Occurrence, identification and phylogenetic analyses of cereal cyst nematodes in Kyrgyzstan

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Summary. The study investigated the status of cereal cyst nematodes (CCN) in the main wheat-growing areas of Kyrgyzstan in 2020. Soil samples were taken from 69 different wheat fields located in Chuy and Issyk-Kul provinces. CCN were found in thirty-one out of the sixty-nine locations surveyed. The highest occurrence of CCN was in the Tyup location in Issyk-Kul province with 81 cysts (250 cm^3 soil)$^{-1}$. The CCN populations were identified by both morphological and molecular analyses. According to the results, all populations were identified as *Heterodera filipjevi*. No variations in rDNA-ITS sequencing data were detected among the 31 cyst nematode populations, and the phylogenetic tree showed that Kyrgyz populations clustered with *H. filipjevi* populations from Belgium, Spain and Turkey, and separated populations from Germany, Iran, UK, Tajikistan, France and Russia. Therefore, the findings suggested the presence of only one species of CCN in the study areas of Kyrgyzstan, currently.

Key words: *Heterodera filipjevi*, incidence, morphometrics, rDNA-ITS sequences, wheat.

Wheat (*Triticum aestivum* L. and *T. durum* L.) is a vital food source, accounting for over one-third of the whole consumable grain produced globally (FAOSTAT, 2020). In Kyrgyzstan, wheat and barley are the main crops, followed by potato, cotton and maize. Chuy and Issyk-Kul are the most important wheat production areas in Kyrgyzstan. In 2020, the total Kyrgyz wheat production reached 559 thousand tons, which is 18.41% below the previous 10-year average (2010-2020) (FAOSTAT, 2020). Wheat production is still insufficient and does not meet Kyrgyzstan's needs. Cereal cultivation in Kyrgyzstan often suffers various constraints such as the lack of sufficient fertilisation and irrigation water, as well as the high incidence of insect pests and soil-borne diseases, including plant-parasitic nematodes (PPN) as in the nearest area of neighbouring country Kazakhstan (Dababat *et al.*, 2020; İmren *et al.*, 2021).

Within the soil profile, PPN are a dominant component of the soil community and are by far the most abundant animals on Earth (Bardgett & van der Putten, 2014). Among PPN genera that attack plants and make significant losses are *Heterodera*, *Pratylenchus* and *Meloidogyne* (Nicol & Rivoal, 2008; Skwıercz *et al.*, 2018; Subbotin *et al.*, 2018). Cereal cyst nematodes (CCN), *Heterodera avenae* group, are globally widespread and recognised as one of the major yield-limiting factors in cereal crops (Subbotin *et al.*, 2010; İmren *et al.*, 2016). They are sedentary PPN that infect wheat, barley and oat (Long *et al.*, 2013; Kumar *et al.*, 2014; Wu *et al.*, 2014). CCN have been reported to be the cause of significant yield losses of wheat throughout the world, especially from dryland cultivations (Nicol *et al.*, 2003; Subbotin *et al.*, 2010; Dababat *et al.*, 2015; Skwıercz *et al.*, 2018; Mehalaine *et al.*, 2020; Özarslandan *et al.*, 2020; Seid *et al.*, 2021). The *H. avenae* group contains 12 species; among which, *H. avenae*, *H. filipjevi* and *H. latipons* are the most studied and cause significant economic losses in small grain crops globally (Smiley *et al.*, 2017; Rumpenhorst *et al.*, 2003).

There have been many reports regarding the taxonomy of *H. avenae* group **(**Golden, 1986; Ferris *et al.*, 1994; Wouts & Sturhan, 1995; Wouts *et al.*, 1995; Subbotin *et al.*, 2010). Identification of the species in this group using solely morphological methods is complicated and sometimes misleading (Subbotin *et al.*, 2003), which can harm the

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breeding programmes by breeding for resistance to the wrong species. However, to develop sound management strategies, proper and precise identification methods are needed to differentiate genetically variable populations of CCN in different geographical regions. For this purpose, the molecular method based on the internal transcribed spacer regions (ITS) of ribosomal DNA has been considered a valuable tool to separate the species of CCN (Ferris *et al.*, 1993a, 1994; Bekal *et al.*, 1997).

Several studies have been carried out to estimate the diversity of PPN in soil of Kazakhstan, Kyrgyzstan, Tajikistan, Turkmenistan, and Uzbekistan. Madzhidov (1991) reported the presence of *H. filipjevi* in many regions of Tajikistan. Subbotin *et al.* (1996) compared several populations of cereal cyst nematodes from Russia, Ukraine and Germany with *H. filipjevi* collected in Tajikistan. So far, several studies have been carried out to evaluate the diversity of PPN in the soil of various crops in different territories of Kazakhstan (Balbaeva & Chinasilov, 1981; Dababat *et al.*, 2020; İmren *et al.*, 2021) and Azerbaijan (Dababat *et al.*, 2019). CCN were reported from different territories mainly based on their morphology (Kirjanova & Sagitov, 1975; Kirjanova *et al.*, 1976; Subbotin *et al.*, 2003, 2010). There was almost no comprehensive study on the presence of CCN in Kyrgyzstan. Therefore, the main objective of the study is to investigate the presence, distribution, and species composition of the CCN species in wheat-growing areas of Chuy and Issyk-Kul provinces of Kyrgyzstan. To achieve this objective, CCN species were described by morphological and morphometrical features of mature cysts and the second-stage infective juveniles (J2). In addition, molecular characterisation of the Kyrgyz populations was performed by ITS sequencing comparatively with international populations to acquire a database on the molecular features and the variability of the separate geographical populations of the CCN members.

MATERIAL AND METHODS

Nematode samples. Soil sampling survey was conducted in 2020 growing season from different localities representing major wheat-growing areas in Chuy and Issyk-Kul provinces located in the north and northeast Kyrgyzstan, respectively (Fig. 1). A total of 69 soil samples were collected. Each sample, which comprised 20 subsamples, was collected from a 0.5 ha area of each field visited. Each sub-sample was taken at a depth of 15-20 cm by using a soil probe in a zigzag sampling pattern from each visited field to form the main sample of 1 kg soil including roots from the wheat rhizosphere. The soil samples were kept in cool and light-proof containers in labelled plastic bags before being transferred to the laboratory and kept at around 5°C until analysis (Hooper, 1986).

A 250 cm^3 soil sample was taken for mature cyst extraction using the floatation method (Shepherd, 1986). Water suspensions containing debris and cysts were firstly poured onto a filter disc that lain on a sieve to drain the water for a day, and then filter paper ingredients were inspected to collect cysts with a binocular stereo microscope at the 25× objective (Stemi 508, Carl Zeiss, Oberkochen, Germany).

Morphology and morphometric studies. The architecture of the cysts' vulval cones, as well as J2 measures and morphometric traits, were used to identify each *Heterodera* population. According to the method of Hooper (1986), vulval cone slides were made by fixing ten cysts from each population in formalin-glycerol fixative, mounting them on glycerol, and inspecting them with a light microscope. The vulval slit was measured, as well as the width of the vulval bridge, the width of the fenestra, the length of the fenestra, and the length and width of the underbridge.

Using a Leica DFC295 digital camera mounted on a Leica DM5000 B optical microscope and Leica Application Suite (LAS) software v.4.1.0, the presence or absence of underbridge and bullae analysis on the cyst perineal pattern was investigated and assessed (Handoo, 2002). Ten juveniles were gently heated, fixed in triethanolamine formalin solution, embedded in glycerol, and placed on permanent slides for each population (Handoo, 2002). The length of the body, the length of the stylet, the distance from the anterior region to the pharyngeal bulb junction, the distance from the anterior region to the pharyngeal bulb base, the tail length, tail breadth, and the length of the hyaline part of the tail were all measured. The a, b', c, and c' ratios, as well as the hyaline component to stylet length ratio, were determined as well as the most important aspects of J2 identification (Handoo, 2002). Previously known descriptions and diagnostic criteria for cyst and J2 physical traits were used to identify CCN populations (Mulvey & Golden, 1983; Handoo, 2002).

Molecular identification. To confirm and validate the morphological and morphometric identification, *H. filipjevi* cysts were subjected to DNA extraction and PCR analysis to amplify the ITS1-5.8S-ITS2 of rRNA using the Direct PCR

Master kit (Jena Bioscience) following the manufacturer's recommendations. PCR amplification was carried out with the primers TW81 (5'-GTT TCC GTA GGT GAA CCT GC-3') and AB28 (5'-ATA TGC TTA AGT TCA GCG GGT-3') in a T100 thermal cycler (Bio-Rad) as described by Subbotin *et al.* (2001). The PCR was performed at initial denaturation of 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 60°C for 45 s, and extension at 72°C for 1.5 min. The program was completed with a final extension step for 10 min at 72°C. The amplification products were visualised on 1.5% agarose gel using the G: BOX F3 gel doc system (Syngene) after ethidium bromide staining. The PCR amplicons were purified using a PCR purification kit (Qiagen) and bidirectionally sequenced with the same primers by a commercial company (Macrogen Inc., Seoul, South Korea).

The sequences were read and edit checked manually using MEGA X software (Kumar *et al.*, 2018), and analysed by BLAST search to identify the closest available reference sequences in the complete National Center for Biotechnology Information (NCBI) nucleotide collection (http://blast.ncbi.nlm.nih.gov). The sequences were deposited in GenBank under accession numbers: MZ425512 to MZ425542. MAFFT, the online sequence alignment tool (https://mafft.cbrc.jp/alignment/server/large.html), was used to construct multiple sequence alignment (Katoh *et al.*, 2019). Phylogenetic analysis on the ITS sequences obtained from this study and corresponding published sequences from the *Avenae* group derived from GenBank was done using PAUP (Phylogenetic Analysis Using Parsimony) version 4.0b10 (Wilgenbusch & Swofford, 2003) for Maximum Likelihood analyses and visualised using Tree-finder version of March 2011 and MEGA X. Phylogenetic analysis of *H. filipjevi* populations from Belgium, Spain, Turkey, Germany, Iran, UK, Tajikistan, France and Russia were included. *Heterodera avenae* (Nematoda: Heteroderidae) was used as an outgroup (accession numbers: AY148358 and AY148374). In the bootstrap test (1,000 repetitions), the proportion of replicate trees in which the connected taxa clustered together is given next to the branches.

To find any notable differences across the 41 populations, data were analysed using analysis of variance (ANOVA) procedures in the SPSS 17.0 program for Windows (SPSS Inc., Chicago (IL), USA). To determine the significant variance between populations, a standard test of means was used ($P \leq 0.05$).

RESULTS

The obtained results indicated that CCN were detected in 31 out of the 69 soil samples collected from Chuy and Issyk-Kul provinces in Kyrgyzstan. This shows that cyst nematodes occur in 45% of the surveyed areas (Table 1). The highest CCN abundance was obtained from Tyup, Issyk-Kul province, with 81 cysts (250 cm^3 soil)$^{-1}$, followed by Shapak, Issyk-Kul province, with 36 cysts (250 cm^3 soil)$^{-1}$. The least abundance of CCN was found in Taldyk samples, Kalininskoe and Dzhayilma locations in Chuy province and Orgochor location in Issyk-Kul province of Kyrgyzstan. The abundance of CCN averaged at 18.1 and 9.9 cysts (250 cm^3 soil)$^{-1}$ in Chuy and Issyk-Kul provinces, respectively (Table 2). The cyst nematodes frequency of occurrence was found to be 34.6% and prominence value of 21.2 cysts (250 cm^3 soil)$^{-1}$ in Issyk-Kul province and 32.4% frequency of occurrence and a prominence value of 14.2 cysts (250 cm^3 soil)$^{-1}$ in Chuy province (Table 2).

Morphology and morphometric studies. The morphological identification of CCN populations obtained during this investigation was based on the standard descriptions and measurements of cysts and J2. Tables 2 and 3 show the measurements of cyst vulval cone for the tested populations. Table 4 shows the morphometrical observations taken for the J2 for the different CCN populations. J2 of the Kyrgyz population had a cylindrical shape, with a slightly offset head and a tapering-round tail ending. The stylets appeared to be firm with shallow, anteriorly concave basal knobs. For the Issyk-Kul population, the length of juvenile bodies ranged from 489.8 to 532.5 µm and the length of stylet was 21.2 to 28.4 µm (Table 4) with moderately concave stylet knobs. In addition, lateral fields had four lines, however only the two inner lines were usually distinct. Tail length and hyaline length were 44.6 to 61.24 and 22.4 to 31.6 µm, respectively (Table 4; Fig. 2). The Chuy population of *H. filipjevi* had juvenile body lengths ranging from 467.8 to 548.5 µm, stylet length ranging from 20.9 to 24.7 µm, and shallowly concave anteriorly knobs. Tail length range was 42.8-63.6 µm with a round ending, and width at anus ranged from 42.8 to 63.6 µm. The hyaline tail-terminal length ranged from 21.4 to 39.4 µm (Fig. 2).

Molecular identification. The sequencing and phylogenetic analysis of the ITS sequences were performed for molecular diagnosis of the Kyrgyz cyst populations. PCR analysis successfully yielded a single fragment of around 1060 bp. No PCR products were detected in the negative control.

Table 1. Soil samples collected from wheat fields in north and northeast Kyrgyzstan.

Code	Location	Province	Coordinates		Density*	Accession no.
1	Sokuluk	Chuy	42°50'34"N	73°58'38"E	6	MZ425512
2	Sokuluk	Chuy	42°49'12"N	73°46'27"E	0	–
3	Kalininskoe	Chuy	42°49'44"N	73°46'01"E	21	MZ425516
4	Kalininskoe	Chuy	42°50'06"N	73°45'15"E	7	MZ425525
5	Taldyk	Chuy	42°52'03"N	73°45'34"E	3	MZ425538
6	Kara-Dobo	Chuy	42°54'04"N	73°45'17"E	20	MZ425518
7	Kara-Dobo	Chuy	42°55'02"N	73°39'48"E	6	MZ425528
8	Dzhayilma	Chuy	42°50'58"N	73°39'11"E	10	MZ425523
9	Kayindi	Chuy	42°48'13"N	73°44'12"E	13	MZ425522
10	Kayindi	Chuy	42°48'37"N	73°45'32"E	3	MZ425539
11	Kayindi	Chuy	42°47'21"N	73°47'17"E	15	MZ425520
12	Kayindi	Chuy	42°45'43"N	73°48'02"E	5	MZ425532
13	Kyizil-Dyikan	Chuy	42°43'24"N	74°00'26"E	5	MZ425533
14	Kyizil-Dyikan	Chuy	42°43'51"N	73°58'57"E	0	–
15	Kosh-Korgon	Chuy	42°44'44"N	73°57'41"E	6	MZ425529
16	Sokuluk	Chuy	42°50'34"N	73°58'38"E	4	MZ425535
17	Sokuluk	Chuy	42°49'12"N	73°46'27"E	0	–
18	Kalininskoe	Chuy	42°49'44"N	73°46'01"E	0	–
19	Kalininskoe	Chuy	42°50'06"N	73°45'15"E	3	MZ425540
20	Taldyk	Chuy	42°52'03"N	73°45'34"E	0	–
21	Kara-Dobo	Chuy	42°54'04"N	73°45'17"E	0	–
22	Kara-Dobo	Chuy	42°55'02"N	73°39'48"E	0	–
23	Dzhayilma	Chuy	42°50'58"N	73°39'11"E	3	MZ425541
24	Kayindi	Chuy	42°48'13"N	73°44'12"E	0	–
25	Kayindi	Chuy	42°48'37"N	73°45'32"E	0	–
26	Kayindi	Chuy	42°47'21"N	73°47'17"E	0	–
27	Kayindi	Chuy	42°45'43"N	73°48'02"E	0	–
28	Kyizil-Dyikan	Chuy	42°43'24"N	74°00'26"E	4	MZ425536
29	Kyizil-Dyikan	Chuy	42°43'51"N	73°58'57"E	36	MZ425514
30	Kosh-Korgon	Chuy	42°44'44"N	73°57'41"E	6	MZ425530
31	Kyzyl-Tuu	Chuy	42°48'09"N	74°25'20"E	0	–
32	Kyzyl-Tuu	Chuy	42°48'26"N	74°25'19"E	0	–
33	Tokbay	Chuy	42°48'50"N	74°24'51"E	0	–
34	Takbay	Chuy	42°49'09"N	74°24'20"E	0	–
35	Tokbay	Chuy	42°49'31"N	74°24'01"E	0	–
36	Tokbay	Chuy	42°57'22"N	74°28'17"E	0	–
37	Takbay	Chuy	42°57'52"N	74°28'08"E	0	–
38	Tokbay	Chuy	42°58'26"N	74°27'01"E	21	MZ425517
39	Tokbay	Chuy	43°02'43"N	74°25'03"E	0	–
40	Takbay	Chuy	43°09'18"N	74°23'58"E	0	–
41	Tokbay	Chuy	43°09'55"N	74°23'43"E	0	–
42	Tokbay	Chuy	43°12'29"N	74°23'29"E	0	–
43	Takbay	Chuy	43°01'43"N	74°26'05"E	0	–
44	Tokbay	Chuy	42°55'54"N	74°27'57"E	6	MZ425531
45	Tokbay	Chuy	42°54'30"N	74°24'35"E	0	–
46	Chelpek	Issyk-Kul	42°28'52"N	78°20'57"E	0	–
47	Chelpek	Issyk-Kul	42°28'38"N	78°19'55"E	7	MZ425526
48	Yrdyk	Issyk-Kul	42°28'31"N	78°17'51"E	5	MZ425534
49	Baltabay	Issyk-Kul	42°29'19"N	78°14'57"E	0	–
50	Lipenka	Issyk-Kul	42°27'38"N	78°12'23"E	0	–
51	Tilekmat	Issyk-Kul	42°25'22"N	78°08'27"E	0	–
52	Shalba	Issyk-Kul	42°23'17"N	78°04'19"E	0	–
53	Jalgyz-Oruk	Issyk-Kul	42°19'46"N	77°58'14"E	0	–
54	Orgochor	Issyk-Kul	42°21'40"N	78°02'12"E	3	MZ425542
55	Jeti-Oguz	Issyk-Kul	42°27'53"N	78°13'29"E	0	–
56	Zhan-Aryk	Issyk-Kul	42°32'31"N	78°23'11"E	0	–
57	Tegizchil	Issyk-Kul	42°33'36"N	78°23'38"E	0	–
58	Tepke	Issyk-Kul	42°34'41"N	78°23'27"E	0	–
59	Tepke	Issyk-Kul	42°35'55"N	78°25'27"E	8	MZ425524
60	Karakol-Sovhoz	Issyk-Kul	42°36'38"N	78°31'30"E	7	MZ425527
61	Orlinoe	Issyk-Kul	42°35'53"N	78°34'24"E	0	–
62	Shapak	Issyk-Kul	42°35'58"N	78°41'57"E	35	MZ425515
63	Boz-Uchuk	Issyk-Kul	42°34'45"N	78°43'42"E	4	MZ425537
64	Sary-Kamysh	Issyk-Kul	42°31'51"N	78°38'15"E	0	–
65	Teploklyuchenka	Issyk-Kul	42°29'58"N	78°28'02"E	0	–
66	Tyup	Issyk-Kul	42°45'02"N	78°20'05"E	81	MZ425513
67	Ak-Bulun	Issyk-Kul	42°46'51"N	78°13'12"E	17	MZ425519
68	Oruktu	Issyk-Kul	42°45'31"N	78°09'15"E	0	–
69	Oital	Issyk-Kul	42°44'14"N	77°55'36"E	14	MZ425521

* Cysts (100 cm^3 soil)$^{-1}$.

Table 2. Occurrence of nematode in wheat fields of Chuy and Issyk-Kul provinces.

Number of cysts		Chuy (45 samples)	Issyk-Kul (24 samples)
Cysts (250 g cm^3 soil)$^{-1}$	P.D. ± S.D	9.95 ± 0.6 b	18.1 ± 1.2 a*
	F.O. %	32.4	34.6
	P.V.	14.2	21.2

P.D. (Population Density) = Total number of nematodes per 100 cm^3 soil or plant root system.
F.O. % (Frequency of Occurrence %) = (Number of samples contained a nematode / Total sample collected) × 100.
P.V. (Prominence Value) = Population density √ Frequency of Occurrence.
*Means in the same raw followed by the same letter(s) were not significantly different according to Tukey HSD test ($P \leq 0.05$).

Table 3. Vulval cone area measurements of the Kyrgyzstan cereal cyst nematode populations. Units in µm, n = 15, mean ± S.D., range.

Characters	Chuy	Issyk-Kul	İmren *et al.* (2015)
Fenestral length	52.25(a) ± 1.80 (39.2-65.8)	62.22 (b) ± 1.40 (42.4-69.8)	54.08 (a)* ± 4.24 (44.8-67.2)
Semifenestral width	20.60 (a) ± 1.10 (17.2-25.2)	25.20 (b) ± 1.30 (18.6-29.4)	21.00 (a) ± 1.34 (16-24)
Vulval bridge width	13.14 (ab) ± 0.80 (7.8-16.2)	16.19 (b) ± 0.30 (9.28-18.44)	12.16 (a) ± 1.50 (7.2-16)
Vulval slit length	24.60 (a) ± 1.20 (11.1-26.2)	26.60 (ab) ± 0.60 (16.2-27.5)	24.60 (a) ± 0.80 (9.6-26.8)

*The same letters (a, ab and b) denote no significant differences at $P \leq 0.05$ in a row, by ANOVA.

Table 4. Second-stage juveniles (J2) of the cyst nematode populations, measurements in µm, mean ± S.D., range.

Characters	Chuy	Issyk-Kul	İmren *et al.* (2015)
Body length	518.9 (ab) ± 6.6 (467.8-548.5)	524.3 (b) ± 4.6 (489.8-532.5)	516.3 (a)* ± 6.2 (476.8-547.0)
Stylet length	23.6 (a) ± 0.6 (20.9-24.7)	26.6 (b) ± 0.7 (21.2-28.4)	24.2 (ab) ± 0.2 (23.2-25.6)
Tail length	53.4 (a) ± 4.5 (42.8-63.6)	55.2 (ab) ± 1.6 (44.6-61.24)	53.9 (a) ± 2.3 (41.6-64.0)
Hyaline length	25.8 (a) ± 1.5 (21.4-39.4)	28.6 (b) ± 1.8 (22.4-31.6)	26.4 (ab) ± 1.6 (22.4-40.0)
a (body length/body width)	18.2 (ab) ± 0.4 (15.6-20.6)	20.8 (b) ± 0.6 (15.2-21.1)	17.2 (a) ± 0.3 (15.2-22.6)
c (body length/tail length)	7.29 (a) ± 0.4 (6.52-8.80)	9.59 (b) ± 0.2 (7.24-10.95)	7.05 (a) ± 0.2 (6.14-9.15)
c' (tail length/anus diameter)	3.35 (a) ± 0.2 (2.42-4.62)	4.23 (b) ± 0.3 (2.58-4.70)	3.13 (a) ± 0.3 (2.38-4.50)
L/MBV (body length/distance anterior end to median bulb)	6.88 (a) ± 0.4 (6.49-8.15)	8.28 (b) ± 0.2 (6.49-8.60)	6.44 (a) ± 0.5 (6.49-8.15)

*The same letters (a, ab and b) denote no significant differences at $P \leq 0.05$ in a row, by ANOVA.

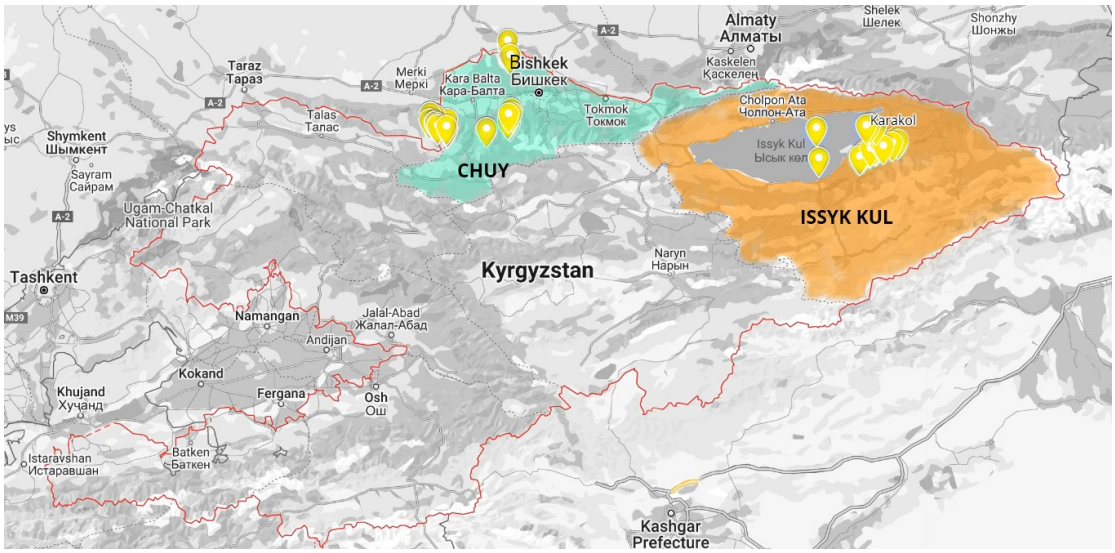

Fig. 1. Geographical locations surveyed in north and northeast Kyrgyzstan.

The consensus sequence was generated and finally used as BLAST queries against the NCBI nucleotide database and compared with those of closely related cyst samples in GenBank. Most cyst populations had 99-100% similarity with the related cyst samples recorded in the GenBank database. Thirty-one cyst samples were identified as *H. filipjevi* based on their ITS sequences. Nucleotide sequences of the populations were deposited in GenBank under accession numbers listed in Table 1.

The phylogenetic tree of the cyst nematode populations from Chuy and Issyk-Kul provinces in Kyrgyzstan is shown in Figure 3. One consensus sequence was derived from bidirectional sequences and used for comprehensive phylogenetic analysis. Sequencing of the internal transcribed spacer (ITS) of the rDNA region was used to identify nematode species and reveal intraspecific genomic variability of the *Heterodera* populations to compare them with the sequences available in the GenBank database (https://www.ncbi.nlm.nih.gov/genbank/) and to determine phylogenetic relationships among themselves. Samples from Kyrgyzstan (Chuy and Issyk-Kul provinces) were grouped to the main cluster of *H. filipjevi*. A phylogenetic tree was created from sequence alignments, which were rearranged globally with random replications. There was some minor intraspecific polymorphism in the *H. filipjevi* populations, which could be clustered in one group within the phylogenetic tree, and representative populations from GenBank, supported by a moderate bootstrap value. As a result, no variations in rDNA-ITS sequencing data were detected among the thirty-one cyst nematode populations in Kyrgyzstan. The sequences of Kyrgyz populations could be distinguished from those of the *H. filipjevi* populations from Germany, Iran, UK, Tajikistan, France and Russia, and grouped with the populations from Belgium, Spain and Turkey. The ITS sequences of Kyrgyz cyst nematode populations confirmed the morphological identification of the studied populations at species level.

DISCUSSION

This study has important findings on CCN that is the first report for Kyrgyzstan on the occurrence and distribution of *H. filipjevi* in the major wheat-growing regions in the north and northeast Kyrgyzstan. The resulting study can be used by the breeding programmes for developing resistant germplasm against *H. filipjevi*. This study reported the presence of *H. filipjevi* infecting wheat in Chuy and Issyk-Kul provinces for the first time. The highest incidence of *H. filipjevi* was found in the Tyup location of Issyk-Kul province where wheat has been monocultured. Similarly, İmren *et al.* (2021) reported that *H. filipjevi* was found infecting wheat fields in Karaganda, Oskemen, and Almaty provinces in Kazakhstan at an incidence of 35%. Also, Dababat *et al.* (2020) stated that *H. filipjevi* was the most widely distributed cyst nematode species with a distribution of 48% in Astana, Kokshetau, Kostanay and Petropavl provinces in northern Kazakhstan. Our study showed a higher prevalence of *H. filipjevi* in Chuy province, which may indicate that *H. filipjevi* populations may develop more in light, well-draining soils when compared to the heavy soil in Issyk-Kul province.

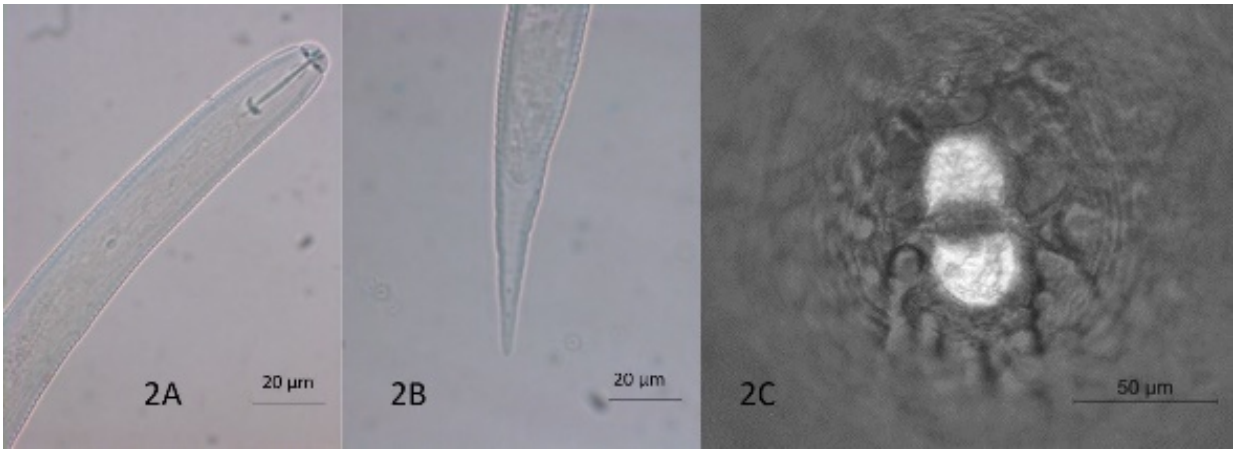

Fig. 2. Light micrographs of *Heterodera filipjevi* from north and northeast Kyrgyzstan. A: Head region, B: Tail region, C: Fenestra region.

Differences in the features and measurements of vulval cone of the cyst and J2 were detected between the Kazakhstan CCN populations and the Turkish CCN populations as reported by İmren *et al.* (2021). When comparing cyst populations from Chuy and those from Turkey they are clearly not similar, these differences ranged from minor to significant. However, there were significant differences between Issyk-Kul and Turkish populations. For example, the length of fenestra, width of semifenestra, width vulval bridge and the length of vulval slit are different, whereas Chuy population are more similar to the Turkish population when compared to the Issyk-Kul population (Table 3).

The ITS region is the most widely sequenced DNA region in the molecular ecology of PPN and has been recommended as the universal PPN barcode sequence (Ferris *et al.*, 1993b, 1994; Subbotin *et al.*, 2010). The findings of the ITS region sequencings confirmed the species identification of the Kyrgyz populations as *H. filipjevi*. Based on the results, *H. filipjevi* populations showed no intraspecific polymorphism, clustering the populations into one group in the phylogenetic tree (Fig. 3), which is consistent with the result of Subbotin *et al.* (2018), who determined minor intraspecific polymorphism between *H. filipjevi* populations collected from different geographical areas. Also, İmren *et al.* (2012) reported that *H. filipjevi* populations from Iran and Turkey were clustered into one group within the phylogenetic tree. Similarly, Subbotin *et al.* (2003) reported that the ITS sequence alignment of *H. filipjevi* populations from Iran and Russia clustered together with 100% nucleotide similarity using the minimum evolution method. By contrast, Toktay *et al.* (2015) reported intraspecific variation among *H. filipjevi* populations obtained from the Eastern Anatolian Region of Turkey. Recently, Dababat *et al.* (2020) reported that there was an intraspecific variation among *H. filipjevi* populations collected from northern Kazakhstan.

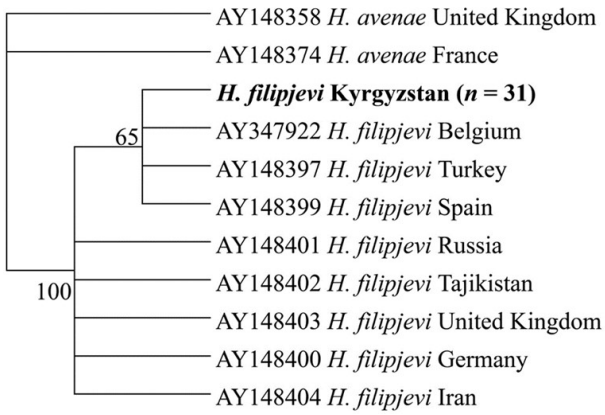

Fig. 3. Phylogenetic relationships of *Heterodera filipjevi* from the north and northeast Kyrgyzstan with other populations.

This study highlighted the eco-regional distribution of the cereal cyst nematode species *H. filipjevi* in the north and northeast Kyrgyzstan. To avoid damage and crop loss caused by *H. filipjevi* it is recommended after this study to diversify wheat cultivars by including durum wheat due to its greater resistance to cyst nematodes, to follow cultural practice particularly crop rotation, and to breed for germplasm with a high level of resistance. Further surveys in Kyrgyzstan and comprehensive pathotype studies on *H. filipjevi* populations from different areas are recommended.

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A. Saleh, Ş. Yıldız, G. Özer, F. Mokrini, T.E. Uulu, M. İmren and A.A. Dababat. Встречаемость, идентификация и филогенетический анализ злаковых нематод в Кыргызстане.

Резюме. В исследовании оценивалось состояние злаковых цистообразующих нематод (ЗЦН) в основных районах возделывания пшеницы в Кыргызстане в 2020 г. Образцы почвы были взяты с 69 различных пшеничных полей, расположенных в Чуйской и Иссык-Кульской областях. ЗЦН были обнаружены в 31 из 69 исследованных мест. Наиболее высокая встречаемость цистообразующих нематод отмечена в Тюпском участке Иссык-Кульской области – 81 циста на 250 см^3 почвы. На основании морфологического и молекулярного анализов все популяции ЗЦН были идентифицированы как *Heterodera filipjevi*. Среди 31 популяции этих нематод не было обнаружено вариаций в данных секвенирования ITS рДНК, а филогенетическое дерево показало, что кыргызские популяции сгруппированы с популяциями *H. filipjevi* из Бельгии, Испании и Турции и отдельными популяциями из Германии, Ирана, Великобратании, Таджикистана, Франции и России. Таким образом, полученные данные свидетельствуют о наличии в настоящее время только одного вида цистообразующих нематод на исследуемых территориях Кыргызстана.