Original article (Orijinal araştırma)

Phytophagous nematodes in cereal fields in Niğde Province, Turkey

Niğde ili tahil alanlarında bulunan fitofag nematodlar

Halil TOKTAY¹ Mustafa İMREN² Badel G. AKYOL²
Emre EVLİCE³ Ian T. RILEY¹ Abdelfattah A. DABABAT⁴

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, Meloidogyne, Pratylenchus, Aphielenchus, Aphielenchoides, Tylenchus, Helicotylenchus, 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: Heterodidae). 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’deki başlıca tahil yetiştirme alanlarında bulunan fitofag nematodların yoğunluk ve dağılımı belirlenmiştir, tahil kist nematodu türlerinin morfolojik ve moleküler yöntemlerle tanımlanmıştır. Fitofag nematodlar 12 cins olarak toprak örneklerinin %95’inde tespit edilmiştir. En yaygın fitofag nematodlar sırasıyla, Heterodera, Ditylenchus, Meloidogyne, Pratylenchus, Aphielenchus, Aphielenchoides, Tylenchus, Helicotylenchus, Pratylenchoides, Filenchus ve Xiphinema cinsleridir (bulunan yoğunlüğuna göre sıralanmıştır). Incelenen toprak örneklerinin %75’inde tahil 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: Heterodidae) olarak belirlenmiştir. Heterodera filipjevi’nin Tür ici varyasyonlarına bakıldığında hepsinin aynı gurupta olabileceği değerlendirilmiştir. Buna ilave olarak tahil kist nematodları yanında kök lezyon nematodlarının, Pratylenchus türlerinin de rotasyon yapılan/yapılmayan Niğde ili tarım alanlarında yüksek yoğunlukta bulunduğunu sonucuna varılmıştır.

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

¹ Niğde Omer Halisdemir University, Faculty of Agricultural Sciences and Technologies, Department of Plant Production and Technologies, 51240, Niğde, Turkey
² Bolu Abant Izzet Baysal University, Faculty of Agriculture and Natural Sciences, Department of Plant Protection, 14100, Bolu, Turkey
³ Plant Protection Central Research Institute, 06170, Ankara, Turkey
⁴ International Maize and Wheat Improvement Center (CIMMYT), 06172, Ankara, Turkey
* Corresponding author (Sorumlu yazar) e-mail: h.toktay@ohu.edu.tr

Received (Alınış): 03.09.2020 Accepted (Kabul ediliş): 08.11.2020 Published Online (Çevrimiçi Yayın Tarihi): 13.11.2020
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) Stelzer, 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 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 Ulukişla 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.

![Map of Niğde Province, Turkey surveyed for phytophagous nematodes](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 4°C 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).
Phytophagous nematodes in cereal fields in Niğde Province, Turkey

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 | Akınlar 1 | 38°94'88.3" 34°27'97.0" |
|           | Central    | 38°76'71.4" 34°89'07.0" |
|           | Çömlekçı   | 38°05'67.1" 34°29'71.8" |
|           | Ulukışla   | 38°04'88.3" 34°21'77.3" |
|           | Yakacak    | 38°02'95.3" 34°34'36.5" |
|           | 2          | 38°00'08.4" 34°31'86.5" |
| Bor       | Bahçeli    | 38°26'51.9" 34°66'77.0" |
|           | Central    | 37°09'67.6" 34°55'06.3" |
|           |            | 38°20'11.4" 34°21'77.3" |
|           |            | 38°16'71.4" 34°35'88.3" |
|           |            | 38°36'27.1" 34°06'79.0" |
|           |            | 38°50'61.0" 34°54'09.3" |
|           |            | 38°90'23.4" 34°12'09.3" |
|           |            | 38°56'87.4" 34°66'56.3" |
| Çamlırdı  | Central    | 38°07'14.6" 34°82'62.3" |
|           |            | 38°06'82.6" 34°85'08.3" |
|           |            | 38°07'71.7" 34°91'65.8" |
|           |            | 38°06'73.4" 34°93'82.6" |
|           |            | 38°04'86.0" 34°96'66.8" |
|           |            | 38°03'18.8" 34°99'12.1" |
|           |            | 37°88'99.8" 35°09'58.2" |
| Kavlaktepe | 7          | 38°99'43.3" 35°06'43.4" |
|           |            | 37°97'66.8" 35°08'45.1" |
| Central   | Ağcaşar    | 38°31'97.0" 34°71'51.6" |
|           | Alay       | 38°26'31.2" 34°69'18.7" |
|           |            | 38°26'73.1" 34°67'91.2" |
|           |            | 38°27'52.7" 34°65'80.2" |
|           |            | 38°26'66.3" 34°71'62.6" |
|           |            | 38°28'54.7" 34°72'90.6" |
|           |            | 38°28'06.6" 34°73'69.8" |
|           |            | 38°27'11.2" 34°74'23.6" |
|           |            | 38°26'95.8" 34°73'42.7" |
|           |            | 38°26'71.4" 34°73'72.5" |
|           |            | 38°28'03.8" 34°69'51.3" |
|           |            | 38°27'15.4" 34°33'20.2" |
|           | Edikli     | 38°19'01.4" 34°96'76.5" |
|           | Konaklı    | 38°17'29.4" 34°94'06.8" |
|           | Orhanlı    | 38°28'36.3" 34°89'33.2" |
|           |            | 38°29'62.4" 34°88'79.9" |
|           |            | 38°29'01.1" 34°88'32.0" |
|           |            | 38°30'62.2" 34°67'77.3" |
|           |            | 38°32'46.3" 34°83'95.3" |
|           |            | 38°33'52.5" 34°82'86.2" |
| Ulukışla  | Güney      | 37°10'42.8" 34°35'11.3" |
|           |            | 37°10'51.9" 34°09'54.3" |
|           |            | 37°06'89.9" 34°68'11.3" |

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≤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'-CGTAAACAGTAGCTGAG-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 | Genera |
|----------|----------|-----------------|--------|
| Altunhisar | Akmanlar | 1 | - - - - 1 1 - - - 3 |
| Central | 1 | - 1 1 - 1 1 - - - 4 |
| Çömlerce | 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 - 8 |
| Çamardı | Central | 7 | 2 2 4 - 1 7 1 3 1 3 10 |
| Kavaktepe | 2 | - - 2 - 1 2 1 1 1 - - 7 |
| Central | Ağçaşar | 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 - - - 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                                    |
| Çamardi   | 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 Çamardi Central (38°07'71.7" N, 34°91'65.8" E and 38°04'86.0" N, 34°96'66.8" E).

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

Only two populations of *H. latipons* from Çamardi 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    | 22.8±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).

![Phylogenetic tree](image1.png)

**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*, *Aphelenchoides*, *Tylencylus*, *Helicotylenchus*, *Trophurus*, *Pratylenchoides*, *Filenchus* and *Xiphinema* (in decreasing order of incidence). *Amphimerlinus*, *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), *Amphimerlinius vicia* (Saltukoğlu, 1973) Siddiqi, 1976 (Tylenchida: Dolicohororidae), *Paratrophurus striatus* Castillo, Siddiqi & Gomez-Barcina, 1989 and *Paratrophurus acrystylus* Siddiqi & Siddiqi, 1983 (Tylenchida: Belonolaimidae) previously determined in wheat-growing areas of the Southeast Anatolian Region of Turkey (Imren & Elekçiöğlu, 2008). Öcal & Elekçiöğlu (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 *Scutylencylus quadriber* (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 Çamardi 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 Elazığ, Erzincan, Erzurum, Iğdir, 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. avenae* (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 find any genetic differentiation between populations of *H. filipjevi* and *H. latipons*. Similarly, Imren et al. (2015) did not find 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, Çamardi, Bor, Central and Ulusla, 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.

References

Abidou, H., A. El-Ahmed, J. M. Nicol, N. Bolat, R. Rivoal & A. Yahyaoui, 2005. Occurrence and distribution of species of the *Heterodera avenae* group in Syria and Turkey. Nematologica Mediterranea, 33 (2):195-201.

Bekal, S., J. P. Gauthier & R. Rivoal, 1997. Genetic diversity among a complex of cereal cyst nematodes inferred from RFLP analysis of the ribosomal internal transcribed spacer region. Genome, 40 (4): 479-486.

Dababat, A. A. & R. W. Foure, 2018. “Nematode Parasites of Cereals. 163-221”. In: Plant Parasitic Nematodes in Subtropical and Tropical Agriculture, 3rd Edition (Eds. R. A. Sikora, D. Coyne, J. Hallmann & P. Timper), CABI, Walliford, UK, 877 pp.

Dababat, A. A., M. Imren, G. Erginbaş-Orakçı, S. Ashrafi, E. Yavuzsloanoglu, H. Toktay & T. Mekete, 2015. The importance and management strategies of cereal cyst nematodes, *Heterodera* spp., in Turkey. Euphytica, 202 (2): 173-188.

Dababat, A. A., G. E. Orakçi, H. Toktay, M. İmren, B. Akin, H. J. Braun & A. I. Morgunov, 2014. Resistance of winter wheat to *Heterodera filipjevi* in Turkey. Turkish Journal of Agriculture and Forestry, 38 (2): 180-186.

FAO, 2018. FAOSTAT database collections. Rome: Food and Agriculture Organization of the United Nations. (Web page: www.fao.org/faostat/en/#home) (Date accessed: 25 June 2018).

Franklin, M. T., 1969. *Heterodera latipons* n. sp., a cereal cyst nematode from the Mediterranean region. Nematologica, 15 (4): 535-542.

Handoo, Z. A., 2002. Key and compendium to species of the *Heterodera avenae* group (Nematode: Heteroderidae), Journal of Nematology, 34 (3): 250-262.

Holtermann, M., A. Van der Wurff, S. Van den Elsen, Van Megen, H. Bongers, T. Holovachov, O. Bakker & J. Helder, 2006. Phylum-wide analysis of SSU rDNA reveals deep phylogenetic relationships among nematodes and accelerated evolution toward crown clades. Molecular Biology and Evolution, 23 (9): 1792-1800.

Hooper, D. J., 1986. “Extraction of Nematodes from Plant Material, 59-80”. In: Laboratory Methods for Work with Plants and Soil Nematodes. (Ed. J. F. Southey) Ministry of Agriculture, Fisheries and Food, London, UK, 202 pp.

Imren, M. & I. H. Elekcióglü, 2008. Diyarbakır ili buğday, sebzeye ve bağ alanlarında önemli bitki parazitleri, bitkilerin ve bitkilerin araştırılması. ÇÜ Fen ve Mühendislik Bilimleri Dergisi, 17 (2): 116-121.

Imren, M., H. Toktay, A. Özarslan, A. A. Dababat & I. H. Elekcióglü, 2012. *Heterodera avenae* Wollenweber (Tylenchida: Heteroderidae) yumurtalarının inkübasyonu üzerine sıcaklık etkisi ile en uygun deneme yöntem ve materyallerinin araştırılması. Turkish Journal of Entomology, 36 (4): 585-593.

Imren, M., H. Toktay, H. Kutuk & A. A. Dababat, 2016. Occurrence and identification of cereal cyst nematode, *Heterodera filipjevi* (Nemata: Heteroderidae), in Bolu Province of Turkey. Nematropica, 46 (2): 154-161.

Imren, M., L. Waeyenberge, N. Viaene, I. H. Elekcióglü & A. A. Dababat, 2015. Morphological and molecular identification of cereal cyst nematodes from the Eastern Mediterranean Region of Turkey. Turkish Journal of Agriculture and Forestry, 39 (1): 91-98.

Imren, M., Ş. Yıldız, V. Çiftçi & A. A. Dababat, 2020. Effect of cereal cyst nematode *Heterodera filipjevi* on wheat yields in Turkey. Turkish Journal of Agriculture and Forestry, 44 (1): 39-45.

Imren, M., Ş. Yıldız, H. Toktay, N. Duman & A. A. Dababat, 2018. Morphometric and genetic variability among Mediterranean cereal cyst nematode (*Heterodera latipons*) populations in Turkey. Turkish Journal of Zoology, 42 (6): 625-636.
Joyce, S. A., A. M. Burnell & T. O. Powers, 1994. Characterization of *Heterorhabditis* isolates by PCR amplification of segments of mtDNA and rDNA genes. Journal of Nematology, 26 (3): 260-270.

Kimura, M., 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution, 16 (2): 111-120.

Kumar, S., G. Stecher & K. Tamura, 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution, 33 (7): 1870-1874.

Madzhidov, A. R., 1981. *Bidera filipjevi* n. sp. (Heteroderina: Tylenchida) from Tadzhikistan. Izvestiya Akademii Nauk Tadzhikskoi SSR, Biologicheskie Nauki, 83 (2): 40-44.

Mulvey, R. H. & A. M. Golden, 1983. An illustrated key to the cyst-forming genera and species of *Heteroderidae* in the western hemisphere with species morphometrics and distribution. Journal of Nematology, 15 (1): 1-59.

Nicol, J. M., 2002. “Important Nematode Pests, 345-366”. In: Bread Wheat: Improvement and Production (Eds. B. C. Curtis, S. Rajaram & H. Gómez Macpherson). FAO, Rome, Italy, XI+554 pp.

Nyaku, S. T., A. Affokpon, A. Danquah & F. C. Brentu, 2017. “Harnessing Useful Rhizosphere Microorganisms for Nematode Control, 153-182”. In: Nematology-Concepts, Diagnosis and Control (Eds. M. M. Shah & M. Mahamood) InTech, London, UK, 194 pp.

Öcal, A. & I. H. Elekçoğlu, 2015. “Plant Parasitic Nematode Species Associated with Barley (*Hordeum vulgare*) and Wheat (*Triticum* spp. L.) in Adiyaman Province, 149-158”. In: Nematodes of Small Grain Cereals. Current Status and Research. (Eds. A. A. Dababat, H. Muminjanov & R. W. Smiley). Food and Agricultural Organization of the United Nations, Ankara, Turkey, 384 pp.

Rivoal, R. & R. Cook, 1993. “Nematode Pests of Cereals, 259-303”. In: Plant Parasitic Nematodes in Temperate Agriculture (Eds. K. Evans, D. L. Trudgill & J. M. Webster). CAB INTERNATIONAL, London, UK, 656 pp.

Rivoal, R., S. Valette, S. Bekal, J. P. Gauthier & A. Yahyaoui, 2003. Genetic and phenotypic diversity in the graminaceous cyst nematode complex, inferred from PCR-RFLP of ribosomal DNA and morphometric analysis. European Journal of Plant Pathology, 109 (3): 227-241.

Rumpenhorst, H. J., İ. H. Elekçoğlu, D. Sturhan, G. Öztürk & S. Ennelli, 1996. The cereal cyst nematode *Heterodera filipjevi* (Madzhidov) in Turkey. Nematologia Mediterranea, 24 (1): 135-138.

Şahin, E., J. M. Nicol, İ. H. Elekçoğlu, Ö. Yorgancılar, A. F. Yıldırım, A. Tülecke, H. Hekimhan, A. Yorgancılar, A. T. Kılınc, N. Bolat & G. Erginbaş-Orakçı, 2009. “Frequency and Diversity of Cereal Nematodes on the Central Anatolian Plateau of Turkey, 100-105”. In: Cereal Cyst Nematodes: Status, Research and Outlook (Eds. I. T. Riley, J. M. Nicol & A. A. Dababat). CIMMYT, Ankara, Turkey, XXI+270 pp.

Smiley, R. W. & J. M. Nicol, 2009. “Nematodes Which Challenge Global Wheat Production, 171-187”. In: Wheat Science and Trade (Ed. B. F. Carver). Wiley Blackwell, Ames, IA, USA, 619 pp.

Subbotin, S. A., M. Mundo-Ocampo & J. G. Baldwin, 2010. “Description and Diagnosis of *Heterodera* Species, 354-449”. In: Systematics of Cyst Nematodes (Nematoda: Heteroderinae), Part B. (Eds. S. A. Subbotin, M. Mundo-Ocampo & J. Baldwin). Brill, Leiden, Netherlands, 512 pp.

Subbotin, S. A., D. Sturhan, H. J. Rumpenhorst & M. Moens, 2003. Molecular and morphological characterisation of the *Heterodera avenae* species complex (Tylenchida: Heteroderidae). Nematology, 5 (4): 515-538.

Tamaka, K. & M. Nei, 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution, 10 (3): 512-526.

Toktay, H., M. İmren, İ. H. Elekçoğlu & A. A. Dababat, 2015. Evaluation of Turkish wild Emmer (*Triticum dicoccoides* Koern.) and wheat varieties for resistance to the root lesion nematodes (*Pratylenchus thornei* and *Pratylenchus neglectus*). Turkish Journal of Entomology, 39 (3): 219-227.

TurkStat, 2020. Agricultural Production Statistics. (Web page: www.turkstat.gov.tr) (Date accessed: 15 June 2020).

Valdeolivas, A. & M. D. Romero, 1990. Morphometric relationships of some members of the *Heterodera avenae* complex (Nematoda: Heteroderidae). Nematologica, 36 (1-4): 292-303.

Wouts, W. M. & D. Sturhan, 1995. *Heterodera aucklandica* sp. n. (Nematoda: *Heteroderidae*) from a New Zealand native grass, with notes on the species of the *H. avenae* group. New Zealand Journal of Zoology, 22 (2): 199-207.

Yüksel, H. S., 1974. Doğu Anadolu'da tespit edilen *Pratylenchus* türlerinin dağılımı ve bunlar üzerinde sistematiğe çalışmalar. Atatürk Üniversitesi Ziraat Fakültesi Dergisi, 4 (1): 53-71.