Incidence of cereal cyst nematodes in the East Anatolia Region in Turkey

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Summary. Cereal cyst nematodes (*Heterodera* spp., CCN) are major economic plant-parasitic nematodes of wheat grown in the East Anatolian region of Turkey. Identification of CCN species is essential for choosing the right control strategy. The morphological and molecular characteristics of cyst nematodes (*Heterodera* spp.) were determined for specimens collected from wheat fields from different provinces of the East Anatolia region in Turkey. Fifty-six percent of wheat fields were infested with cereal cyst nematodes. Based on morphological characterisations and molecular techniques, two *Heterodera* species, *H. filipjevi* and *H. latipons*, were identified. *Heterodera filipjevi* was the dominant species in the wheat fields of Elazig, Malatya, Sivas, Erzurum, Erzincan, Igdir and Kars provinces, while *H. latipons* was found only in three provinces (Erzincan, Elazig and Malatya). This is the first detection of *H. latipons* in the East Anatolia region. Genetic dissimilarity was higher within *H. filipjevi* populations than within *H. latipons* populations. While intraspecific polymorphism was not observed within *H. latipons*, it was recorded in *H. filipjevi* populations. Our results showed a clear separation of the two cyst nematode species using both morphological and molecular tests, and confirmed the link between genetic and morphological traits.

Key words: *Heterodera* spp., ITS region, nematode, taxonomy, wheat.

Wheat is the most important and strategic cereal crop in the world, ranking second in total production as a cereal crop behind rice (Nicol *et al*., 2003). Wheat production has steadily increased in Turkey during the last 35 years and recently reached production of 22 million tons/year from 9 million hectares of land (Anonymous, 2013). East Anatolian region of Turkey produces 1.2 million tons of wheat that is 7.1% of total wheat production in the country (Anonymous, 2013).

Plant-parasitic nematodes are distributed worldwide in wheat cultivation areas and cause annual yield loss of around 7% (Sasser, 1987). Among the plant-parasitic nematodes, cereal cyst nematodes (CCN) are widespread worldwide and incur significant economic yield losses in many countries, especially under rainfed conditions (Nicol *et al*., 2003).

The genus *Heterodera* includes 12 species that adversely affect the roots of cereals and grasses. These species of (CCN) are designated as the *Heterodera avenae* group (Rivoal & Cook, 1993). Among these, three species (*H. avenae*, *H. filipjevi* and *H. latipons*) are the most important cyst nematodes attacking wheat, causing economic yield loss worldwide (Handoo 2002; Subbotin *et al*., 2003, 2010). These three species have been reported from different parts of Turkey. *Heterodera avenae* was recorded for the first time in 1974 at Erzurum province (Yüksel, 1973) and is now reported in many cereal-producing regions such as South Anatolia and the Eastern Mediterranean regions (İmren *et al.*, 2012, 2015). High populations of

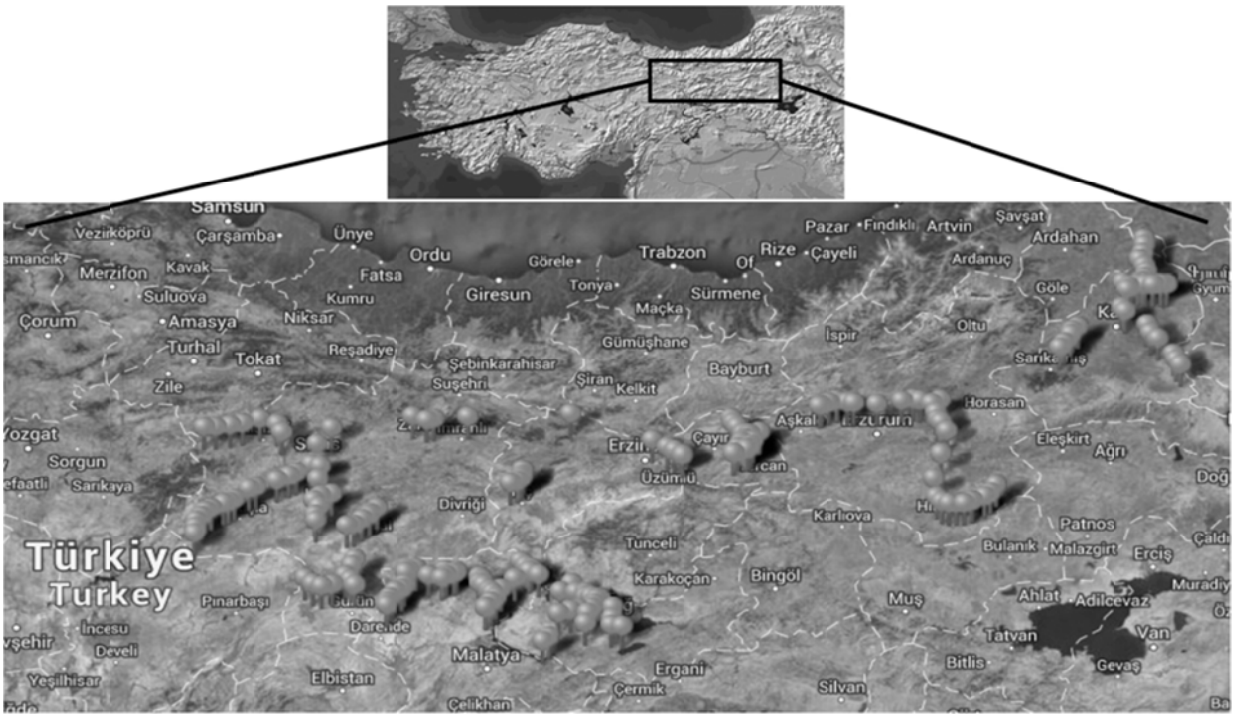

Fig. 1. A map of *Heterodera* populations showing locations in the seven provinces from where the samples were collected.

H. avenae in commercial fields reduced yields of spring wheat up to 27% and occasionally destroyed the second crop of spring wheat in Eastern Mediterranean region of Turkey (İmren & Elekçioğlu, 2014). *Heterodera filipjevi* was reported for the first time from the Central Anatolian region of Turkey (Rumpenhorst *et al*., 1996), while *H. latipons* was observed in different provinces in the south part of Turkey (Dababat *et al*., 2015; İmren *et al*., 2015). There is no study of the diversity and occurrence of CCN in East Anatolian region.

Heterodera filipjevi is closely related to *H. avenae* and *H. latipons*; only minor morphological differences differentiate them (Handoo, 2002; Subbotin *et al*., 2003). Identification of these species based on morphology is a time consuming process and demands great skill and training by the observer. Sequences analysis based on the internal transcribed spacer (ITS) region of the ribosomal (r) DNA repeat unit has provided a reliable tool for quick and precise identification of cyst nematode species and subspecies (Subbotin *et al*., 1999, 2003; Tanha Maafi *et al*., 2003; Madani *et al*., 2004). Comparative analysis of the ITS-rDNA of unknown nematode species sequences with those of known species published or deposited in GenBank facilitates rapid identification of most cyst nematode species. The information from molecular tests strengthens the microscopic identifications based on the analysis of differences in morphology (Handoo, 2002; Subbotin *et al*., 2003), but morphological identification is also needed to support the identification from molecular tests. Genetic differences among species determined from molecular tests generally corresponded with specific morphological characters in the graminaceous cyst nematode complex (Yan & Smiley, 2010).

İmren *et al*. (2015) have reported on occurrence and diversity of CCN species, based on morphological and molecular identification of populations collected from a limited area of the Mediterranean region of Turkey. This area has a warm climate and is mostly coastal, whereas our study included areas from East Anatolian region that has a continental climate and a high altitude far from the Mediterranean region. Therefore, the climate and ecological conditions of the Mediterranean region are very different from those of the Eastern Anatolian region. In addition, our study includes information on population densities and detailed morphological measurements of nematodes. Yüksel (1973) identified *H. avenae* in the East Anatolian region, but it most probably belonged to another species of *H. avenae* group since *H. filipjevi* was known as *H. avenae* before the 1980s (Madzhidov, 1981).

The objectives of the present study were: *i*) to investigate population densities and distribution of

cereal cyst nematodes (*Heterodera* spp.) collected from wheat fields in the East Anatolia Region of Turkey; *ii*) to distinguish the *Heterodera* spp. by sequences of the ITS-rDNA and cyst morphological characters; and *iii*) to examine possible intraspecific variation within *Heterodera* spp. populations based on the ITS region of their rDNA.

MATERIALS AND METHODS

Nematode samples. A survey of wheat growing areas in East Anatolia Region was conducted during July and August of 2013, just after harvest (Fig. 1). Using a spade, soil samples were collected at 15-20 cm deep. From each field, a subtotal of 2 kg soil was taken containing fine roots and rhizosphere soil of wheat plants. Samples were sent directly to Laboratory of Nematology, Nigde University for further processing. Cysts were extracted from soil using the Seinhorst method (Seinhorst, 1964). Cysts were collected using 20× magnification of a stereomicroscope and were sterilised with 0.01% NaOCl for 10 min followed by several rinses with sterilised water. Cleaned cysts were stored at 4°C before molecular and morphological identification.

Morphological and morphometric characterisation. The morphological features and morphometric measurements of the vulval region of adult and second-stage juveniles (J2) were used for diagnosis after fixation in glycerin (Seinhorst, 1959).

Species of *Heterodera* were differentiated from each other by morphological and morphometric features of J2 (body length, stylet length, tail length, hyaline tail length, stylet knob shape and tail terminus shape) as described previously by Subbotin *et al*. (1999). Other morphological and morphometric features such as the b and c ratios of J2, the length of cysts excluding the neck (L'), the width of cysts (B), the L'/B ratio, the presence, size and shape of bullae inside the vulval cone and cyst color were also determined (Subbotin *et al*., 1996, 2003; Handoo, 2002).

Fifteen mature cysts from *Heterodera latipons* and twenty-five cysts from *H. filipjevi* vulval cones were mounted in glycerin, and examined. Identifications were made based on underbridge structure, shape of semi-fenestra in the fenestral area and bullae. Cyst characters measured were the fenestral length, semi-fenestral width, vulval bridge width and vulval slit length (Handoo, 2002; Subbotin *et al*., 2010).

Molecular identification and phylogenetic analysis. DNA extraction. Forty-four *Heterodera* populations from different provinces were selected for DNA extraction. For each population, one cyst was transferred into 45 µl of double distilled water (ddH_2O) in an Eppendorf tube and crushed using a micro homogeniser. After centrifugation of the crushed cyst content, 40 µl of the mix was transferred into a PCR tube (0.2 ml). Fifty µl of worm lysis buffer (WLB) and 10 µl of Proteinase K (20 mg ml^{-1}) were added to each tube (Holterman *et al*., 2006); the tubes were frozen at –80°C for at least 10 min. Then the tubes were incubated at 65°C for 1 h and 95°C for 10 min consecutively in a thermocycler. After incubation, the tubes were centrifuged for 1 min at 16400 g and kept at –20°C until use (Tanha Maafi *et al*., 2003).

PCR amplification. For molecular identification, the ITS-rDNA region was amplified. One ng of DNA was added to the PCR reaction mixture containing 23 µl ddH2O, 25 µl 2× DreamTaq PCR Master Mix (Thermo Scientific, Belgium) and 1 µM of each forward primer (5'-CG TAACAAGGTAGCTGTAG-3') and reverse primer (5'-TCCTCCGCTAAATGATATG-3') (Ferris *et al*., 1993). The DNA thermal cycler program consisted of 5 min at 95°C; 40 cycles of 94°C for 30 s, 45°C for 45 s and 72°C for 45 s; followed by a final elongation step of 8 min at 72°C.

Following PCR amplification, 5 µl of each PCR product was mixed with 1 µl of 6× loading buffer (Fermentas Life Sciences, Germany) and loaded on a 1.5% standard TAE buffered agarose gel. After electrophoresis (100 V for 40 min) the gel was stained with ethidium bromide (0.1 µg ml^{-1}) for 15 min, visualised and photographed under UV-light. The remaining PCR product was stored at –20°C.

Sequencing and phylogenetic analysis. Ninety µl of PCR product was loaded on a 1% agarose gel for electrophoresis (100 V, 40 min). The purification was carried out as described in the manufacturer's instructions (Wizard® SV Gel and PCR Clean-Up System Kit, Promega). Purified PCR-product from each sample was sequenced (Macrogen, Amsterdam, The Netherlands) in both directions to obtain overlapping sequences of both DNA strand. Species were identified using the BLAST tool on the NCBI-website (www.ncbi.com).

For phylogenetic analysis *H. latipons* (Syria, JX024179), *H. hordecalis* (Estonia, AY692356), *H. filipjevi* (Iran, AY148404), *H. ustinovi* (Belgium, AY148407), *H. pratensis* (Russia, AY148351, Germany, AY148384), *H. australis* (Australia, AY148394, AY148396), *H. mani* (Germany, AY148377, AY148378), *H. avenae* (China, EU616697, HM560755), *H. aucklandica* (United Kingdom, AY148380), *H. ciceri* (Syria, AY045758), *H. schachtii* (Morocco, AY166346)

and *H. goettingiana* (Iran, AF498374) were added to our obtained sequences. *Cryphodera brinkmani* (Japan, AF274418) and *Meloidodera alni* (Belgium, AF274419) were also included in the phylogenetic analysis.

All sequences were aligned with ClustalX 1.64 with default options. The evolutionary history was inferred using the Maximum Likelihood method based on the General Time Reversible model (Nei & Kumar, 2008). The tree with the highest log likelihood (–7010,1904) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+*G*, parameter = 1,1014)). The analysis involved 65 nucleotide sequences. There were a total of 1232 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 (Tamura *et al*., 2013).

RESULTS

Field infestation. The survey of cyst nematodes showed that 56% of wheat fields in the East Anatolia Region of Turkey were infested with CCN. Based on morphological characters and molecular techniques, two species, *H. filipjevi* and *H. latipons*, were identified. *Heterodera filipjevi* was the dominant species in the wheat fields of Elazig, Malatya, Sivas, Erzurum, Erzincan, Igdir and Kars provinces, while *H. latipons* had a very limited distribution, being found in only three provinces (Elazig, Malatya and Erzincan). *Heterodera latipons* was detected for the first time in the East Anatolia Region.

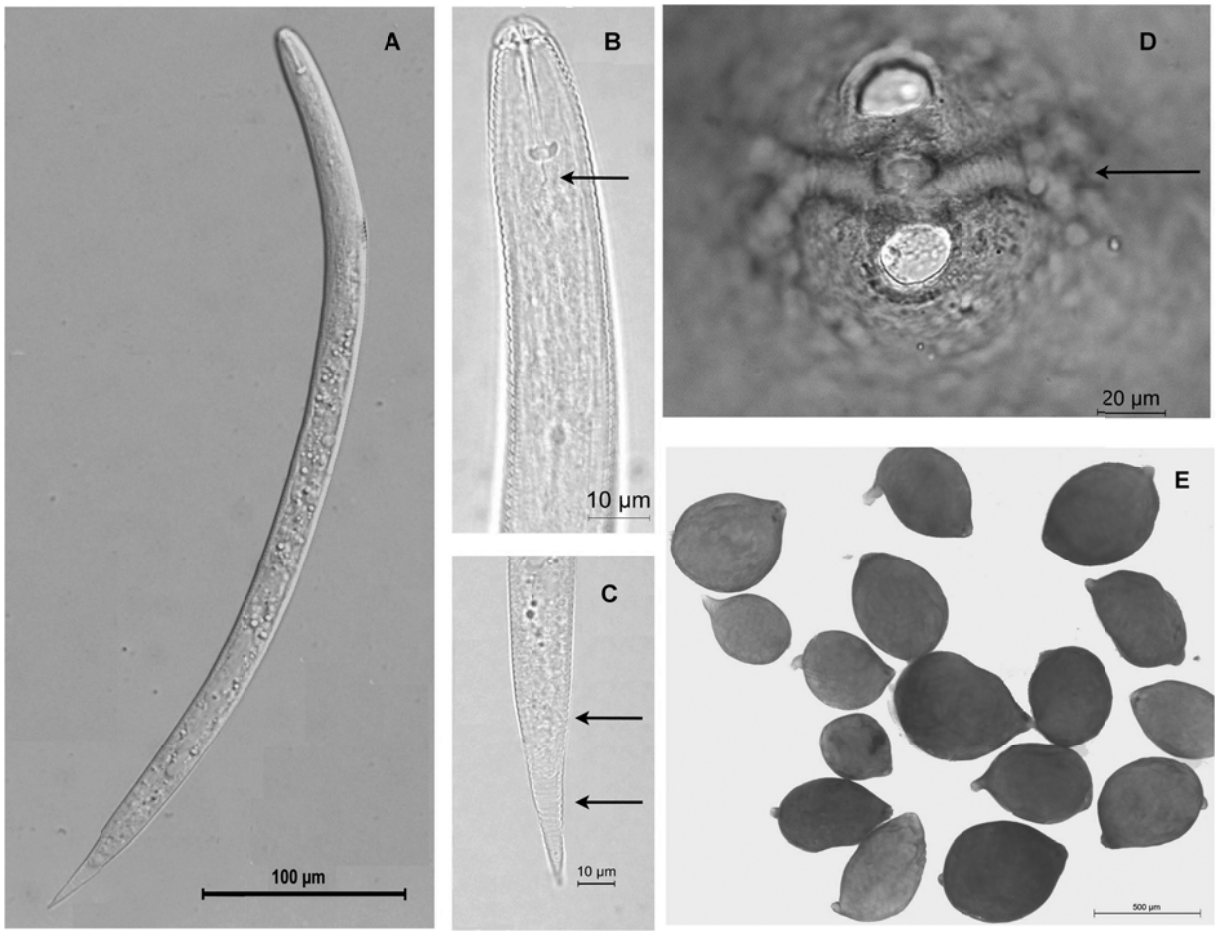

Fig. 2. Light micrographs of the second-stage juveniles of *Heterodera latipons* from Erzincan in East Anatolia Turkey. A: the second stage juvenile; B: stylet & DGO; C: anus and hyaline tail tip; **D**: female fenestra, strong underbridge and bifurcated vulval slit; E: light brown cysts.

Table 1. Occurrence of the cyst nematodes *Heterodera filipjevi* and *H. latipons* in wheat fields surveyed East Anatolian Region in Turkey

Sample no.	Provinces	Number of fields surveyed	Number of infested fields	Infestation rate (%)	Species	Infestation level (average number of cysts per 100 g of soil)
1	Elazig	30	21	70	*H. filipjevi* + *H. latipons*	10
2	Malatya	35	21	60	*H. filipjevi* + *H. latipons*	9
3	Erzurum	40	18	45	*H. filipjevi*	4
4	Erzincan	40	20	50	*H. filipjevi* + *H. latipons*	4
5	Sivas	70	42	60	*H. filipjevi*.	8
6	Kars	40	15	47,5	*H. filipjevi*	3
7	Igdir	25	16	64	*H. filipjevi*	2
Total		280	157	56		

Table 2. Morphological and morphometrical characteristics of cysts of *Heterodera filipjevi* and *H. latipons*

Species character	*Heterodera filipjevi*		*Heterodera latipons*	
	25		15	
	Mean ± S.E.	Range	Mean ± S.E.	Range
Length (excl. neck)	641.1 ± 18.4	490-852	601.6 ± 12.9	510-672
Width	449.1 ± 12.2	309-620	442.3 ± 13.4	364-530
Length/width	1.43 ± 0.02	1.18-1.68	1.37 ± 0.03	1.18-1.57
Fenestral length	51.81 ± 0.74	40-61	62.74 ± 1.17	59-70
Fenestral width	25.85 ± 0.56	19-40	19.89 ± 0.64	17-22
Vulval bridge width	13.37 ± 0.28	11.0-17.5	28.04 ± 4.77	18-35
Underbridge length	80.90 ± 5.50	71.1-94.8	94.50 ± 8.70	87-99
Vulval slit length	9.43 ± 1.02	7.5-13.1	7.70 ± 1.50	5.2-11.0

Cyst nematodes were detected in almost all the fields with monoculture of wheat in Elazig, Malatya, Erzurum, Erzincan, Sivas, Kars and Igdir provinces. Fields in which cyst nematodes were not detected were generally rotated with other crops. High incidence levels were recorded, *e.g.*, 21 of 30 fields were infested in Elazig. The lowest incidence of infested fields was in Kars with 19 of 40 fields infested.

The average number of cysts per 100 g of soil was estimated based on the total samples per province. The greatest numbers were 10 and 9 cysts in Elazig and Malatya provinces, respectively. Low levels of infestation (2 and 3) were recorded in Kars and Sivas as shown in Table 1.

Morphological and morphometric characterisation of *Heterodera* populations. Cysts of *H. filipjevi* were bigger than those of *H. latipons* and were yellow to light-brown in colour. *Heterodera filipjevi* was distinguished from *H. latipons* by its prominent bullae and a clear underbridge, which was very narrow in the centre and located close to the vulval bridge. Cysts of *H. latipons* had no bullaea with a strong underbridge, and vulval slit prominent and bifurcated in both sides. They differed from *H. filipjevi* by a strong and deep underbridge without bullae and greater fenestral length (59-70 µm *vs* 40-61 µm), a larger vulval bridge width (18-35 µm *vs* 8-13 µm), longer vulval slit length (5.3-11 µm *vs* 7.5-13.1), tail length (44.1-53.8 µm *vs* 41.7-64), hyaline part of tail length (24-30 *vs* 25-39 µm), ratio B-J2 (hyaline part of tail length/stylet length) (1.2-1.4 *vs* 1.2-1.8) and ratio C-J2 (total length/tail length) (8.4-10.7 *vs* 8.2-11.9) (Tables 2 & 3).

Heterodera filipjevi and *H. latipons* had cysts of different sizes (490-852 µm *vs* 510-672), were brown and dark in colour and with a round and thin vulval cone top respectively (Figs 2 & 3).

Molecular identification and phylogenetic analysis. PCR amplification of the ITS regions of rDNA of all 45 *Heterodera* populations and species tested produced a single fragment of approximately 1.2 kb. Forty populations from Elazig, Malatya, Sivas, Kars, Erzurum, Erzincan and Igdir were identified as *H. filipjevi*; 4 populations from Elazig,

Malatya and Erzincan were identified as *H. latipons* (Table 4).

Based on the ITS-rDNA sequences, *Heterodera filipjevi* populations showed some intraspecific polymorphism. All *H. filipjevi* populations grouped together and were supported by a bootstrap value of 96%. *Heterodera latipons* populations also showed little intraspecific polymorphism. The *H. latipons* populations analysed here grouped with *H. latipons* populations from Syria (JX024179) (Fig. 4).

DISCUSSION

This survey of CCN from the East Anatolian Region of Turkey showed the presence of both *H. latipons* and *H. filipjevi* in wheat cultivating areas. Both nematodes are reported for the first time from this region. *Heterodera filipjevi* is widely distributed in Elazig, Malatya, Sivas, Kars, Erzurum, Erzincan and Igdir provinces but *H. latipons* was found only in Malatya, Elazig and Erzincan. Finding *H. latipons* in these areas was unexpected, as the majority of fields were in high-elevated land in Turkey and this species is generally found in fields on the Mediterranean coast or having a Mediterranean climate (İmren *et al*., 2015). *Heterodera filipjevi* has been reported in Central and South of Turkey with high altitude wheats fields (Dababat *et al*., 2015).

Our results demonstrate that the highest levels of infestation were found in the Sivas, Elazig and Malatya provinces, where the total number of cysts per 100 g of soil reached 8, 9 and 10, respectively. By contrast, the lowest levels of infestation were found in the Igdir and Kars provinces, where the total numbers of cysts per 100 g of soil recorded were 2 and 3, respectively.

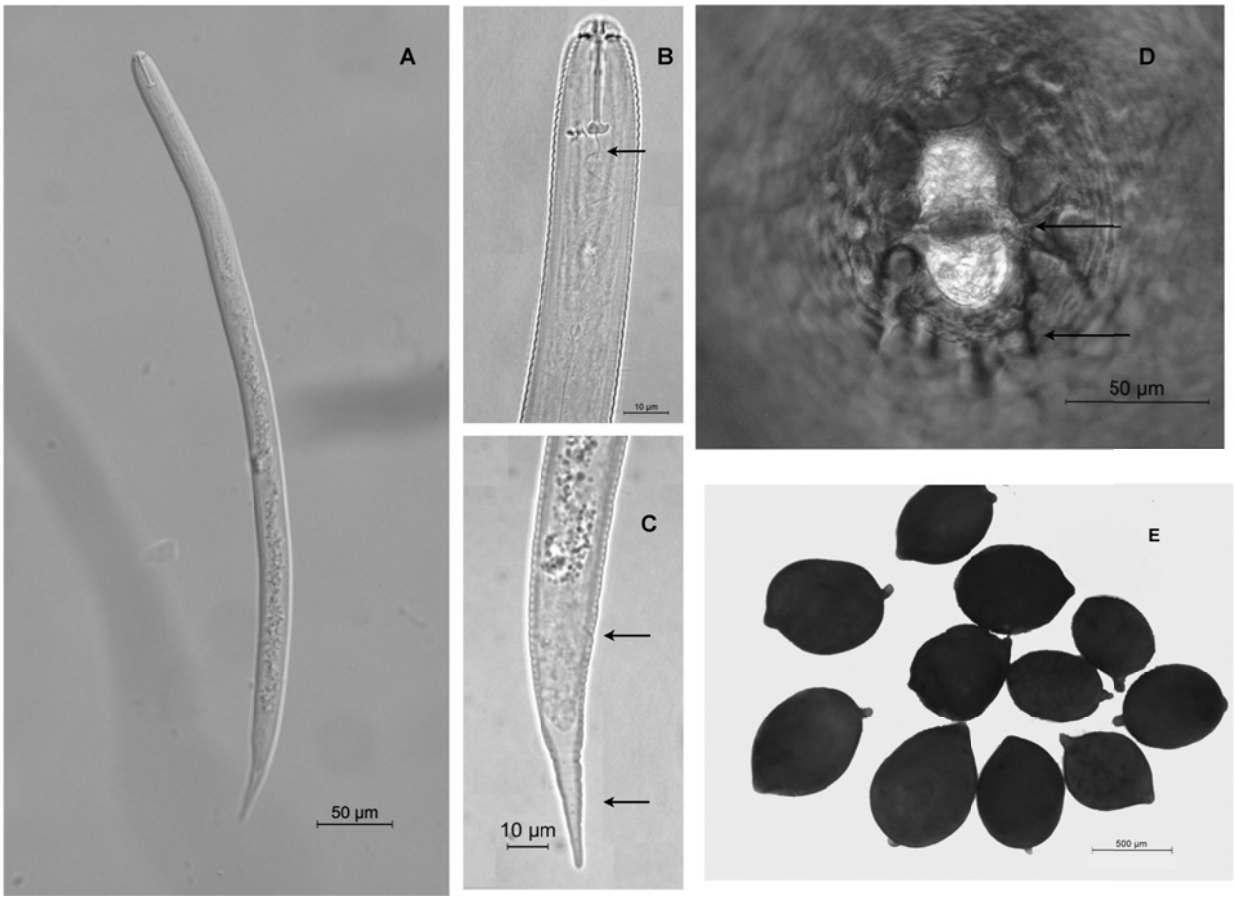

Fig. 3. Light micrographs of *Heterodera filipjevi* from Erzurum in East Anatolia Turkey. A: the second stage juvenile; B: stylet & DGO; C: anus and hyaline tail tip; D: female fenestra, narrow vulval slit & heavy bullae; E: dark brown cysts.

Table 3. Morphological and morphometrical characteristics of second-stage juveniles of *Heterodera filipjevi* and *H. latipons*

Species character	*Heterodera filipjevi*		*Heterodera latipons*	
	25		15	
	Mean ± S.E.	Range	Mean ± S.E.	Range
L	526.6 ± 6.20	477-583	459.6 ± 5.45	424-489
a	25.30 ± 0.59	17.0-29.7	18.95 ± 2.20	16.5-24.17
b	4.39 ± 0.05	3.8-4.7	3.75 ± 0.07	3.4-4.2
c	9.50 ± 0.20	8.2-11.9	9.45 ± 0.15	8.4-10.7
c’	3.71 ± 0.09	3.2-4.3	3.28 ± 0.10	2.4-3.6
Stylet length	22.39 ± 0.30	20-25	21.92 ± 0.31	19.4-24.0
Labial region height	4.04 ± 0.15	3.0-5.4	4.16 ± 0.16	2.74-5.05
Labial region diam.	9.38 ± 0.20	7.9-11.0	9.13 ± 0.14	8.7-10.0
DGO	4.82 ± 0.14	3.5-5.7	4.87 ± 0.19	3.25-6.0
Distance anterior end to median bulb (mb)	73.21 ± 0.98	71.2-81.5	66.85 ± 0.87	62.5-71.0
Excretory pore	103.8 ± 0.86	98-109	102.0 ± 1.27	97-107
Body diameter at mid-body	21.05 ± 0.54	18.4-22.8	20.97 ± 0.50	19.3-24.0
Body diameter at anus	15.19 ± 0.37	12-21	14.96 ± 0.46	13.0-19.3
Tail length	55.85 ± 1.08	41.7-64.0	48.75 ± 0.79	44.1-53.8
Hyalin region (H)	33.85 ± 0.81	25-39	27.33 ± 0.47	24-30
H/stylet length	1.52 ± 0.04	1.20-1.84	1.25 ± 0.02	1.2-1.4
L/mb	6.85 ± 0.08	6.2-7.5	5.98 ± 0.07	5.5-6.2

Measurements are in µm.

In these areas, barley and wheat are continuously cultivated on the same land as monoculture. Although, the incidence and impact of cereal cyst nematode depends on the type of host and soil, nematode pathotype and ecotype and climatic conditions of the area (Rivoal & Cook, 1993), growing cereals as a monoculture has resulted in gradually increasing populations of CCN that influence the amount of yield loss in infested fields. It appears that existence of environmental suitable conditions for the completion of the life cycle of these nematodes can be an important factor for posing a threat to cereal production in Sivas, Elazig and Malatya provinces.

To maintain the population densities of these species of nematodes below damaging levels, appropriate management measures, such as rotational schemes and the use of resistant varieties, are necessary. A number of resistance sources for breeding purposes have been found in domestic cereals and their wild relatives, acting against both *Heterodera* species (Dababat *et al*., 2015). However, the efficiency of the genes involved differs according to the virulence of the populations (pathotypes) of *Heterodera* species, although preliminary studies indicate that several resistance genes of barley or wheat, are to some extent also resistant to populations of *H. filipjevi* or *H. latipons* originating from different sites in North Africa, Europe and Asia (Bekal *et al*., 1997; Mokabli *et al*., 2002). Further investigations are necessary to identify suitable resistance sources to be used in cereal breeding programmes.

Correlations between morphological characters of cysts and juveniles allow differentiation of *H. latipons* and *H. filipjevi* of the *Heterodera avenae* group. For example, useful characters for distinguishing these species are the presence of distinct underbridge in the vulval cone and small bullae situated below the fenestrae of cysts of *H. filipjevi* (Subbotin *et al*., 2003). Morphometric characters, such as the hyaline part of the tail of the J2 and the fenestral length of cysts, are also important characters (Madzhidov, 1981). Differentiation of *H. latipons* could be readily achieved using either the fenestral length or the vulval bridge width of the cyst and either the lengths of the tail or hyaline part of the tail of the J2, in addition to the underbridge and bullae presence in the vulval cone (Wouts *et al.*, 1995). In our study, the distinguishing of specimens based on the development of bullae and presence of an underbridge was successful at low magnification, including populations with mixed species (the Elazig, Malatya and Erzincan populations).

Table 4. Locations of *Heterodera* species in East Anatolia Region of Turkey

No.	Code	Province	Location	Species	Accession no.
1	256	Malatya	Malatya Yazihan Yolu	*H. filipjevi*	KP708712
2	257	Malatya	Yazihan Kirec Ocagi	*H. filipjevi*	KP708745
3	261	Malatya	Fethiye	*H.latipons*	KP708713
4	264	Malatya	Yazihan	*H. filipjevi*	KP708714
5	268	Malatya	Arapkir Yolu	*H. filipjevi*	KP708746
6	275	Malatya	Arapkir Yolu	*H. filipjevi*	KP708715
7	280	Malatya	Pötürge Yolu	*H. filipjevi*	KP708716
8	284	Malatya	Malatya Dogansehir Yolu	*H. filipjevi*	KP708717
9	288	Elazig	Cevizkemer Yolu	*H. filipjevi*	KP708718
10	291	Elazig	Elazig Merkez	*H.latipons*	KP708719
11	293	Elazig	Elazig Sahinkaya Köyü	*H.latipons*	KP708720
12	295	Elazig	Yazikonak ihl	*H. filipjevi*	KP708747
13	296	Elazig	Yazikonak	*H. filipjevi*	KP708721
14	297	Elazig	Molla Kendi	*H. filipjevi*	KP708722
15	300	Elazig	Güntas	*H. filipjevi*	KP708748
16	301	Elazig	Yukaribagi	*H. filipjevi*	KP708723
17	302	Elazig	Degirmen önü	*H. filipjevi*	KP708749
18	303	Elazig	Elazig Yolu	*H. filipjevi*	KP708750
19	304	Elazig	Elazig Yolu	*H. filipjevi*	KP708724
20	325	erzurum	Oltu Ardahan Yolu	*H. filipjevi*	KP708725
21	354	Igdir	Aralik Yolu	*H. filipjevi*	KP708726
22	364	Igdir	Kücük ova yolu	*H. filipjevi*	KP708727
23	371	Erzincan	Erzincan Asagi Carsi	*H. filipjevi*	KP708728
24	378	Erzincan	Erzincan	*H. filipjevi*	KP708728
25	420	Erzurum	Cinsuyu köyü	*H. filipjevi*	KP708751
26	421	Erzurum	Cinsuyu Anayola 2 km	*H. filipjevi*	KP708735
27	425	Erzurum	Erzurum-Askale Eski Yol	*H. filipjevi*	KP708736
28	430	Erzincan	Mercan Otluk Beli Yolu	*H.latipons*	KP708737
29	452	Sivas	İncetas Yolu	*H. filipjevi*	KP708738
30	457	Sivas	Yildizeli Sarkisla Yolu	*H. filipjevi*	KP708739
31	463	Sivas	Yilanhöyük Köyü	*H. filipjevi*	KP708740
32	466	Sivas	Gürün Kangal Yolu	*H. filipjevi*	KP708752
33	472	Sivas	Kangal Kus Kayasi Köyü	*H. filipjevi*	KP708730
34	475	Sivas	Sutasi Köyü	*H. filipjevi*	KP708753
35	480	Sivas	Sivas Kangal Yolu	*H. filipjevi*	KP708741
36	482	Sivas	Ulas Kangal Yolu	*H. filipjevi*	KP708731
37	484	Sivas	Ulas Kangal Yolu	*H. filipjevi*	KP708742
38	488	Sivas	Kabak Cetinkaya	*H. filipjevi*	KP708743
39	490	Sivas	Kangal Divrigi Yolu	*H. filipjevi*	KP708732
40	495	Erzurum	Erzincan İlic Boyalik köyü	*H. filipjevi*	KP708754
41	496	Erzurum	Erzurum Tekman	*H. filipjevi*	KP708744
42	514	Igdir	Yagli Girisi	*H. filipjevi*	KP708755
43	521	Kars	Oluklu köyü giris	*H. filipjevi*	KP708733
44	550	Kars	Cildir Yolu	*H. filipjevi*	KP708734

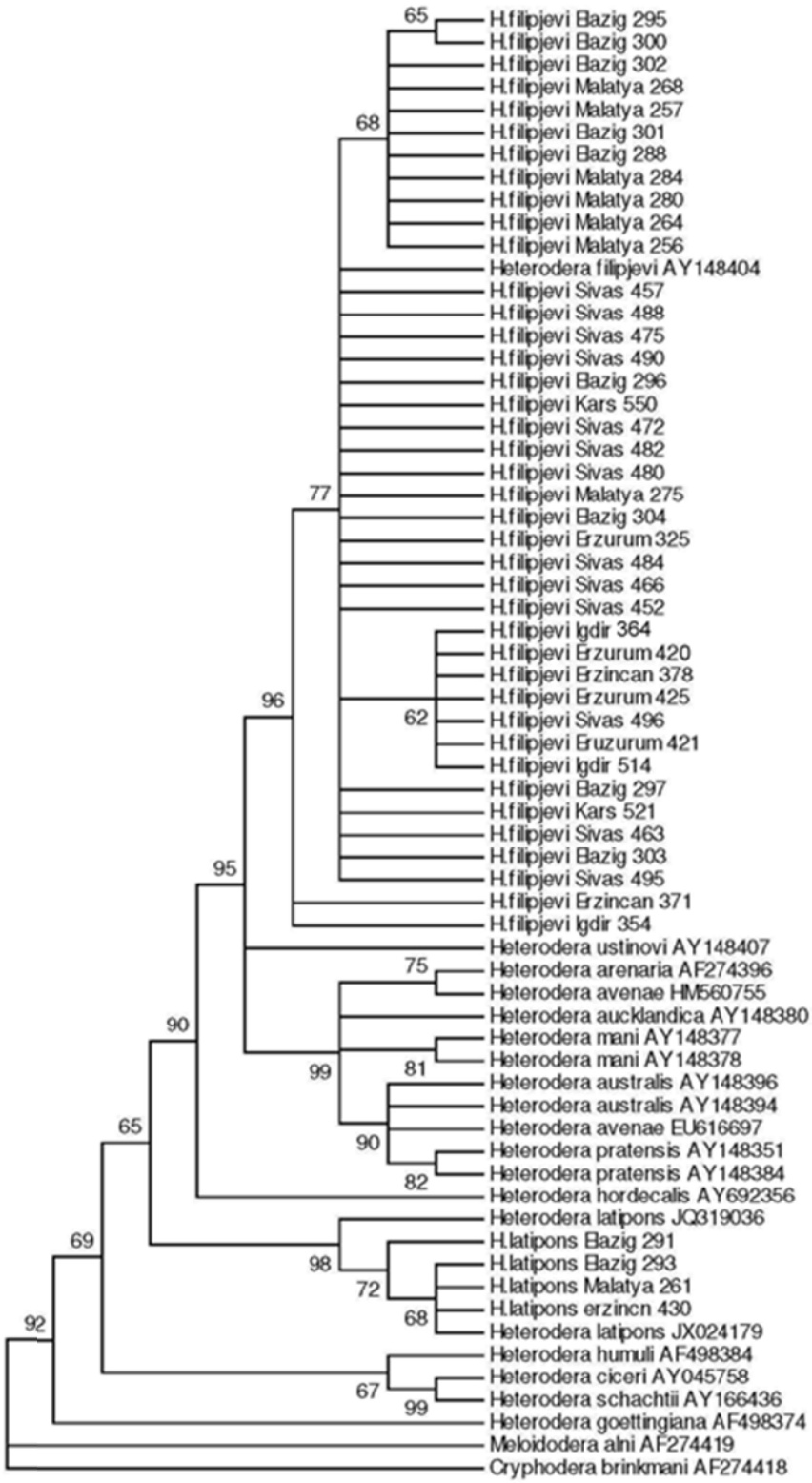

Fig. 4. Phylogenetic tree of *Heterodera* spp. populations from East Anatolian region of Turkey.

Our results showed an intraspecific polymorphism between populations of *H. filipjevi*. Similarly, Subbotin *et al*. (2003) revealed intraspecific polymorphism between *H. filipjevi* populations. However, Bekal *et al*. (1997) did not detect any genetic variation among different populations of this species. Also, İmren *et al*. (2015) did not detect any genetic variation among *H. filipjevi* populations in Mediterranean region of Turkey. Based on our results, differences in ITS sequences were not observed between *H. latipons* populations and were found to be close to the Syrian population of *H. latipons* with bootstrap value 68%. Similar results were obtained by İmren *et al*. (2015), who showed intraspecific differentiation between populations in *H. latipons* in the Mediterranean region of Turkey between Moroccan population and Syrian population of *H. latipons*. Madani *et al*. (2004) and Rivoal *et al*. (2003) showed an intraspecific variation between populations in *H. latipons*. However, in this study, we did not find any intraspecific variation in our Syrian population of *H. latipons*.

The sequences of the ITS region of cyst-forming nematode species is necessary in addition to morphological traits for accurate identification of *Heterodera* spp. attacking crops, especially when estimating pathogenic characterisations and searching for sources of host plant resistance. The present study has indicated there was no polymorphism in the populations of *H. filipjevi* and *H. latipons* concerning molecular parameters.

Variation in the cultivated wheat varieties and alternations in agro-ecosystems could cause the development of new races and resistance breaking populations of CCN (Abdollahi, 2008). Recent work on physiological variation using international differentials revealed the occurrence of two biotypes in the East Mediterranean region of Turkey (İmren *et al*., 2013; Toktay *et al*., 2013). *Heterodera filipjevi* belonged to Ha33 *H. aveae* Ha21 pathotype in Turkey (İmren *et al*., 2013; Toktay *et al*., 2013). There is no report about *H. latipons* pathotype in Turkey.

Further investigations are necessary to identify the pathotypes of the *H. filipjevi* and *H. latipons* populations of the East Anatolian Region of Turkey, as well as suitable resistance sources to be used in cereal breeding programmes.

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H. Toktay, M. İmren, A. Öcal, L. Waeyenberge, N. Viaene and A. Dababat. Встречаемость цистообразующих нематод злаков в регионе Восточной Анатолии, Турция.

Резюме. Цистообразующие нематоды злаков (*Heterodera* spp., ЦОН) – причина основных потерь урожая пшеницы в регионе Восточной Анатолии в Турции. Определение видов ЦОН представляет собой ключевой фактор для выбора правильной стратегии контроля этого вредителя. Определены морфологические и молекулярные особенности цистоообразующих нематод рода *Heterodera*, собранных на пшеничных полях в различных провинциях Восточной Анатолии. Показано, что 56% полей региона поражено этими нематодами. Основываясь на морфологических и молекулярных признаках, выявлено доминирование двух видов *Heterodera – H. filipjevi* и *H. latipons*. *Heterodera filipjevi* был доминантным видом на пшеничных полях в провинциях Элязыг, Малатья, Сивас, Эрзурум, Эрзинджан, Ыгдыр и Карс, тогда как вид *H. latipons* был выявлен лишь в трех провинциях (Эрзинджан, Элязыг и Малатья). Это первое сообщение о присутствии *H. latipons* в регионе Восточной Анатолии. Уровень генетического разнообразия популяций был выше у *H. filipjevi*, чем у *H. latipons*. Внутривидовой полиморфизм был выявлен у *H. filipjevi*, но не у *H. latipons*. Наши результаты показывают наличие четких морфологических и молекулярных различий между двумя видами. Подтверждено соответствие между морфологическими и молекулярными признаками этих видов.