°94'88.3" 34º27'97.0"
	Central	1	38°76'71.4" 34º89'07.0"
	Çömlekçi	1	38°05'67.1" 34º29'71.8"
	Ulukışla	1	38°04'88.3" 34º21'77.3"
	Yakacık	2	38°02'95.3" 34º34'36.5"; 38°00'88.4" 34º31'85.6"
Bor	Bahçeli	1	38°26'51.9" 34º66'77.0"
	Central	7	37°09'67.6" 34º55'06.3"; 38°20'11.4" 34º21'77.3"; 38°16'71.4" 34º35'88.3"; 38°36'27.1" 34º06'79.0"; 38°50'61.0" 34º54'09.3"; 38°90'23.4" 34º12'09.3"; 38°56'87.4" 34º66'56.3"
Çamardı	Central	7	38°07'14.6" 34º82'62.3"; 38°06'82.6" 34º85'08.3"; 38°07'71.7" 34º91'65.8"; 38°06'73.4" 34º93'82.6"; 38°04'86.0" 34º96'66.8"; 38°03'18.8" 34º99'12.1"; 37°88'89.6" 35º09'58.2"
	Kavlaktepe	2	37°99'43.3" 35º06'43.4"; 37°97'66.8" 35º08'45.1"
Central	Ağcaşar	1	38°31'97.0" 34º71'51.6"
	Alay	11	38°26'31.2" 34º69'18.7"; 38°26'73.1" 34º67'91.2"; 38°27'52.7" 34º65'80.2"; 38°26'66.3" 34º71'62.6"; 38°28'54.7" 34º72'90.6"; 38°28'06.6" 34º73'69.8"; 38°27'11.2" 34º74'23.6"; 38°26'95.8" 34º73'42.7"; 38°26'71.4" 34º73'72.5"; 38°28'03.8" 34º69'51.3"; 38°27'15.4" 34º33'20.2"
	Edikli	3	38°19'01.4" 34º96'76.5"; 38°22'27.4" 34º96'43.1"; 38°26'13.1" 34º91'94.5"
	Konaklı	1	38°17'29.4" 34º94'06.8"
	Orhanlı	6	38°28'36.3" 34º89'33.2"; 38°29'62.4" 34º88'79.9"; 38°29'01.1" 34º88'32.0"; 38°30'62.2" 34º87'77.3"; 38°32'46.3" 34º83'95.3"; 38°33'52.5" 34º82'86.2"
Ulukışla	Güney	3	37°10'42.8" 34º35'11.3"; 37°10'51.9" 34º09'54.3"; 37°06'89.9" 34º68'11.3"

To prepare permanent slides, 15 juveniles from the individual cysts were heated, fixed in TAF solution (7% formalin and 40% formalin) and put in glycerol (Handoo, 2002). The most important features of J2s including stylet length, length and width of body, length, width and hyaline part of tail were measured. The morphometric ratios a, b', c and c' were determined (Handoo, 2002). *Heterodera* spp. cyst and J2 were identified using previous studies and identification keys. (Franklin, 1969; Mulvey & Golden, 1983; Handoo, 2002). At least 15 J2s and 15 cysts from each population were examined and photographed using a Leica DFC295 optic camera installed on a DM5500 B light microscope (Leica, Wetzlar, Germany) and measured by Leica software v.4.1.0.

Data were analyzed with SPSS 22.0 for Windows (IBM, Armonk, NY, USA) to determine any significant differences between the 48 populations ($P \leq 0.05$).

Molecular identification of *Heterodera* specimens

Genomic DNA of each population were isolated from one cyst according to method in Holterman et al. (2006). The primers were used for sequencing, AB28 (5'-CGTAACAAGGTAGCTGTAG-3') and TW81 (5'-TCCTCCGCTAAATGATATG-3') to amplify the ITS region of nematode DNA according to protocol of Joyce et al. (1994).

A total of 48 ITS sequences were identified using a BLAST in the GenBank database. After identification of CCN species in Niğde, six population from each district of Niğde were used for phylogenetic relationship DNA sequences of were aligned with Clustal X (Kimura, 1980). Phylogenetic analyses of the CCN populations and reference population available in the GenBank database were performed with MEGA v 7.0 software using ITS regions (Kumar et al., 2016). A neighbor-joining tree was constructed as Tamura & Nei (1993) using 1000 times bootstrap. Gaps were considered as missing data in the sequences. *Heterodera schachtii* Schmidt, 1871 (Tylenchida: Heteroderidae) was used for as an outgroup for character polarization.

Results

Incidence of motile nematodes and cysts

Only 48 samples were evaluated for PPNs because soil samples were hard and dry during harvest. Specimens of motile phytophagous nematode genera were obtained from soil from 46 (96%) of the 48 infested fields sampled (Tables 1 & 2) with an average density 840 nematodes/100 g soil. The PPNs were identified in the samples as shown in Table 2. The highest incidence was for *Ditylenchus* species. (25 fields) followed by species of *Merlinus* (21 fields) and *Pratylenchus* (13 fields). The least common genera were *Filenchus* and *Xiphinema* each only found in a single field. *Heterodera* cysts were found in all of these 48 sampled fields. The economically important PPNs found were *Heterodera* (CCNs) and *Pratylenchus* (root lesion nematode). The cyst nematodes in found in 48 of the 64 fields (Table 3). The species were determined to be *H. filipjevi* and *H. latipons,* both in the *H. avenae* group, based on the morphologic, morphometric and molecular analysis. The fields in which cyst nematodes were detected were mostly used for cereal monocultures without fallow or crop rotation. The highest cyst incidence was in Altunhisar and Çamardı Districts and the lowest in Ulukışla District (Table 3). The average cyst density (cysts/250 g of soil) was calculated for each district, with the highest density in Çamardı District being threefold that found in Bor and Central Districts (Table 3).

Table 2. Genera of phytophagous nematodes extracted from in 48 infested wheat and barley fields in locations within five districts of Niğde Province, Turkey in 2018. Data shown is the number of fields for each the 12 genera extracted and the final column the number of genera extracted at each location including all fields sampled. The nematodes were extracted as motile forms from soil, except for *Heterodera*, which was extracted as cysts

District	Location	Infested fields	*Aphelenchoides*	*Aphelenchus*	*Ditylenchus*	*Filenchus*	*Helicotylenchus*	*Merlinius*	*Heterodera*	*Pratylenchoides*	*Pratylenchus*	*Trophurus*	*Tylenchus*	*Xiphinema*	Genera
Altunhisar	Akmanlar	1	-	-	-	-	-	1	1	-	1	-	-	-	3
	Central	1	-	1	1	-	-	1	1	-	-	-	-	-	4
	Çömlekçi	1	-	1	-	-	-	1	1	-	-	-	-	-	3
	Ulukışla	1	-	-	1	-	-	1	1	-	-	-	-	-	3
	Yakacık	2	-	-	1	-	1	2	2	-	-	-	-	-	4
Bor	Bahçeli	1	-	-	1	-	-	-	1	-	-	-	-	-	2
	Central	7	2	2	2	1	-	1	7	-	3	-	3	-	8
Çamardı	Central	7	2	2	4	-	-	1	7	1	3	1	3	1	10
	Kavlaktepe	2	-	-	2	-	1	1	2	1	1	1	-	-	7
Central	Ağcaşar	1	-	-	1	-	-	1	1	-	-	-	-	-	3
	Alay	11	2	-	4	-	3	6	11	-	4	-	2	-	7
	Edikli	3	1	1	2	-	-	1	3	-	-	1	-	-	6
	Konaklı	1	-	-	-	-	-	1	1	-	-	1	-	-	3
	Orhanlı	6	1	3	4	-	1	1	6	-	-	-	-	-	6
Ulukışla	Güney	3	1	1	2	-	1	2	3	-	1	-	-	-	7
All districts		48	9	11	25	1	7	21	48	2	13	4	8	1	

Morphology and morphometrics of cyst population

The survey yielded 48 *Heterodera* populations, with 46 determined to be *H. filipjevi* and two *H. latipons*.

***Heterodera filipjevi* (Madzhidov, 1981) Stelter (Tylenchida: Heteroderidae)**

Forty-six populations of *H. filipjevi* were detected across the five districts sampled. The morphological and morphometric characters were consistent with those reported by Handoo (2002). The CCN has lemon shaped cysts and their vulval area protruding at the posterior. Fenestral area was bifenestrate and horseshoe shaped with heavy bullae and underbridge (Figure 2). Measurements (mean and range, n = 15) for specimens from four districts (Table 4) were: body length without neck 599 µm (550-632 µm), neck length 85 µm (72-108 µm), fenestra length 50 µm (47-55 µm) and width 21.2 µm (20.4-21.8 µm). J2s head were slightly offset head and its body cylindrical with conical hyaline tail tip. The juveniles have strong stylet and moderately concave basal knobs (Figure 2). The body of *H. filipjevi* varied from 550 to 632 µm long and stylet from 24.2 to 25.7 µm. The body has four lateral lines but usually the two inner lines were prominent.

Table 3. Incidence and population density of cyst nematodes (*Heterodera* spp.) in wheat and barley fields sampled in districts of Niğde Province, Turkey, in 2018. *Heterodera filipjevi* as detected in 46 fields and *Heterodera latipons* in two fields

District	Fields sampled	Infested fields	Incidence (%)	Population density (cysts/250 g soil)
Altunhisar	6	6	100	7
Bor	10	8	80	10
Çamardı	9	9	100	30
Çiftlik	5	0	0	0
Central	30	22	73	10
Ulukışla	4	3	75	4
All	64	48	81	

* The two fields with *H. latipons* were in Çamardı Central (38°07'71.7" N, 34º91’65.8" E and 38°04'86.0" N, 34º96'66.8" E).

***Heterodera latipons* (Franklin, 1969) (Tylenchida: Heteroderidae)**

Only two populations of *H. latipons* from Çamardı District were examined. The morphological and morphometric characters were consistent with those reported by Handoo (2002) (Table 4). The cyst color was light brown, lemon shaped with ridges in a zigzag pattern (Figure 3). Fenestral area was bifenestrate and strong under bridge with no bullae body 470 µm (451-532 µm) without neck, body 293 µm (250-390 µm), neck 65 µm (60-80 µm), fenestra 64 µm (47.3-66.5 µm) and width 21 µm (18-25 µm), underbridge 96 µm (85-115 µm), vulval slit 8 µm (7.4-8.5 µm), vulval bridge width 12.4 µm (9.4-13.4 µm) (Figure 3). J2s (n = 15) had cylindrical body and head with conical tail extended with short hyaline terminal compared with *H. filipjevi* bodies. J2 body had four lateral lines, length 470 µm (412-482 µm), width 19 µm (19-21 µm), length of stylet 24 µm (23-25 µm), tail 60.2 µm (56-63 µm) and hyaline terminal 25 µm (23-29 µm) (Table 4).

Molecular characterization of cyst population

The nematode ribosomal gene of ITS(ITS1-5.8S-ITS2), region of all 48 *Heterodera* populations were successfully amplified for sequencing using AB28, TW81 primers pair and PCR product of nematodes were about 1060 bp. Forty-six sequences were identified as *H. filipjevi* and two *H. latipons* using BLAST program in GenBank database.

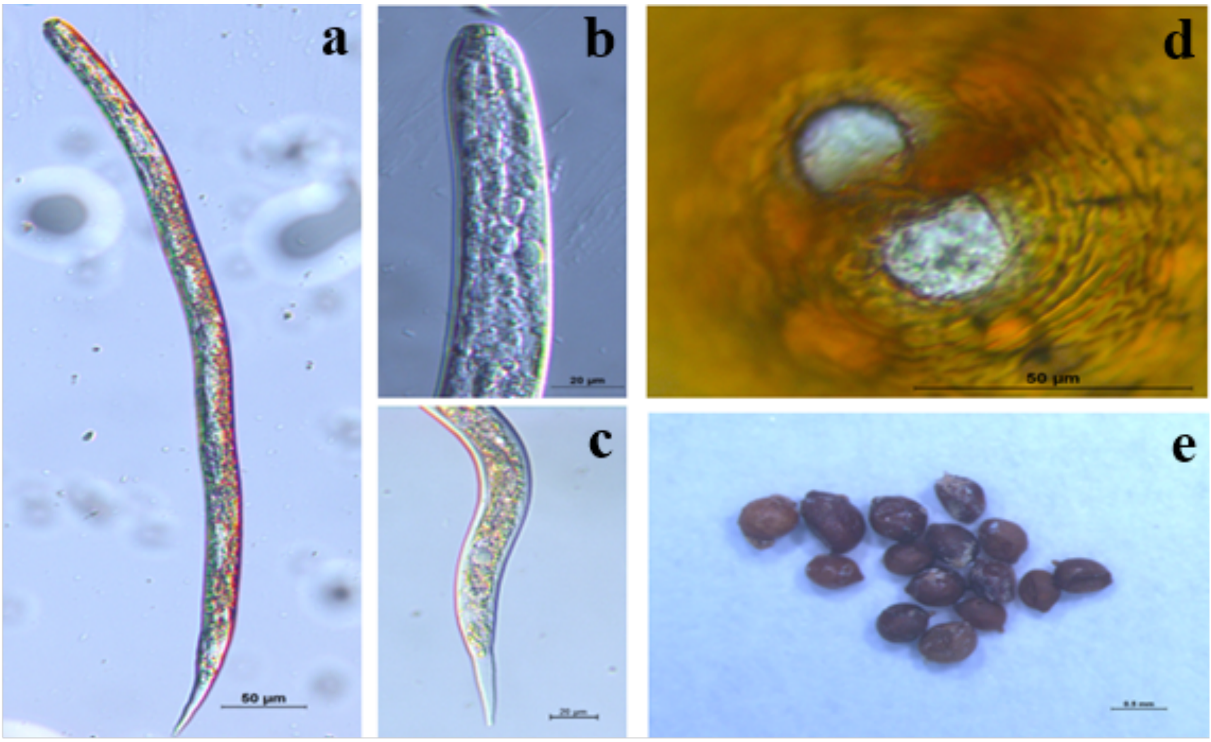

Figure 2. Light micrographs of *Heterodera filipjevi* from northern Niğde Province: a) second-stage juveniles; b) head region; c) tail region; d) fenestral region; and e) cysts of *Heterodera filipjevi.*

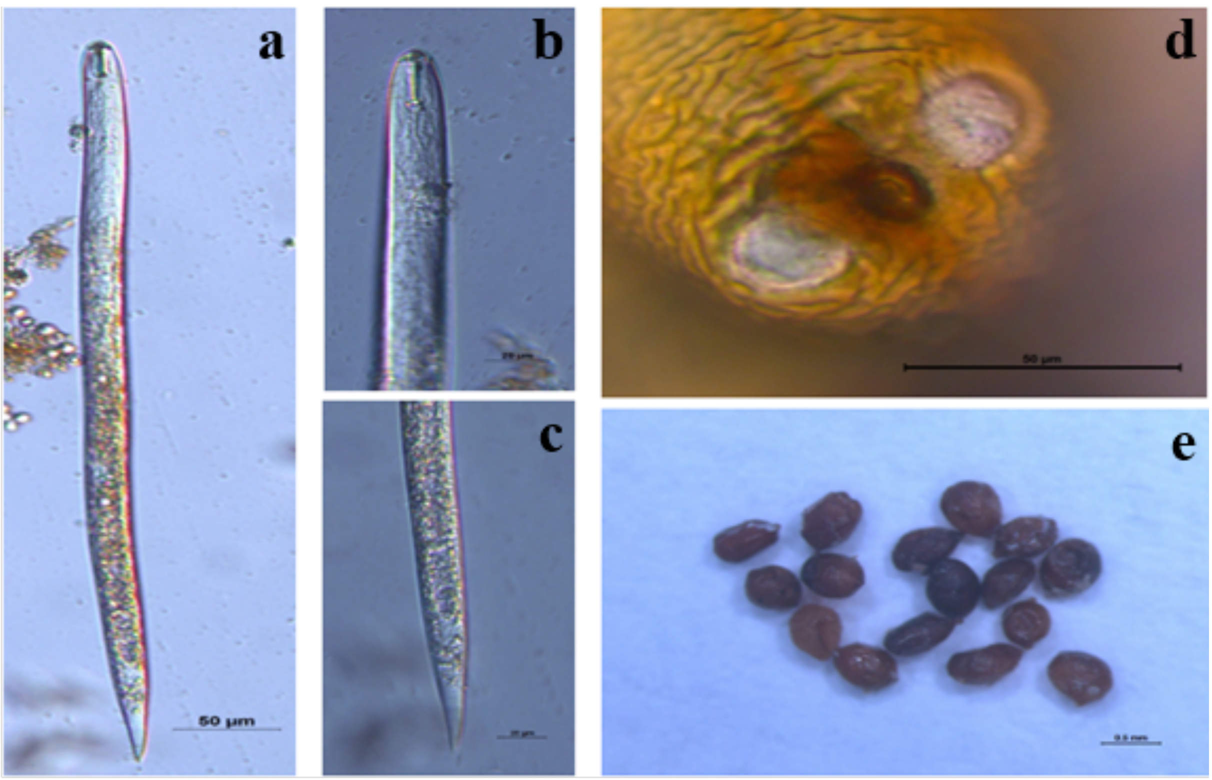

Figure 3. Light micrographs of *Heterodera filipjevi* from North part of Niğde Province: a) second-stage juveniles; b) head region; c) tail region; d) fenestral region; and e) cysts of *Heterodera latipons*.

Table 4. Some morphometric characters of the second-stage juveniles and cysts of *Heterodera filipjevi* and *H. latipons* populations (n = 10) extracted from wheat and barley fields in districts of Niğde Province, Turkey in 2018. All measurements are given in μm ± SE

Character	*H. filipjevi*				*H. latipons*
	Altunhisar	Bor	Central	Ulukışla	Çamardı
	Second-stage juveniles				
Body length	613±4.1	602±2.0	551±3.0	632±1.1	471±5.0
Stylet length	25.7±0.3	25.3±0.2	24.2±0.4	25.4±0.5	22.6±7.0
Tail length	65.1±1.1	63.0±1.9	56.1±1.1	66.1±1.1	49.4±0.18
Hyaline length	42±0.4	37±0.19	38±0.82	40±0.2	25±0.45
	Cysts				
Fenestra length	47.3±3.6	50.1±4.9	55.3±6.7	49.6±9.1	66.5±6.0
Semifenestra width	21.7	21.8	21.1	20.4	17.7±1.1
Vulval bridge width	10.2±1.3	11.9±0.7	13.8±0.2	9.4±1.7	19.8±4.6
Vulval slit length	7.8±1.4	8.2±1.7	8.5±1.1	7.4±1.1	7.0±1.6

A phylogenetic tree was constructed with six population of Niğde from ITS region sequence of CCN populations (Figure 4). Samples from the four different locations; Altunhisar, Central, Bor and Ulukışla Districts in Niğde Province were grouped as *H. filipjevi* and one population from Çamardı as *H. latipons* and the outgroup of *H. schachtii* (Figure 4). *Heterodera filipjevi* populations was compared to international genotypes in the phylogenetic tree. Results indicated that a clear separation of the cyst nematodes, *H. filipjevi* and *H. latipons*, and confirmed the link between genotyping and phenotyping traits. Intraspecific polymorphism was not observed within *H. filipjevi* populations, which were in the same group within the phylogenetic tree and representative isolates from GenBank, supported by a moderate to high bootstrap value (Figure 4).

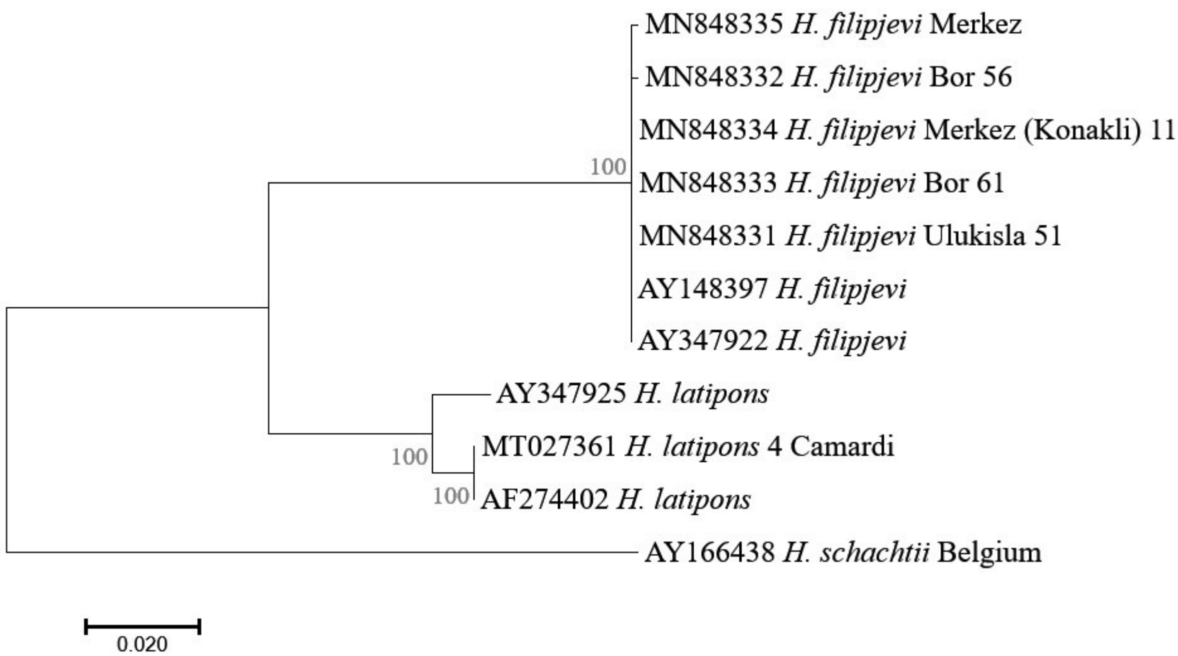

Figure 4. Phylogenetic tree of *Heterodera filipjevi* and *H. latipons* populations from Niğde Province. *Heterodera schachtii* from Belgium was used as an outgroup.

Discussion

This survey was completed in order to determine the incidence of PPNs in cereal fields in Niğde Province. Phytophagous nematodes were detected in 96% of soil samples, and 12 PPN genera were determined, namely *Heterodera, Ditylenchus, Merlinius, Pratylenchus, Aphelenchus, Aphelenchoides, Tylenchus, Helicotylenchus, Trophurus, Pratylenchoides, Filenchus* and *Xiphinema* (in decreasing order of incidence). *Amplimerlinus, Merlinus, Paratrophurus, Pratylenchoides* and the other tylenchid nematodes were the predominant genera of phytophagous nematodes including *Pratylenchoides sheri* Robbins, 1985 (Tylenchida: Hoplolaimidae), *Merlinius brevidens* (Allen, 1955) (Tylenchida: Belonolaimidae), *Amplimerlinius vicia* (Saltukoğlu, 1973) Siddiqi, 1976 (Tylenchida: Dolichodoridae), *Paratrophurus striatus* Castillo, Siddiqi & Gomez-Barcina, 1989 and *Paratrophurus acristylus* Siddiqi & Siddigi, 1983 (Tylenchida: Belonolaimidae) previously determined in wheat-growing areas of the Southeast Anatolian Region of Turkey (Imren & Elekcioğlu, 2008). Öcal & Elekçioğlu (2015) reported that the most common phytophagous nematodes were *Aphelenchus avenae* Bastian, 1865 (Tylenchida: Aphelenchoididae), *H. latipons, M. brevidens, Pratylenchus thornei* Sher & Allen, 1953 (Tylenchida: Hoplolaimidae) and *Scutylenchus quadrifer* (Andrássy, 1954) (Tylenchina: Merliniidae) in the cereal cropping system of Adıyaman Province, Turkey. Consequently, the reported genera are potentially of economic importance for cereal production in Niğde Province.

The high incidence of *Heterodera* (*H. filipjevi* and *H. latipons*) is a key finding of this study, 76% of sampled fields having cyst nematodes. The fields where cyst nematodes were not detected were generally rotated with other crops. The highest incidence (100%) was found in Altunhisar and Çamardı Districts but the lowest was still over 70% in the three other districts sampled. Sahin et al. (2009) were found CCNs (*H. filipjevi* and *H. latipons),* and root lesion nematodes [*P. thornei* and *Pratylenchus. neglectus* (Rensch, 1924) Filipjev & Schuurmans Stekhoven, 1941], were the most widely distributed species. Toktay et al. (2015) reported that 56% of wheat fields were infested with *H. filipjevi* in Elazig, Erzincan, Erzurum, Igdir, Kars, Malatya and Sivas Provinces in the East Anatolia Region of Turkey. Imren et al. (2016) reported that 83% of wheat fields were infested with *H. filipjevi*, in Bolu Province, Turkey. Therefore, cropping practice and environmental conditions in Niğde Province are particularly suited to the persistence of *H. filipjevi* which poses a risk to wheat production. The incidence of *H. latipons* was low, so it must be less competitive than *H. filipjevi* in this context.

This study indicated that there was no polymorphism in the populations of *H. filipjevi* according to the morphological parameters, which confirm differences in measurements of cysts and J2 bodies.

Heterodera filipjevi is similar to *H. avenae* with some minor differences such as less bullae and thinner underbridge in *H. avena*e (Handoo, 2002; Subbotin et al., 2010). However, *H. latipons* is quite different from *H. filipjevi* with a particularly prominent underbridge and no bullae in the fenestral area. *Heterodera filipjevi* and *H. latipons* can be easily separated using differences in underbridge structure and presence of bullae in the fenestral area of *H. latipons* (Wouts & Sturhan, 1995; Rivoal et al., 2003; Subbotin et al., 2003). *Heterodera latipons* J2s have a shorter tail, stylet and hyaline compared to *H. filipjevi* J2s. Briefly some morphometric features are easily distinguishable in these species such as cyst length, color and shape, fenestra length, J2s tail and hyaline length (Madzhidov, 1981; Valdeolivas & Romero, 1990; Wouts & Sturhan, 1995).

This study confirmed the functionality of CCN cysts and J2 body dimensions, and rDNA sequences for identifying populations of *H. latipons* and *H.* filipjevi*.* Specimens within the *H. filipjevi* population can be clearly grouped by morphological and molecular data. Likewise, Bekal et al. (1997) did not found any genetic differentiation between populations of *H. filipjevi* and *H. latipons*. Similarly, Imren et al. (2015) did not found any genetic differentiation between *H. filipjevi* populations in the Mediterranean Region of Turkey. However, Subbotin et al. (2010) reported intraspecific polymorphism between *H. filipjevi* populations and Toktay et al. (2015) also found intraspecific variation between populations in *H. filipjevi* from the Eastern Anatolian Region of Turkey.

This study determined the incidence of phytophagous nematodes in the five main cereal-growing districts, Altunhisar, Çamardı, Bor, Central and Ulukışla, of Niğde Province, particularly the high incidence and population densities of *H. filipjevi*. These findings indicated that detailed investigation of the pathotypes of *H. filipjevi* and *H. latipons* in comparison to other areas of Turkey would be justified. It is therefore recommended that policymakers and researchers consider diversification of wheat genotypes cultivated in Niğde, including durum wheat given its higher resistance to cyst nematodes; follow cultural practices especially crop rotation. Also, the is a need to breed wheat germplasm adapted to the areas with high levels of resistant to the cereal cyst nematodes, and to increase the awareness of advisors and growers of the potential impact of phytophagous nematodes on cereal productions in the province.

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