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## Occurrence and biodiversity of cyst nematodes in wheat and barley cultivation areas of Uşak Province, Türkiye

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**Abstract:** Plant parasitic nematodes (PPNs) are one of the major threats to various crops including cereals. In wheat and barley production, the profitability of cultivation heavily depends on the management of cyst nematodes (CNs), especially on *Heterodera avenae*, *H. filipjevi*, and *H. latipons*. In this study, field surveys were conducted in wheat and barley cultivation systems of Uşak Province to reveal the presence and diversity of PPNS. In total, 80 wheat and barley fields were surveyed, and samples (soil and root) were processed for the isolation and identification of PPNS. The PPN genera were identified to the species level based on morphological and morphometric characters. CNs were further analyzed using molecular methods. In total, 17 species belonging to 10 genera were identified. *Helicotylenchus* Steiner, *Pratylenchoides* Winslow, *Merlinius* Siddiqi, and *Heterodera* Schmidt were the most prevalent and abundant genera. The Shannon diversity index averaged at 2.51 in the wheat and barley fields, indicating a moderate level of biodiversity. The evenness value of 0.798 suggests a high degree of uniformity among migratory PPNS. The results indicate that PPNS have a serious impact on reducing grain yield and quality in the wheat- and barley-growing areas of Uşak Province.

**Key words:** Biodiversity, cereal, identification, phytonematodes, Uşak

### 1. Introduction

Plant parasitic nematodes (PPNs) have become increasingly prevalent worldwide and are recognized as one of the primary contributors to yield reductions and quality deterioration in cereal crops (Chitwood, 2003). Cyst nematodes (CNs) (*Heterodera* spp.) and root-knot nematodes (RKNs) (*Meloidogyne* spp.) belonging to the family Heteroderidae are obligate root parasites (Dababat et al., 2014; Dababat et al., 2015). These two genera alone account for 80% of annual crop losses caused by nematodes, highlighting their global importance (Chitwood, 2003). Other economically significant cereal PPNS include the seed gall nematode (*Anguina tritici*), stem and bulb nematode (*Ditylenchus dipsaci*), and root lesion nematode (*Pratylenchus* spp.), (İmren and Elekcioglu, 2014).

The cereal CN (CCN), *Heterodera avenae*, is a significant cereal pest in Türkiye (Dababat et al., 2015;

Imren et al., 2015). Another notable pest species is *Meloidogyne naasi*, which is prevalent in the southern region of Türkiye (Dababat and Fourie, 2018). *Ditylenchus dipsaci* is primarily found in oats and can be managed by cultivating resistant cultivars (French and Cairns, 1960). Additionally, *Pratylenchus* species, particularly *P. fallax*, have been linked to barley patches exhibiting stunted growth (Corbett, 1972).

Cadet and Spaul (2005) conducted a study that showed *Paratrichodorus anemones* having a negative impact on the growth of spring barley and wheat. However, there are contradictory reports regarding the significance of other migratory PPNS and their ability to cause damage to cereal crops. For instance, there are many reports of *Pratylenchus* species causing damage to cereal crops, i.e. *P. neglectus* and *P. thornei* in Australia and Mediterranean countries (Jones, 1979). On the other hand, barley plants experiencing early

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leaf yellowing were found to be associated with *Longidorus* and *Tylenchorhynchus* species, whereas *Tylenchorhynchus dubius* specifically exhibited a significant detrimental impact on wheat yield (Cadet and Spaul, 2005). A previous study by Sharma (1971) reported yield reductions caused by *T. dubius*, while another investigation conducted by Saynor (1979) did not establish a correlation between nematode population density and yield. *Longidorus leptcephalus* and *L. elongatus*, reduce barley yields (Sykes, 1979; Boag et al., 1990). The identification of *Heterodera* spp. and *Pratylenchus* spp. populations from various wheat cultivation systems was accomplished by considering their morphological characteristics as well as utilizing molecular techniques in Türkiye (Şahin et al., 2009; Toktay et al., 2015; İmren et al., 2018, Imren et al., 2021; Dababat et al., 2020). Despite Uşak Province being a significant wheat-producing region in the Aegean region of Türkiye, knowledge about the PPN species composition in this region is still insufficient. Therefore, this study aimed to accomplish specific objectives: 1) to investigate the presence and prevalence of these important PPN genera in the Aegean region, 2) to identify *Heterodera* spp. using both morphological/morphometric and molecular data, and 3) to examine the genetic relationships and identify *Heterodera* spp. using morphological/morphometric data among populations.

## 2. Materials and methods

### 2.1. Nematode sampling

In Uşak Province, surveys were conducted to recover migratory and sedentary nematodes during the wheat and barley tillering/flowering, and harvesting time, respectively. Forty samples (soil and roots) were taken

from the rhizospheres of barley and wheat along important wheat and barley fields in Banaz, Karahallı, Ulubey, Sivasslı, and the Central districts of Uşak Province during the 2020–2021 growing season (Figure 1). The collected samples for this study comprised of 15 to 20 cores, each with a diameter of 2.5 cm and a depth of 20 cm. To form composite samples for each field, these cores were combined. In the field, the samples were placed into a cooler containing ice cubes (approximately 14 °C) and then transported to the laboratory. Upon arrival, the samples were appropriately stored by refrigerating them at 4 °C until further processing was initiated. Migratory and sedentary nematodes were obtained from the soil using a modified petri-dish Baermann funnel method and the Fenwick can method with 60-mesh (0.25 mm) and 100-mesh (0.015 mm) sieves, respectively (Hooper, 1986). Nematode genera were identified based on morphological and morphometrical characters using a light microscope (DM1000, Leica Microsystems, Wetzlar, Germany) at 100× magnification and a stereomicroscope (Zeiss Stemi 305, Carl Zeiss, Jena, Germany). The occurrence frequency of each PPN at each sampling site was established by division of the ratio of the positive samples to the total number of samples.

### 2.2. Morphological identification

Permanent slides of the nematodes were prepared by following the method described by Seinhorst (1962). Adult male nematodes obtained from the soil samples and juveniles hatched from cysts were carefully selected and subjected to gentle heat. These nematodes were individually placed on temporary glass slides and covered with a glass cover supported by a wax ring. Subsequently, the nematodes were examined using a stereo microscope to identify the species.

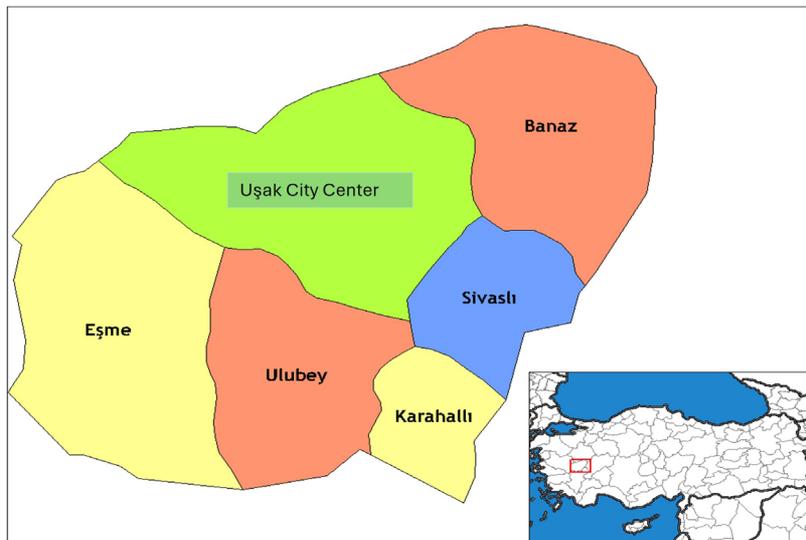


Figure 1. Locations in Uşak Province surveyed for PPNs.

The morphological characteristics encompassed various aspects such as the head shape, number of lip region annules, body and stylet length, stylet knob shape, lateral field incisures, presence or absence and structure of the spermathecal in females, shape of the female tail terminus, as well as the shape and length of the spicules and gubernaculum (Handoo, 2002).

The process of identifying species within the *Heterodera* genus relied on a number of morphological characteristics of second-stage juveniles and features of the vulval plate (Mathews, 1970; Mulvey, 1972). The species level identification of the nematodes was conducted by observing the prepared permanent slides using an Axio Lab A1 model light microscope (Carl Zeiss AG, Oberkochen, Germany). To obtain measurements, ZEN lite software was utilized along with an AxioCam ERc5s digital camera (Carl Zeiss AG, Oberkochen, Germany).

### 2.3. Molecular identification of CNS

DNA extraction was carried out based on the method of Waeyenberge et al. (2000). Individual second-stage juveniles (J2) obtained from an individual cyst of each population were placed into separate Eppendorf tubes, each containing 25 µL of double-distilled water (ddH<sub>2</sub>O) and 25 µL of nematode lysis buffer. The nematode lysis buffer had a final concentration of 200 mM of sodium chloride, 200 mM of Tris-hydrochloride (pH 8), 1% mercaptoethanol, and 800 µg of proteinase K. The tubes were then subjected to incubation at 65 °C for 1.5 h, followed by a subsequent incubation at 99 °C for 5 min. This process was conducted according to the protocol defined by Holterman et al. (2006). The resulting DNA suspension was either stored at -20 °C for future use or instantly employed for DNA amplification.

The amplification of the internal transcribed spacer ribosomal RNA (ITS-rDNA) region was performed using primers 5'-CGT AAC AAG GTA GCT GTA G-3' (F) and 5'-TCC TCC GCT AAA TGA TAT G-3' (R), as described by Ferris et al. (1993). Polymerase chain reaction (PCR) was carried out in a 25-µL reaction volume, consisting of 2.5 µL of Dream Taq Buffer 10X, 1 µL of deoxynucleotide triphosphate (2 mM), 2 µL of each primer (10 µM), 1 µL of bovine serum albumin (20 mg mL<sup>-1</sup>), 1.5 mM of magnesium dichloride, 0.5 µL of Dream Taq polymerase (5 U µL<sup>-1</sup>, Thermo Fisher Scientific Inc., Waltham, MA, USA), and 5 µL of the DNA extract. The amplification process involved an initial denaturation step at 95 °C for 5 min, followed by 40 cycles of denaturation at 94 °C for 30 s, annealing at 45 °C for 45 s, extension at 72 °C for 45 s, and a final extension at 72 °C for 7 min.

The PCR products were visualized by electrophoresis on a 1.6% agarose gel prepared in Tris-borate-ethylenediaminetetraacetic acid 0.5X buffer. GelRed™

stain (Biotium, Fremont, CA, USA) was used to visualize the PCR products. To purify the PCR products, NucleoSpin gel and a PCR Clean-up Kit (Macherey-Nagel Inc., Allentown, PA, USA) were employed. The purified PCR products were then sent to Macrogen (Seoul, South Korea) for bidirectional sequencing. The nucleotide sequences were aligned using BioEdit software (Hall, 1999). Comparative analyses were carried out to evaluate differences in the expressed genes with existing *Heterodera* sequences in the GenBank database.

### 2.4. Genetic diversity

A phylogenetic analysis of the ITS-rDNA sequences from *Heterodera* species obtained in this study, along with sequences retrieved from the GenBank database, was performed using Bayesian inference (BI) analysis. MrBayes 3.2 software, developed by Ronquist and Huelsenbeck (2003), was employed for this analysis. Sequence alignment was accomplished using the MUSCLE algorithm (Edgar, 2004) implemented in MEGA6 software, developed by Tamura et al. (2013).

To determine the most suitable DNA model of evolution, jModelTest 2 was utilized, and the best model (K80 + G) was chosen based on the Bayesian information criterion (BIC) (Guindon and Gascuel, 2003; Darriba et al., 2012). The resulting phylogenetic tree was created using FigTree 1.4.3. To enhance the strength of the analysis, at least one sequence from previous peer-reviewed publications was included for each *Heterodera* species employed in the phylogenetic analysis.

### 2.5. Biodiversity

The Shannon diversity index is a method employed to assess and quantify the level of species diversity within a given community. It is computed using the formula  $H = -\sum \pi \times \ln(\pi)$ , where  $\Sigma$  is the summation,  $\ln$  refers to the natural logarithm, and  $\pi$  represents the proportion of the community made up of a particular species  $I$  (Shannon, 1948). A greater  $H$  value indicates a greater diversity of species within a specific community, while a lower  $H$  value suggests a lower diversity. The Shannon equitability index was employed to assess the evenness of species distribution within a community (Shannon, 1948). The term 'evenness' reflects the similarity in abundance among different species in the community. The evenness (EH) is computed as  $EH = H/\ln(S)$ , where  $H$  represents the Shannon diversity index and  $S$  is the total count of distinct species. The EH value ranges from 0 to 1, with 1 indicating a state of complete evenness (Shannon, 1948).

## 3. Results

The identified PPNs were 17 species belonging to 10 genera, as follows: *Filenchus thornei* (Andrássy, 1954) Andrássy, 1963; *Filenchus cylindricauda* Wu, 1969 Siddiqi 1986; *Filenchus filiformis* Bütschli, 1873 Meyl, 1961;

*Ditylenchus myceliophagus* Goodey, 1958; *Ditylenchus dipsaci* Kuhn, 1857; *Ditylenchus valveus* Thorne & Malek, 1968; *Ditylenchus clarus* Thorne & Malek, 1968; *Safianema lutanense* Siddiqi, 1980; *Helicotylenchus tunisiensis* Siddiqi, 1964; *Pratylenchoides alkani* Yüksel, 1977; *Pratylenchoides crenicauda* Winslow, 1958; *Bitylenchus dubius* Filipjev, 1936; *Merlinius brevidens* (Allen, 1955) Siddiqi, 1970; *Scutylenchus cylindricaudatus* Ivanova, 1968; *Scutylenchus koreanus* Choi & Geraert, 1971; *Heterodera filipjevi* Madzhidov, 1981; and *Aphelenchus avenae* Bastian, 1865.

Among the identified nematodes *Helicotylenchus* Steiner, *Pratylenchoides* Winslow, *Merlinius* Siddiqi, and *Heterodera* Schmidt showed the most frequent and abundant genera.

The occurrence of PPN genera on cereal at 40 sites from Banaz, Karahallı, Ulubey, Sivashlı, and the Central districts of Uşak Province of Türkiye is given in Table 1. The majority of the samples (86%) had PPNs, with an average abundance of 96 individuals per gram of soil (Table 2).

### 3.1. Morphological identification

*Heterodera filipjevi* was the only recognized CN within the 13 populations found from Banaz, Karahallı, Ulubey, Sivashlı and the Central districts. The second-stage juveniles: cylindrical body shape, with a faintly angled head and tapered, rounded tail tip, body length ranged from 488 to 531 µm, stylet length 24–25 µm, robust, exhibiting moderately concave stylet knobs (Table 3). Hyaline length and tail length were 40.6 to 72.4 µm and 21.4 to 39.4 µm, respectively. The second-stage juveniles of *H. filipjevi* slightly differed statistically from other *Heterodera* species by their slightly longer tail length (40.6 to 72.4 µm versus 42 to 67 µm) and slightly longer hyaline length (21.4 to 39.4 µm versus 20 to 36 µm) except for *Heterodera avenae*.

The cysts exhibited a golden-brown color and had a lemon-like shape, characterized by a posterior protuberance. The vulval cone of *H. filipjevi* exhibited a bifenestrate structure, along with well-developed bullae and a narrow underbridge positioned near the center and

**Table 1.** Distribution of plant-parasitic nematodes in cereal fields across districts and locations of Uşak Province.

District	Location	Nematode genera
Karahallı	Karayakuplu	<i>Merlinius</i> , <i>Safianema</i> , <i>Helicotylenchus</i>
Karahallı	Alfaklar	<i>Pratylenchoides</i> , <i>Ditylenchus</i> , <i>Heterodera</i>
Banaz	Kızılhisar	<i>Pratylenchoides</i> , <i>Scutylenchus</i> , <i>Filenchus</i> , <i>Heterodera</i>
Sivashlı	Kökez	<i>Ditylenchus</i> , <i>Heterodera</i>
Sivashlı	Hacım	<i>Aphelenchus</i> , <i>Pratylenchoides</i> , <i>Heterodera</i>
Ulubey	Avgan	<i>Ditylenchus</i> , <i>Pratylenchoides</i> , <i>Heterodera</i>
Central district	İkisaray	<i>Bitylenchus</i>
Central district	Koyunbeyli	<i>Ditylenchus</i>
Central district	Kırka	<i>Filenchus</i> , <i>Ditylenchus</i>
Central district	Ortaköy	<i>Bitylenchus</i> , <i>Ditylenchus</i>
Central district	Çarık	<i>Bitylenchus</i> , <i>Ditylenchus</i> , <i>Filenchus</i> , <i>Scutylenchus</i>
Central district	Gücer	<i>Aphelenchus</i> , <i>Filenchus</i> , <i>Heterodera</i>
Central district	Yoncalı	<i>Ditylenchus</i> , <i>Pratylenchoides</i>
Central district	Susuzören	<i>Merlinus</i> , <i>Pratylenchoides</i>

**Table 2.** Plant-parasitic nematode genera collected from cereal fields in Uşak Province.

Nematode genera	Infested fields (%) *	Nematode abundance **
<i>Ditylenchus</i>	42.5	380 ± 42.22 (200–800)
<i>Pratylenchoides</i>	30.5	220 ± 22.50 (180–400)
<i>Filenchus</i>	55	250 ± 25 (200–555)
<i>Bitylenchus</i>	25	290 ± 13 (200–650)
<i>Aphelenchus</i>	35	340 ± 14 (180–520)
<i>Scutylenchus</i>	25	140 ± 10 (100–320)
<i>Merlinius</i>	45	330 ± 14 (240–620)
<i>Helicotylenchus</i>	62.5	320 ± 10 (260–710)
<i>Safianema</i>	62.5	220 ± 10 (80–240)
<i>Heterodera</i>	32.5	14 cysts (10–34)

\* (Nematode infested samples / total samples) × 100. \*\* Average number of nematodes / 100 g of soil ± SD (min and max).

**Table 3.** Morphometric and morphological analysis of the second-stage juveniles and cysts from the *Heterodera filipjevi* populations (n = 10), measured in  $\mu\text{m}$ .

Character	Sivaslı	Karahallı	Ulubey	Banaz
Body length	528.3 $\pm$ 4.85 (490.6–550.7)	573.3 $\pm$ 3.74 (465.7–592.6)	518.9 $\pm$ 6.62 (466.6–548.5)	560.3 $\pm$ 6.55 (481.8–583.8)
Stylet length	22.9 $\pm$ 0.88 (22.2–25.6)	25.5 $\pm$ 0.32 (22.5–28.9)	24.4 $\pm$ 1.56 (21.6–27.6)	24.2 $\pm$ 1.74 (21.6–28.8)
Tail length	48.34 $\pm$ 2.36 (40.7–67.8)	52.66 $\pm$ 3.35 (41.6–64.6)	54.68 $\pm$ 3.94 (45.6–66.8)	51.24 $\pm$ 4.52 (42.5–67.6)
Hyaline tail tip length	25.6 $\pm$ 1.32 (21.4–34.5)	28.2 $\pm$ 1.43 (20.9–35.5)	26.7 $\pm$ 1.14 (23.2–36.4)	24.8 $\pm$ 1.34 (22.3–34.5)
Fenestral length	55.24 $\pm$ 1.45 (48.8–65.2)	58.34 $\pm$ 3.4 (43.8–68.4)	54.54 $\pm$ 2.32 (45.2–63.2)	48.34 $\pm$ 1.64 (40.2–59.8)
Semifenestral width	20.4 $\pm$ 2.43 (15.2–26.6)	25.2 $\pm$ 2.7 (20.4–27.4)	24.7 $\pm$ 1.21 (19.9–26.9)	21.4 $\pm$ 1.34 (16.4–25.2)
Vulval bridge width	13.43 $\pm$ 1.14 (9.3–16.8)	13.98 $\pm$ 1.32 (9.5–14.12)	14.34 $\pm$ 1.45 (9.45–17.33)	16.67 $\pm$ 1.34 (10.6–18.67)
Vulval slit length	20.6 $\pm$ 1.67 (16.2–24.68)	21.7 $\pm$ 1.21 (15.4–24.9)	21.34 $\pm$ 1.45 (16.7–24.6)	17.2 $\pm$ 1.34 (14.6–26.2)

in close proximity to the vulval bridge. The length of the fenestra and the width of the semifenestra of *H. filipjevi* ranged from 49 to 63  $\mu\text{m}$  and 17 to 22  $\mu\text{m}$ , respectively. Additionally, the vulval cone displayed a bifenestrate pattern along with an underbridge, as indicated in Table 3. The width of the vulval bridge ranged from 9.3 to 18.67  $\mu\text{m}$ , while the length of the vulval slit varied from 14.6 to 26.2  $\mu\text{m}$ . *H. filipjevi* cysts slightly varied statistically from other *Heterodera* species by their weak underbridge with bullae and higher fenestral length (42.1 to 63.2  $\mu\text{m}$  versus 40 to 59  $\mu\text{m}$ ), slightly larger vulval bridge width (7.28 to 22.6  $\mu\text{m}$  versus 9 to 17  $\mu\text{m}$ ), and longer vulval slit length (11.4 to 29.6  $\mu\text{m}$  versus 7.3 to 13.4  $\mu\text{m}$ ) (Table 3).

The Shannon diversity index (H) is commonly classified into three categories: low diversity ( $H < 2$ ), moderate diversity ( $2 < H < 4$ ), and high diversity ( $H > 4$ ) (Lumeran, 2019). In the present study, the Shannon diversity index (H) was computed as  $H = 2.51295$  for the 17 identified nematode species in wheat and barley fields. The calculated value of H falls within the range of 2–4, indicating a moderate diversity of nematode species in the genus. Furthermore, the evenness index (EH) was determined as 0.798931. These findings suggest that nematode evenness is high in areas where wheat and barley are cultivated, as evidenced by the EH value approaching 1 (Table 4).

### 3.2. Molecular identification

The rDNA-ITS sequences of *H. filipjevi* from Uşak Province, Türkiye, were subjected to sequencing and phylogenetic analysis for molecular diagnosis. The DNA sequences were generated using TW81 and AB28 primers to identify the cyst populations. The achieved sequences were then compared to the National Center for Biotechnology Information nucleotide database using Basic Local Alignment Search Tool searches (Figure 2). The cyst populations from Banaz, Karahallı, Ulubey, Sivaslı, and the Central districts were compared to thoroughly related cyst samples available in the GenBank database.

The majority of the cyst populations showed a high similarity of 99%–100% with the connected cyst samples already documented in GenBank. Based on the analysis of their ITS sequences, 13 cyst samples were accurately identified as *Heterodera filipjevi*. The nucleotide sequences of the ITS region were submitted to GenBank and assigned accession numbers ranging from OR214981 to OR214993.

Figure 3 presents the phylogenetic tree showing the CN populations found in Uşak Province. The construction of the phylogenetic tree involved globally randomized sequence alignments with repeated replications. Within the tree, minor intraspecific polymorphism within the *H. filipjevi* populations were detected, leading to their classification into two separate clades. These groupings were supported by illustrative populations from GenBank, with a moderate bootstrap value (Figure 3).

*H. filipjevi* populations were compared to national and international cereal CN species, *H. avenae*, *H. latipons*, and *H. filipjevi* populations with an outgroup of *Meloidogyne incognita* in the phylogenetic tree (Figure 3). As a result, one main consensus sequence, except for the samples of OR214986, was obtained from bidirectional sequencing and served as the basis for conducting a thorough phylogenetic analysis (Figure 3).

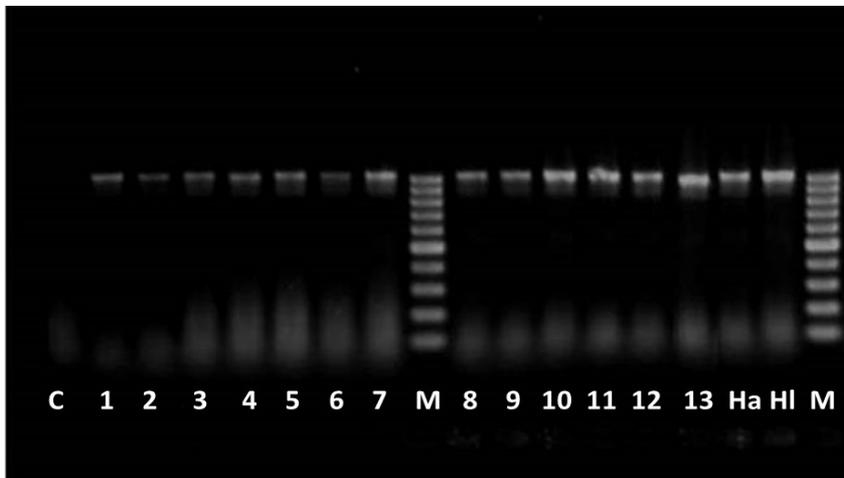
### 4. Discussion

This study aimed to research the incidence of PPNs in the wheat and barley cropping system of Uşak Province. Keçici et al. (2022) reported that the most predominant PPNs in cereal soils in Sakarya Province were *Helicotylenchus*, *Merlinius*, *Heterodera*, *Pratylenchus*, *Pratylenchoides*, *Trophurus*, *Paratrophurus*, *Tylenchus*, *Filenchus*, *Scutylenchus*, *Amplimerlinius*, *Boleodorus*, and *Basiria*. Toktay et al. (2020) stated that the PPNs found in cereal soils predominantly encompassed nematode genera *Ditylenchus*, *Pratylenchus*, *Heterodera*, *Aphelenchus*, *Merlinius*, *Xiphinema*, *Aphelenchoides*, *Tylenchus*, *Pratylenchoides*, *Helicotylenchus*, *Filenchus*, and *Trophurus* in Niğde Province. In the current study,

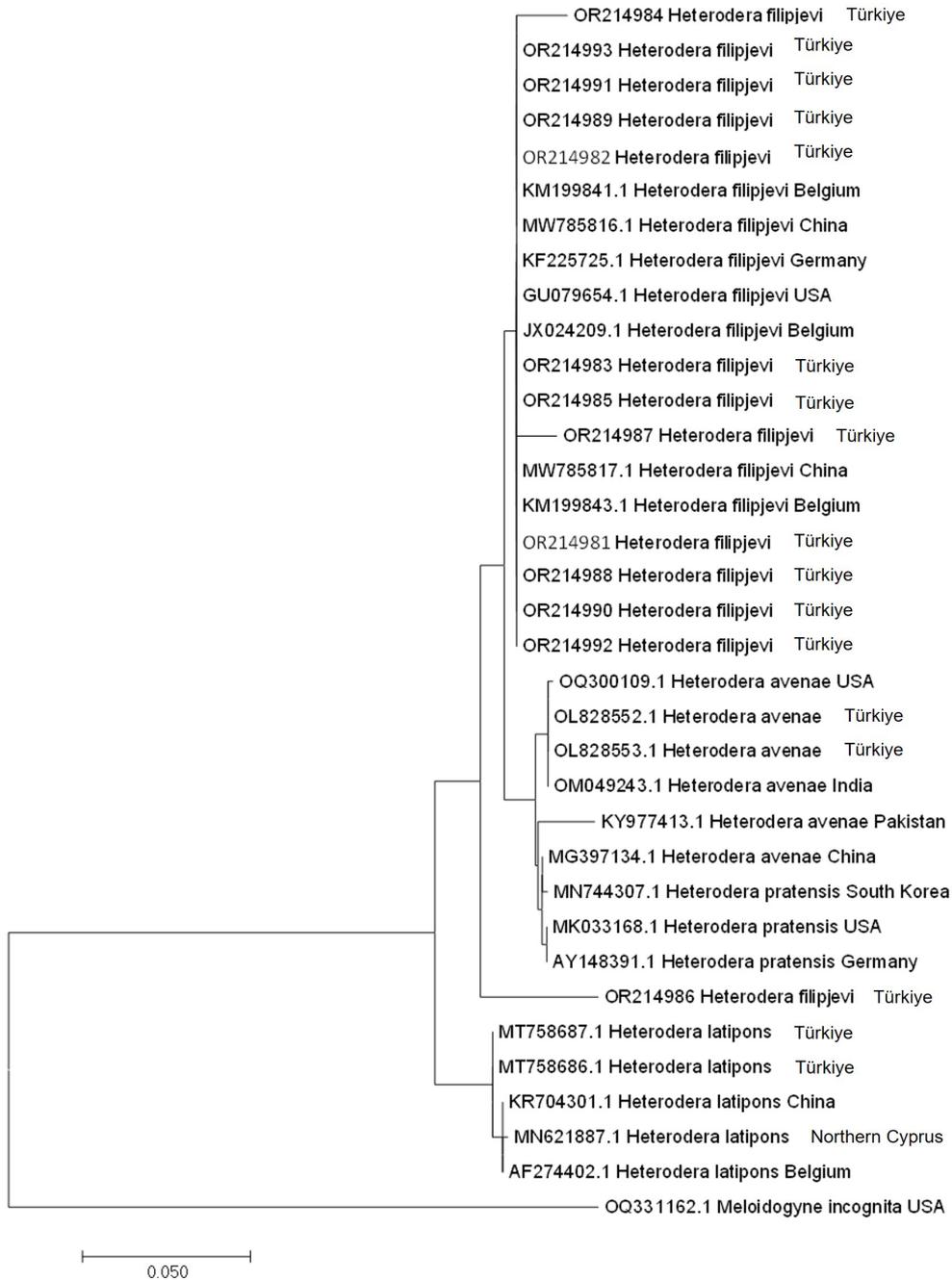
**Table 4.** Assessment of the nematode diversity in wheat and barley fields using the Shannon diversity index.

Nematode species	Average number of nematodes/100 g of soil (abundance)	of $p_i$	$\ln(p_i)$	$p_i * \ln(p_i)$
<i>Aphelenchus avenae</i>	40	0.044743	-3.10683	-0.13901
<i>Bitylenchus dubius</i>	60	0.067114	-2.70136	-0.1813
<i>D. clarus</i>	20	0.022371	-3.79997	-0.08501
<i>D. dipsaci</i>	100	0.111857	-2.19054	-0.24503
<i>D. myceliophagus</i>	200	0.223714	-1.49739	-0.33499
<i>D. valveus</i>	60	0.067114	-2.70136	-0.1813
<i>F. cylindricauda</i>	20	0.022371	-3.79997	-0.08501
<i>F. filiformis</i>	60	0.067114	-2.70136	-0.1813
<i>F. thornei</i>	20	0.022371	-3.79997	-0.08501
<i>Helicotylenchus tunisiensis</i>	20	0.022371	-3.79997	-0.08501
<i>H. filipjevi</i>	14	0.01566	-4.15665	-0.06509
<i>Merlinius brevidens</i>	40	0.044743	-3.10683	-0.13901
<i>P. alkani</i>	120	0.134228	-2.00821	-0.26956
<i>P. crenicauda</i>	60	0.067114	-2.70136	-0.1813
<i>S. cylindricaudatus</i>	20	0.022371	-3.79997	-0.08501
<i>Safianema lutonense</i>	20	0.022371	-3.79997	-0.08501
<i>Scutylenchus koreanus</i>	20	0.022371	-3.79997	-0.08501
Total	894	1	-53.4717	-2.51295
			H = 2.51295	
			$E_H = 0.798931$	

\*H: Shannon diversity index,  $E_H$ : evenness measure,  $p_i$ : proportion of species  $i$  within the entire community, and  $\ln$ : natural logarithm.



**Figure 2.** PCR products of the rDNA-ITS of the second-stage juveniles of 13 cereal CN (numbered from 1 to 13) populations showing an amplified fragment of 1200 bp. ddH<sub>2</sub>O was used as a negative control (C). Ha and Hl indicate the positive controls: Ha: *Heterodera avenae* (Accession number: KM199803). Hl: *Heterodera latipons* (Accession number: KY352335). M: DNA ladder. The codes correspond to those in Table 1.



**Figure 3.** Construction of the maximum-likelihood phylogenetic tree using IQ-TREE ITS sequences of the *Heterodera* populations. Bootstrap values are indicated at each node on the left, while posterior probabilities are displayed on the right.

some plant-parasitic genera identified in the studied areas were *Pratylenchoides*, *Helicotylenchus*, *Merlinius*, and *Heterodera*.

Dababat et al. (2019) and Kasapoğlu Uludamar et al. (2018) discovered that *Pratylenchoides sheri*, *Amplimerlinus vicia*, *Merlinus brevidens*, *Paratrophurus acristylus*, and *Paratrophurus striatus* were the most common PPNs in

southeast Anatolia, Türkiye. Additionally, *Scutylenechus quadrifer*, *Merlinius brevidens*, *H. latipons*, *Aphelenchus avenae*, and *P. thornei* were reported as the most frequent PPNs on cereal in Adiyaman Province, Türkiye (Öcal and Elekcioğlu, 2015). Moreover, Keçici et al. (2022) reported that genera *Helicotylenchus*, *Heterodera*, *Merlinius*, *Pratylenchoides*, *Pratylenchus*, *Trophurus*, *Paratrophurus*,

*Filenchus*, *Tylenchus*, *Scutylenchus*, *Amplimerlinius*, *Boleodorus*, and *Basiria* were detected in cereal growing areas in Sakarya Province, Türkiye.

The results herein demonstrated an important occurrence of CCN species *H. filipjevi* in various cereal-growing areas in Banaz, Karahallı, Ulubey, Sivaslı, and the Central districts of Uşak Province, Türkiye. The results revealed that approximately 32.5% of the sampled locations in Uşak Province were infested with *H. filipjevi*. In an extensive field survey in Türkiye, Toktay et al. (2015) reported that more than half of the sampled wheat fields were contaminated with *H. filipjevi*. Similarly, İmren et al. (2016) reported a high infestation rate of *H. filipjevi* (83%) in the wheat production areas of Bolu Province. In a study conducted by Keçici et al. (2022), it was found that 24% of wheat fields located in Sakarya Province, Türkiye, were infected by *H. filipjevi*.

The results of this study revealed that there is no noticeable variation in the populations of *H. filipjevi* based on morphological characteristics, confirming that measurements of cysts and second-stage juveniles differ. *H. filipjevi* is thoroughly related to *H. avenae*, and they share slight morphological variances that distinguish them (Handoo, 2002; Tanha Maafi et al., 2007; Subbotin et al., 2010). *H. filipjevi* cysts have minor bullae and a thin underbridge that is observable but not prominent.

The Shannon diversity index is used to assess the species diversity and relative abundance within a community, providing insights into the diversity of species existing (Shannon, 1948). In the case of nematodes in wheat and barley fields, the Shannon diversity index indicated a moderate level of diversity. This suggests that the presence of wheat and barley has an impact on the PPN community. Typically, undisturbed ecosystems not influenced by human activities tend to exhibit higher nematode richness in the soil. However, the moderate diversity identified in the current study could be ascribed to the practice of monoculture agriculture.

The phylogenetic relatedness of *H. filipjevi* populations was examined and compared to international sources. A slight level of within species genetic variation was detected among the *H. filipjevi* populations, leading to their clustering into two distinct groups in the phylogenetic tree. This clustering was supported by a modest bootstrap value and comparison with representative populations from GenBank (Figure 3). The *H. filipjevi* populations could be classed as a separate group, according to the molecular evidence. This finding aligns with previous studies by İmren et al. (2016) and Toumi et al. (2018), in which similar findings were observed, indicating restricted genetic diversity within *H. filipjevi* populations in the Mediterranean region of Türkiye. However, certain variations within the population of *H. filipjevi* indicate intraspecific polymorphism (Subbotin, 2015). The intraspecific diversity of *H. filipjevi* populations was also reported in the south Anatolian region of Türkiye in earlier studies (Dağlı et al. 2023).

The primary aim of this research was to define the ecological dissemination of main PPN genera in Uşak Province, focusing specifically on the CCN species. Furthermore, it is recommended that wide-ranging surveys be conducted in Uşak Province, along with comprehensive investigations into the pathotypes of *H. filipjevi*, and other PPNs involving collaboration with various local government branches. Based on the findings, the subsequent recommendations are proposed to local technicians and researchers: diversify the selection of wheat cultivars by including durum wheat in regions with high cyst populations, as durum wheat exhibits greater resistance compared to spring wheat; implement cultural practices, particularly the rotation of crops strategies; prioritize breeding programs aimed at developing high resistance germplasm potential against CCNs; and lastly, provide further education and training to technical personnel focusing on soil-borne disease research in the field.

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