RESEARCH/ INVESTIGACIÓN

DISTRIBUTION AND DIVERSITY OF CYST NEMATODE (NEMATODA: HETERODERIDAE) POPULATIONS IN THE REPUBLIC OF AZERBAIJAN, AND THEIR MOLECULAR CHARACTERIZATION USING ITS-RDNA ANALYSIS

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ABSTRACT

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Cereal cyst nematodes (*Heterodera* spp.) are a significant threat to global cereal production systems and choosing the correct management strategy requires knowledge and identification of various species. We conducted a survey across the main cereal-growing regions of the Republic of Azerbaijan in 2017. Cyst-forming nematodes were detected in 34 samples (44.7%), of which 28 were identified as *H. filipjevi* or *H. avenae* using internal transcribed spacer (ITS)-rDNA sequencing. Six populations were unidentifiable to species level and were recorded as belonging to the *H. avenae* group. *H. filipjevi* was the dominant species, found in 19 samples (25%) from the provinces of Qobustan, İsmailli, Oguz, Sheki, Barda, and Kurdamir. *H. avenae* was detected in 9 samples (11.8%) across the provinces of İsmailli, Oguz, and Sheki. ITS-rDNA phylogenetic analyses showed that populations of *H. filipjevi* clustered in one group with two subgroups, all supported by high bootstrap values. Populations of *H. avenae* also clustered in one group with two subgroups. Genetic dissimilarities were higher within populations of *H. filipjevi* when compared to populations of *H. avenae*. The density of many of these cyst populations approached or exceeded the maximum threshold level for economic losses. This is the first report on *H. filipjevi* and *H. avenae* in Azerbaijan. The knowledge of cereal cyst nematode presence is extremely important for Azerbaijan's agricultural industry when assessing the occurrence and distribution of soilborne diseases. Management measures to control cereal cyst nematodes should be directed towards breeding for resistant germplasm, crop rotation, and implementing other management practices.

Key words: cereal cyst nematodes, morphology, phylogenetic, sequence

RESUMEN

Dababat, A. A., H. Muminjanov, G. Erginbas-Orakci, G. Ahmadova Fakhraddin, L. Waeyenberge, Ş. Yildiz, N. Duman, y M. Imren. 2019. La distribución y la división de nematodos de quistes (Nematoda: Heteroderıdae) en las poblaciones de la República de Azerbaiyán, y su caracterización molecular utilizando análisis de rDNA. Nematropica 49:18-30.

Los nematodos de quistes de cereales (*Heterodera* spp.) son una amenaza importante para los sistemas de producción de cereales a nivel mundial, y elegir la estrategia de manejo correcta requiere el conocimiento y la identificación de varias especies. Realizamos una encuesta en las principales regiones productoras de cereales de la República de Azerbaiyán en 2017. Se detectaron nematodos formadores de quistes en 34 muestras (44,7%), de las cuales 28 se identificaron como *H. filipjevi* o *H. avenae* utilizando un espaciador transcrito interno (ITS) -rDNA secuenciación. Seis poblaciones no fueron identificables a nivel de especie y se registraron como pertenecientes al grupo *H. avenae*. *H. filipjevi* fue la especie dominante, encontrada en 19 muestras (25%) de las provincias de Qobustan, İsmailli, Oguz, Sheki, Barda y Kurdamir. Se detectó *H. avenae* en 9 muestras (11.8%) en las provincias de İsmailli, Oguz y Sheki. Los análisis filogenéticos de ITS-ADNr mostraron que las poblaciones de *H. filipjevi* se agruparon en un grupo con dos subgrupos, todos con el apoyo de valores altos de bootstrap. Las poblaciones de *H. avenae* también se agruparon en un grupo con dos subgrupos. Las diferencias genéticas fueron mayores dentro de las poblaciones de *H. filipjevi* en comparación con las poblaciones de *H. avenae*. La densidad de muchas de estas poblaciones de quistes se acercó o excedió el nivel de umbral máximo para pérdidas económicas. Este es el primer informe sobre *H. filipjevi* y *H. avenae* en Azerbaiyán. El conocimiento de la presencia de nematodos en quistes de cereales es extremadamente importante para la industria agrícola de Azerbaiyán al evaluar la aparición y distribución de enfermedades transmitidas por el suelo. Las medidas de manejo para controlar los nematodos de los quistes de cereales deben dirigirse hacia la reproducción de germoplasma resistente, la rotación de cultivos y la implementación de otras prácticas de manejo.

Palabras clave: filogenética, morfología, nematodos de quistes de cereales, secuencia

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the third most important food crop globally and constitutes approximately 34.5% of per capita calorie intake in Western Asia (http://faostat.fao.org). The Caucasian region is considered important comprising the primary center of origin of cultivated wheat (Bonjean and Angus, 2001). Wheat production is an important component of Azerbaijan's agricultural sector and economy and has steadily increased in the last 20 years, resulting in gross production of approximately 1.8 million tons over 600,000 hectares in 2017 (http://faostat.fao.org). Wheat production is far below its potential in Azerbaijan, and the country must import a further 1.6 million tons of grains to meet consumption demands (http://www.fao.org/europe/news/detail-news/en/c/275265/). Meanwhile, wheat farming in Azerbaijan faces the combined challenges of abiotic (drought and heat) and biotic (fungal diseases and nematodes) stresses (Dababat *et al*., unpublished) as well as poor crop management.

Cereal cyst nematodes (CCN) of the *Heterodera avenae* group are predominant and widespread throughout cereal-growing regions. These nematodes can significantly reduce the quality and quantity of wheat and barley crops, particularly in rainfed systems or when combined with other biotic and abiotic stresses such as drought or fungal pathogens (Holdeman and Watson, 1977; Meagher, 1977; Brown *et al.*, 1985; Nicol *et al*., 2002; Dababat *et al*., 2015). Globally, CCN can cause wheat yield losses of up to 40% (Nicol and Rivoal, 2007).

The *H. avenae* group comprises 12 described and several more undescribed species (Subbotin *et al*., 1999; Handoo, 2002). Within the group, three species – *H. avenae*, *H. latipons*, and *H. filipjevi* –

have been reported as the most prominent and widely distributed in many wheat-growing regions (Rivoal and Cook, 1993; Evans and Rowe, 1998). *H. avenae* is distributed worldwide, whereas *H. latipons* is found primarily in Mediterranean countries and *H. filipjevi* is usually reported in Eastern Europe and West Asia (Sikora, 1988; Rivoal and Cook, 1993; Sturhan and Rumpenhorst, 1996; Subbotin *et al.*, 1996).

Plants attacked by CCN display yellowing, weak tillering, stunted growth, and form patchy areas in fields (Dababat *et al.*, 2011). Leaves may appear to be smaller, curved, and with a brownish-yellow coloring (Rivoal and Cook, 1993). Wheat and barley plants attacked by CCN also display primary root elongations and have a 'bushy-knotted' appearance. White cysts that darken in time can be observed on roots. CCN complete one generation per growing season. Mature brown cysts contain approximately 350-400 eggs (Meagher, 1968) and are able to survive harsh conditions during hot and dry summers. Cysts develop a facultative quiescence stage and hatching is facilitated by low temperature pre-treatment of mature cysts, in combination with moisture, either naturally in winter soils or artificially through refrigeration (Agrios, 1978). Approximately 70-80% of eggs within cysts hatch in each favorable season, while the remainder may remain in the soil for several seasons (Wherrett and Vanstone, 2010).

Correct identification of CCN species, abundance, and scale of potential damage is a crucial element in evaluating and applying appropriate management tools (Nicol *et al.*, 2002). *Heterodera* species closely resemble each other but can be distinguished by skilled nematologists via slight morphological and morphometrical differences (Handoo, 2002; Subbotin *et al.*, 2003). Biotechnological tools can also be used to discriminate closely associated nematode species (Romero *et al.*, 1996; Rumpenhorst *et al.*, 1996; Rivoal *et al.*, 2003; Subbotin *et al.*, 2003). The internal transcribed spacer (ITS) region of nematode rDNA differs among CCN species and is therefore a useful tool for identifying nematodes at the species level. ITS-rDNA of undescribed nematodes species can be sequenced and compared with previously published or GenBank reserved sequences to accurately and efficiently identify most CCN species (Ferris *et al.*, 1994; Thiery and Mugniery, 1996; Orui, 1997; Szalanski *et al.*, 1997; Sabo *et al.*, 2001; Subbotin *et al.*, 2001; Tanha

Maafi *et al.*, 2003).

The distribution, virulence, and pathogenicity of CCN make them a major threat to the world's wheat supply. Yet CCN diversity, occurrence, and potential yield losses have yet to be studied in cereal cropping areas of Azerbaijan. Therefore, the aim of this study was to: i) investigate CCN population density and distribution in wheat and barley fields of Azerbaijan; ii) identify *Heterodera* species via ITS-rDNA sequencing; and iii) examine possible intraspecific variation within *Heterodera* spp., based on the ITS-rDNA region.

MATERIALS AND METHODS

Nematode populations

A diagnostic survey was conducted during the 2017 cereal-growing season to collect soil and root samples from Azerbaijan's primary wheat and barley growing regions (Fig. 1; Table 1). We arbitrarily selected 76 field sites approximately 15-20 km apart. Soil and root samples were taken at crop maturity stage and/or just after harvesting. At the crop maturity stage, samples were taken from areas exhibiting nematode damage, such as crop stunting and chlorotic/weak plants. Up to 20 samples were taken from within each field, using a zigzag pattern and sampling at a depth of 10-15 cm (once the top 3 cm of soil had been removed). Subsamples were then combined to form one representative sample, as per Bridge and Starr (2007).

Cysts were extracted using decanting and sieving (Cobb, 1918) and the Fenwick-Can technique (Fenwick, 1940). Extracted materials (including cysts) were gently rinsed with tap water then transferred into a glass beaker, as per Dababat *et al.* (2014). Cysts were collected under a stereomicroscope, surface sterilized with 0.5% NaOCl for 10 min, and rinsed several times with distilled water. Cysts were held at 4°C until molecular identification.

Molecular characterisation and phylogenetic analysis preparation of DNA templates

Cysts were crushed to release juvenile nematodes. From each cyst, 3-5 juveniles were hand-picked and placed into a 10 μl drop of 1× PCR reaction buffer (16 mM $[NH_4]_2SO_4$, 67 mM Tris-HCl pH 8.8, 0.1% Tween-20) containing 60
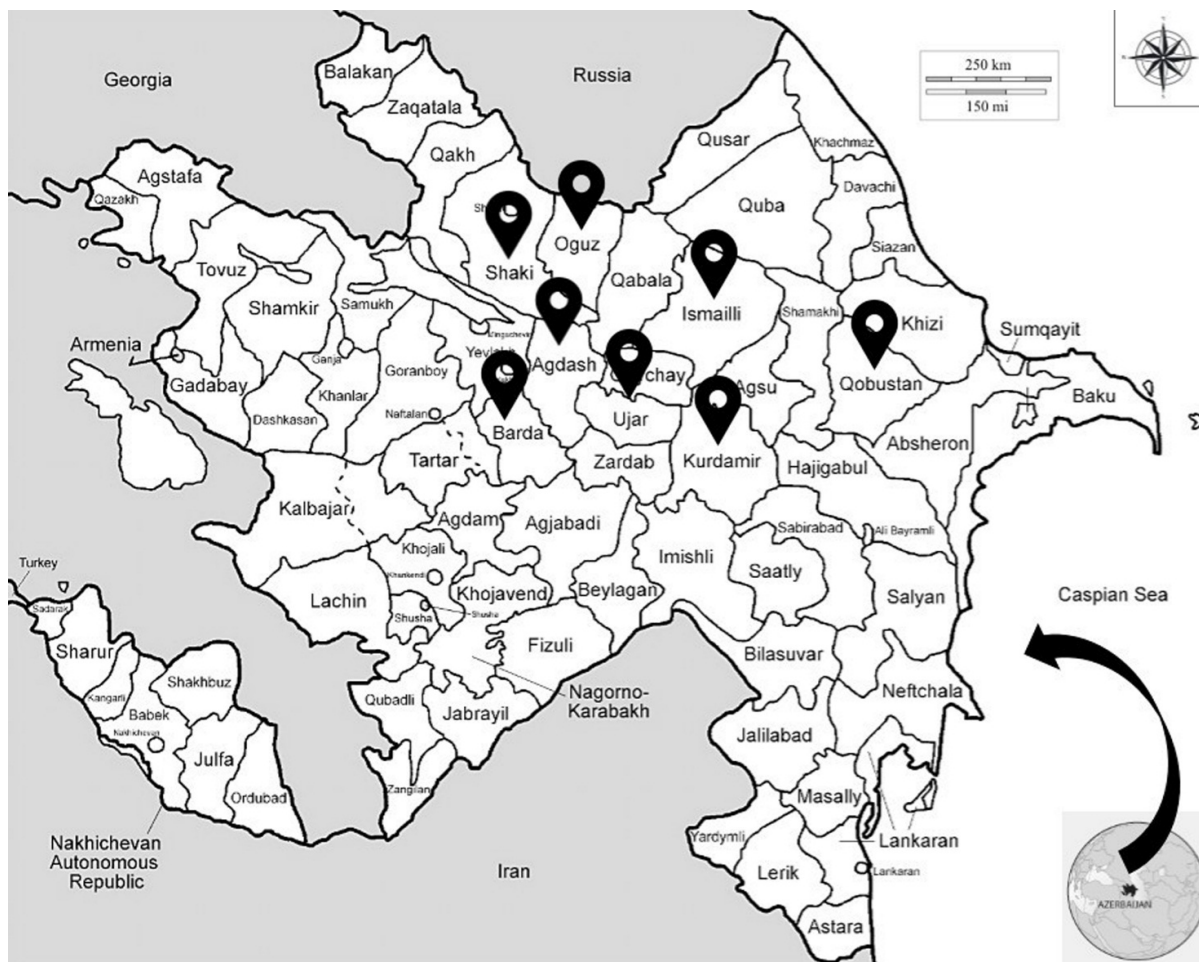
Figure. 1. Map of the Republic of Azerbaijan showing the different agro-geographical regions and sampling sites during June 2017.

µg/ml Proteinase K in a sterile PCR tube. Tubes were then incubted at 60°C for 15 min followed by an additional 5 min at 95°C. Lysate was stored at -80°C.

Amplification of ITS-rDNA

We used ITS primers (ITS1 and ITS2), AB28 (5′-CGTAACAAGGTAGCTGTAG-3′), and TW81 (5′- TCCTCCGCTAAATGATATG-3′) to amplify the ITS-rDNA, as described by Joyce *et al.* (1994). The 25 µl PCR reaction volume comprised 5 µl of nematode lysate with 0.5 µM of each primer, dNTPs (deoxynucleotide triphosphates) each at 200 µM final concentration, 1× Taq reaction buffer, 1.5 mM $MgCl_2$, and 1 µl Taq polymerase. The mixture was processed through 30 cycles of denaturation at 94°C for 20 sec, annealing at 55°C for 30 sec, and extension at 72°C for 45 sec, followed by a 2 min incubation period at 72°C.

DNA sequencing

PCR products containing the ITS-rDNA regions were purified and sequenced by Gene Research and Biotechnology (Ref-Gen, Turkey), using an ABI 3500xL Genetic Analysis with 24 capillaries. Thirty-four Azerbaijan CCN populations were sequenced, and good quality sequences were obtained. These sequences were then identified using the BLASTn algorithm on the NCBI website (https://www.ncbi.nlm.nih.gov/; Table 2). New sequences derived from this study were deposited into GenBank and received the accession numbers shown in Table 2.

Sequence analysis

ITS-rDNA sequences of indigenous isolates were phylogenetically aligned with selected reference sequences of *H. goettingiana*, *H. mani*,

Table 1. Occurrence and population density of cyst nematodes, *Heterodera* spp., in wheat and barley fields in Azerbaijan.

No.	Provence	Cultivar	Coordinates		Cysts/ 100 g soil	Average # J2 per cyst
			Latitude	Longitude		
1	Qobustan	Wheat	40º 30' 28 N	48º 59' 21 E	2	152
2	İsmailli	Wheat	40º 40' 39 N	48º 32' 70 E	5	162
3	İsmailli	Wheat	40º 43' 19 N	48º 18' 95 E	3	114
4	İsmailli	Barley	40º 47' 10 N	48º 10' 71 E	6	116
5	İsmailli	Wheat	40º 46' 46 N	48º 07' 81 E	0	
6	İsmailli	Wheat	40º 40' 46 N	48º 04' 86 E	2	142
7	İsmailli	Wheat	40º 46' 34 N	48º 01' 97 E	2	148
8	İsmailli	Wheat	40º 43' 33 N	48º 00' 85 E	0	
9	İsmailli	Wheat	40º 42' 63 N	47º 59' 11 E	0	
10	İsmailli	Barley	40º 42' 65 N	47º 57' 33 E	0	
11	Oguz	Barley	40º 56' 07 N	47º 34' 72 E	3	220
12	Oguz	Wheat	40º 59' 42 N	47º 28' 58 E	3	128
13	Oguz	Wheat	41º 01' 82 N	47º 26' 59 E	3	145
14	Oguz	Wheat	41º 02' 42 N	47º 26' 19 E	0	
15	Oguz	Barley	41º 02' 42 N	47º 26' 19 E	4	186
16	Sheki	Barley	41º 02' 42 N	47º 26' 19 E	2	145
17	Sheki	Barley	41º 04' 79 N	47º 18' 09 E	0	
18	Sheki	Barley	41º 07' 01 N	47º 15' 83 E	1	102
19	Sheki	Barley	04º 07' 60 N	47º 8.' 83 E	0	
20	Sheki	Barley	04º 08' 62 N	47º 08' 58 E	2	156
21	Sheki	Barley	41º 03' 91 N	47º 08' 42 E	0	
22	Sheki	Barley	41º 03' 91 N	47º 08' 47 E	0	
23	Sheki	Barley	41º 02' 33 N	47º 09' 33 E	11	228
24	Sheki	Wheat	41º 02' 34 N	47º 09' 36 E	19	342
25	Sheki	Barley	41º 00' 11 N	47º 07' 21 E	1	102
26	Sheki	Barley	41º 00' 09 N	47º 07' 27 E	2	124
27	Sheki	Wheat	90º 57' 02 N	47º 08' 66 E	0	
28	Sheki	Barley	40º 37' 04 N	47º 08' 67 E	0	
29	Sheki	Barley	40º 53' 61 N	47º 11' 19 E	0	
30	Sheki	Barley	40º 53' 62 N	47º 11' 62 E	1	98
31	Sheki	Barley	40º 50' 91 N	47º 13' 13 E	5	159
32	Sheki	Barley	40º 50' 92 N	47º 13' 17 E	1	104
33	Sheki	Barley	40º 50' 92 N	47º 13' 15 E	0	
34	Sheki	Barley	40º 45' 92 N	47º 11' 45 E	2	142
35	Sheki	Barley	40º 45' 92 N	47º 11' 45 E	0	
36	Barda	Wheat	40º 19' 84 N	47º 07' 76 E	1	122
37	Barda	Wheat	40º 19' 84 N	47º 07' 76 E	1	132
38	Barda	Wheat	40º 17' 00 N	47º 11' 48 E	0	
39	Barda	Wheat	40º 16' 95 N	47º 11' 53 E	4	16
40	Barda	Wheat	40º 16' 61 N	47º 11' 36 E	0	
41	Barda	Wheat	40º 16' 61 N	47º 11' 36 E	1	178
42	Barda	Wheat	40º 15' 05 N	47º 13' 49 E	0	
43	Barda	Wheat	40º 15' 05 N	47º 13' 49 E	0	
44	Barda	Wheat	40º 15' 43 N	47º 15' 29 E	0	
45	Barda	Wheat	40º 15' 43 N	47º 15' 29 E	1	156
46	Agdas	Barley	40º 16' 11 N	47º 12' 24 E	0	
47	Agdas	Barley	40º 16' 11 N	47º 12' 24 E	0	
48	Ujar	Wheat	40º 30' 55 N	47º 32' 96 E	0	
49	Ujar	Wheat	40º 29' 08 N	47º 47' 36 E	0	
50	Ujar	Wheat	40º 27' 78 N	47º 51' 14 E	2	186
51	Ujar	Barley	40º 27' 45 N	47º 51' 94 E	1	128
52	Ujar	Barley	40º 24' 90 N	47º 58' 18 E	1	124
53	Kurdamir	Barley	40º 18' 68 N	48º 08' 38 E	0	
54	Kurdamir	Wheat	40º 26' 95 N	48º 08' 11 E	0	

Table 1. Continued

No.	Provence	Cultivar	Coordinates		Cysts/ 100 g soil	Average # J2 per cyst
			Latitude	Longitude		
55	Kurdamir	Wheat	40º 26' 95 N	48º 08' 11 E	0	
56	Kurdamir	Barley	40º 26' 95 N	48º 08' 11 E	0	
57	Kurdamir	Wheat	40º 13' 48 N	48º 06' 12 E	0	
58	Kurdamir	Barley	40º 13' 48 N	48º 06' 12 E	0	
59	Kurdamir	Barley	40º 08' 84 N	48º 06' 00 E	0	
60	Kurdamir	Wheat	40º 08' 84 N	48º 06' 00 E	0	
61	Kurdamir	Wheat	40º 06' 93 N	48º 06' 56 E	0	
62	Kurdamir	Barley	40º 06' 41 N	48º 07' 81 E	0	
63	Kurdamir	Wheat	40º 06' 41 N	48º 07' 81 E	0	
64	Kurdamir	Barley	40º 05' 90 N	48º 08' 91 E	0	
65	Kurdamir	Wheat	40º 05' 64 N	48º 09' 21 E	0	
66	Kurdamir	Barley	40º 05' 64 N	48º 09' 21 E	1	88
67	Kurdamir	Barley	40º 05' 07 N	48º 10' 43 E	0	
68	Kurdamir	Wheat	40º 05' 07 N	48º 10' 43 E	0	
69	Kurdamir	Wheat	40º 05' 12 N	48º 11' 56 E	0	
70	Kurdamir	Barley	40º 05' 12 N	48º 11' 56 E	2	96
71	Kurdamir	Wheat	40º 05' 19 N	48º 12' 47 E	0	
72	Kurdamir	Wheat	40º 05' 19 N	48º 12' 47 E	2	104
73	Kurdamir	Wheat	40º 14' 74 N	48º 26' 37 E	0	
74	Kurdamir	Wheat	40º 14' 74 N	48º 26' 37 E	7	152
75	Kurdamir	Barley	40º 12' 88 N	48º 31' 19 E	0	
76	Kurdamir	Barley	40º 12' 88 N	48º 31' 19 E	1	143

H. humuli, *H. hordecalis*, and *H. schachtii* (Subbotin *et al.*, 2001; Tanha Maafi *et al.*, 2003; Yan and Smiley, 2009; Madani *et al*., 2010; Subbotin *et al*., 2010). A non-cyst forming heteroderid, *Cryphodera brinkmani*, was used as an out-group species. Finally, the sequences of the Azerbaijan populations were included. Sequence alignments were edited to contain only ITS regions.

Phylogenetic analysis

ClustalX 2.1 (with default options) (Thompson *et al.*, 1997) was used to align sequences to achieve the best formation for phylogenetic relationships. Sequence distance analysis of indigenous isolates and selected *Heterodera* species was performed using MEGA5 (Tamura *et al.,* 2011). We calculated the degree of base variations in a sequence and the base substitutions per site among sequences, the latter using the Tamura-Nei model (Tamura and Nei, 1993). Evolutionary history was inferred using the Maximum Likelihood method based on the Hasegawa-Kishino-Yano model (Hasegawa *et al*.,

1985). Figure 2 shows the phylogenetic tree with the highest log likelihood (-4161.9099), where branches corresponding to partitions reproduced in less than 60% bootstrap replicates have been collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches (Felsenstein, 1985). 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 approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites. The analysis involved 46 nucleotide sequences. All positions with gaps or missing data were eliminated, resulting in a total of 771 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar *et al*., 2015).

RESULTS

CCN were found in 34 (44.7%) of the 76 samples collected from wheat and barley fields in

Azerbaijan, of which 28 (36.8%) were identified as *H. filipjevi* or *H. avenae* using ITS-rDNA sequencing. Six populations were unidentifiable to species and were recorded as belonging to the *H. avenae* group. *H. filipjevi* was the dominant species, found in 19 samples (25%) from six provinces (Qobustan, İsmailli, Oguz, Sheki, Barda, and Kurdamir). *H. avenae* was detected in 9 samples (11.8%) across three provinces (İsmailli, Oguz, and Sheki).

Heterodera spp. were associated with sites in Qobustan, İsmailli, Oguz, Sheki, Barda, Ujar, and Kurdamir (Table 1). CCN were detected in most fields, but especially in İsmailli, Oguz, Sheki, and Barda provinces, where extension officers report widespread barley or wheat monocropping. Fields without CCN seem to utilize cultural practices such as rotation with other crops, e.g., in Kurdamir province where only 5 of 25 fields were infested. There was high cyst incidence in Oguz (4 of 5 fields were infested), where only cereals such as barley and wheat are cultivated. The highest CCN population densities were found in İsmailli, Oguz, and Sheki provinces, where the total number of cysts per 100 g of soil reached 7, 9, and 11, respectively. The lowest CCN population densities were found in the Kurdamir and Qobustan provinces, with 1 and 2 cysts, respectively, per 100 g soil (Table 1).

DNA sequences were obtained from the ITS-rDNA PCR products acquired using TW81 and AB28 primers. The rDNA-ITS regions of all prepared samples were successfully amplified using these primers. All samples yielded a single fragment of approximately 1060 bp. Conversely, no PCR products were obtained from the negative control lacking a DNA template. DNA sequences of the cysts isolated from Azerbaijan are closely related (similarity range of 97–99%) to cyst sequences in the GenBank (Table 2). Nucleotide sequences of the ITS region of isolated cyst species were deposited in GenBank database under the accession numbers listed in Table 2. Sequences acquired from the isolates of barley and wheat samples resembled each other, though they displayed significant variations when compared to the other sequences, which were, in turn, all significantly different from each other.

Figure 2 shows the phylogenetic tree of CCN species isolated from wheat and barley growing areas in Azerbaijan. Based on the ITS-rDNA phylogenetic analyses, we conclude that populations of *H. filipjevi* clearly clustered in two distinct sub-groups (sub-group I and II) with high bootstrap values of 99% and 95%, respectively. Sub-group I includes 13 isolates (numbers 36, 37, 39, 41, 45, 50, 51, 52, 66, 70, 72, 74, and 76) whereas sub-group II contains 6 isolates (numbers 1, 20, 28, 31, 32, and 34). Another six isolates (numbers 3, 2, 23, 24, 25, and 30) could not be identified to species but – based on the phylogenetic tree – they seem to belong to the *H. avenae* group, especially *H. filipjevi* sequences. Sequence analysis of the ITS-rDNA region showed significant similarities with 99% in sub-group I for *H. filipjevi* with 552/557 identities, 95% in sub-group II for *H. filipjevi* with 530/557 identities. These *H. filipjevi* isolates are closely related to the Turkish isolate (KR704308) as there were only slight nucleotide differences within the 549 bp alignment of the BLASTn result.

All *H. avenae* populations also formed one group with a moderate bootstrap value of 71%, with two distinct sub-groups (sub-group I and II) supported by a moderate bootstrap value of 76% for isolates 11 and 18 (sub-group I) and a high bootstrap value (97%) for isolates 12, 13, and 15 (sub-group II). Based on the phylogenetic tree, genetic dissimilarity was generally higher within *H. filipjevi* populations than within *H. avenae* populations. PCR Amplicons' sequences had a 97-100% match with the sequences of corresponding closely related cyst species documented in GenBank, allowing us to conclude homology between the species in our study and those in the GenBank database (Fig. 2).

DISCUSSION

This is the first comprehensive study conducted to investigate CCN distribution and occurrence in Azerbaijan's cereal production areas. The wheat-growing regions in Azerbaijan are infested with both *H. avenae* and *H. filipjevi*. Several CCN belonging to species of the *Heterodera avenae* group were also found. These populations are most likely undescribed pathotypes of *H. filipjevi*, though this will need to be confirmed using morphological, molecular (alternative gene region), and biological tests. Greco *et al*. (2002) reported that the most economically important CCN species damaging winter cereals are *H. avenae*, *H. Hordecalis,* and *H.*

Table 2. *Heterodera* species identified using ITS-rDNA from wheat and barley fields in Azerbaijan with their Genbank accession numbers.

No	Location	Species	Accession number
1	Qobustan	*Heterodera filipjevi*	MG066501
2	İsmailli	*H. avenae* group	MG066502
3	İsmailli	*H. avenae* group	MG066503
4	İsmailli	*H. avenae*	MG066504
6	İsmailli	*H. avenae*	MG066505
7	İsmailli	*H. avenae*	MG066506
11	Oguz	*H. avenae*	MG066507
12	Oguz	*H. avenae*	MG066508
13	Oguz	*H. avenae*	MG066509
15	Oguz	*H. avenae*	MG066510
16	Sheki	*H. avenae*	MG066511
18	Sheki	*H. avenae*	MG066512
20	Sheki	*H. filipjevi*	MG066513
23	Sheki	*H. avenae* group	MG066514
24	Sheki	*H. avenae* group	MG066515
25	Sheki	*H. avenae* group	MG066516
26	Sheki	*H. filipjevi*	MG066517
30	Sheki	*H. avenae* group	MG066518
31	Sheki	*H. filipjevi*	MG066519
32	Sheki	*H. filipjevi*	MG066520
34	Sheki	*H. filipjevi*	MG066521
36	Barda	*H. filipjevi*	MG066522
37	Barda	*H. filipjevi*	MG066523
39	Barda	*H. filipjevi*	MG066524
41	Barda	*H. filipjevi*	MG066525
45	Barda	*H. filipjevi*	MG066526
50	Ujar	*H. filipjevi*	MG066527
51	Ujar	*H. filipjevi*	MG066528
52	Ujar	*H. filipjevi*	MG066529
66	Kurdamir	*H. filipjevi*	MG066530
70	Kurdamir	*H. filipjevi*	MG066531
72	Kurdamir	*H. filipjevi*	MG066532
74	Kurdamir	*H. filipjevi*	MG066533
76	Kurdamir	*H. filipjevi*	MG066534

filipjevi (though this is often confused with *H. avenae*). McDonald and Nicol (2005) stated that *H. avenae* is the dominant species on temperate cereals, though *H. filipjevi* (formerly known as the Gotland strain of *H. avenae*) is also an important species with a wide and increasing distribution.

CCN symptoms become obvious under rain-fed conditions (Nicol and Rivoal, 2007). In drought conditions, plants cannot uptake water and nutrients from the soil due to nematode infection (Williamson and Gleason, 2003). The areas surveyed in this study are dominated by
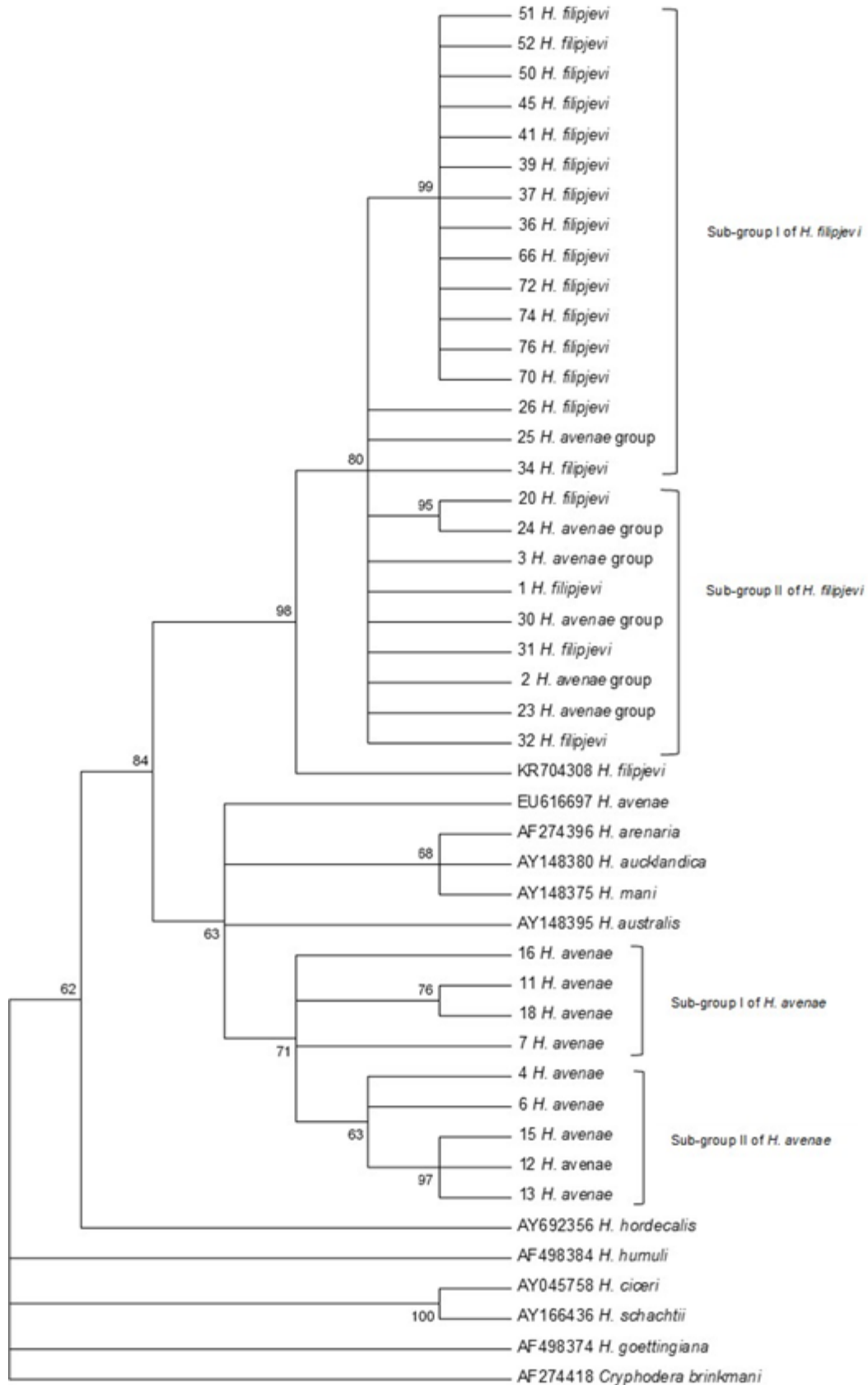
Figure 2. Phylogenetic tree formed using inferred from partial nucleotide sequences of ribosomal DNA Consensus Maximum Likelihood from the samples collected in the Republic of Azerbaijan. Bootstrap values >60 are given.

monocropping systems. Barley and wheat crops are cultivated in the same fields without rotation with other crops. This was most obvious in Sheki province, where the effect of monocultures not only caused problems with CCN, but also with the dry land crown rot caused by *Fusarium* spp. Monocropping enables nematodes to increase npopulations in the soil, ultimately reducing nematode populations in the soil, ultimately reducing yield and quality. Rivoal and Cook (1993) reported that CCN damage potential depends on factors such as host type, soil features, climatic conditions, and the nematode's pathotype and ecotype. The environmental conditions in İsmailli, Oguz, Sheki, and Barda provinces appeared to facilitate CCN life-cycle completion and increase nematode density, exacerbating the damage for the successive crop.

This study demonstrates intraspecific polymorphism between populations of *H. filipjevi*. Similarly, Subbotin *et al.* (2003) reported intraspecific polymorphism among *H. filipjevi* populations from Germany, Iran, Russia, Spain, Tajikistan, Turkey, and the United Kingdom. However, Bekal *et al.* (1997) did not detect any genetic variation among different *H. filipjevi* populations collected from areas of the Mediterranean, which concurs with the results obtained by Imren *et al.* (2015), who did not find any genetic diversity among restricted *H. filipjevi* populations collected from Kahramanmaras province in Turkey's eastern Mediterranean region.

We detected differences in ITS sequences (intraspecific variation) between *H. avenae* populations, which were close to the European *H. avenae* population (EU616697). Similar results were obtained by Imren *et al.* (2015), who showed intraspecific differentiation between populations of *H. avenae* from Adana and Hatay provinces in Turkey's Mediterranean region, as well as between Moroccan populations and Syrian populations. Meanwhile Madani *et al.* (2004) reported intraspecific variation between populations of *H. avenae* from France, Israel, Morocco, and Turkey. Rivoal *et al.* (2003) detected genetic polymorphism among 30 populations of *H. avenae* from Mediterranean areas, except for isolates from Australia, China, India, and Russia. Subbotin *et al.* (1999) revealed intraspecific polymorphism in *Heterodera* populations and identified two types of ITS regions among *H. avenae* populations: type A for most European populations and type B for

Indian populations. A unique restriction fragment length polymorphism profile with heterogeneity in the ITS region of an Australian *H. avenae* population has also been observed (Subbotin *et al.*, 2002) and confirmed based on sequences of the ITS-rDNA and random amplified polymorphic DNA data (Subbotin *et al.*, 2003). Our findings are also supported by Bekal *et al.* (1997), who detected intraspecific genetic variation among *H. avenae* populations from Australia, Saudi Arabia, and the Middle East, and concluded that *H. avenae* most likely originated from cereal-growing regions of the Middle East and could have been introduced to Australia from West Asia.

Our study found a high degree of homology among CCN populations from Azerbaijan with those deposited in GenBank. It seems that only a small degree of evolution has occurred and that the *Heterodera* isolates have not been exposed to extreme environmental factors that would force them to widen their species genetic diversity to promote their survival (Ganley and Kobayashi, 2007).

To minimize yield loss, CCN populations must be maintained under damage threshold levels. Populations can be controlled by utilizing management measures such as crop rotation, use of resistant cultivars, and adequate fertilization. The CCN population densities often exceeded or approached the threshold level for economic loss. Several sources of *Heterodera* resistance suitable for breeding programs have been identified in cereal landraces and their wild ancestors (Dababat *et al.,* 2015), as well as in winter wheat accessions (Dababat *et al.*, 2014). However, the resistance to CCN varies depending on the virulence (pathotype) of the *Heterodera* nematode population encountered. To improve host-plant resistance in breeding programs, further research is needed to identify adequate resistance sources against CCN.

This is the first report of *H. filipjevi* and *H. avenae* in Azerbaijan. This knowledge will inform targeted resistance breeding programs, as well as management practices such as crop rotation. To better understand the diversity of cyst nematode species in Azerbaijan, further studies are needed to produce detailed descriptions of the *H. filipjevi* and *H. avenae* populations, investigate possible pathotypes, and identify sources of resistance in wheat and barley germplasm. Based on the results of this study, the Soil Borne Diseases Program at the International Maize and Wheat Improvement

Center (CIMMYT), Turkey, has already screened the 34 bread and durum wheat varieties cultivated in Azeri provinces against *H. filipjevi*. A few lines have been identified as resistant to *H. filipjevi* and can be used in breeding programs for Azerbaijan's wheat farming systems.

ACKNOWLEDGMENTS

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