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# Population genetics of the cereal cyst nematode *Heterodera avenae* reveal geographical segregation and host adaptation

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## Abstract

Cereal cyst nematodes (CCNs) lead to major losses in the cereal crop industry worldwide and have been reported in many provinces of China. However, this plant nematode's distribution and genetic differences are not fully understood. In the present study, 821 soil and host root samples were collected from 16 provinces in 2019–2022 to investigate the distribution of the CCNs. *Heterodera avenae* was detected in 56.39% of the total samples, primarily in Hubei, Henan, Hebei, Shandong, Shanxi, Gansu, Beijing, Tianjin, Inner Mongolia, Ningxia, Xinjiang, Qinghai, Anhui, Shaanxi, and Jiangsu. *H. filipjevi* was present in 21 samples, with a detection rate of 2.60%, and it was found mainly in Henan, Anhui, Jiangsu, Shandong, Shanxi, and Qinghai. A phylogenetic analysis of the internal transcribed spacer (ITS) region of the rRNA gene indicated that significant evolutionary and genetic differences existed between the Chinese populations and populations from other countries. Our results indicate that ITS1 can be used as a phylogenetic analysis and genetic target for *H. avenae* populations. The haplotypes of the ITS1 sequences of *H. avenae* populations from 14 countries were analyzed, and we speculate that *H. avenae* originated in a Middle East hotspot, then spread westwards to Europe and the United States and eastwards to China and Australia. Genetic differences between Asian and European populations suggest that the Himalayas and Kunlun Mountains formed a barrier that resulted in the formation of a separate evolutionary group in China. The phylogenetic and haplotype analysis results from different hosts showed significant differences among populations isolated from different hosts, and those isolated from weeds were distinct from those from other hosts, indicating that the rich genetic diversity of *H. avenae* populations is related to the large number of available hosts. Above all, geographic barriers, time of origin, and host adaptation might explain the current known distribution patterns of Chinese *H. avenae* populations.

**Keywords** Cereal cyst nematode, Distribution, Haplotype, Geographic barrier, Host adaptation

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## Background

*Heterodera avenae* and *H. filipjevi* are the most important and damaging nematode pests parasitizing wheat, barley, oats, and certain wild grasses (Rivoal and Cook 1993; Rivoal and Cook 1993; Nicol 2022) worldwide. *H. avenae* was first discovered in Germany in 1874 (Kuhn 1874) and was subsequently found in more than 40 countries, including the United Kingdom, Russia, Norway, Australia, Canada, Japan, India, the United States, New Zealand, and China (Baldwin and Mundo 1991; Rivoal and Cook 1993), where it severely affected the production of cereal crops, particularly wheat crops. In China, *H. avenae* was first reported in Hubei Province in 1989 (Wang et al. 1991; Chen et al. 1992). It has now been confirmed to be widely distributed in several provinces (Peng et al. 1995, 1996, 2009; Long et al. 2013). *H. filipjevi* was first found in Henan Province (Peng et al. 2010) and was then found in Shandong, Anhui, and Hebei (Peng et al. 2016; Zhen et al. 2018; Cui et al. 2021). The estimated yield losses caused by *H. avenae* and *H. filipjevi* reached 18–35% in Henan Province, 15–20% in Hebei Province, 11% to 18% in Beijing Suburbs, and 24–28% in Qinghai (Peng et al. 2020).

The use of molecular techniques to explore the internal transcribed spacer (rDNA-ITS) sequence characteristics of different populations has become a popular research tool for the molecular diagnosis of cyst nematodes in recent years. The ITS sequence is a versatile genetic marker that is located between repeating clusters of 18S and 28S ribosomal DNA genes and is separated by the 5.8S ribosomal DNA genes (Subbotin et al. 2001). Subbotin et al. (1999) analysed the ITS regions in the rDNA of cereal cyst nematode populations from several countries and regions of the world and found heterogeneity in the ITS region of CCNs. Accordingly, CCNs were categorized as ITS 'A' and ITS 'B' types. The isolates of *H. avenae* from China were thought to form a distinct group within the *H. avenae* complex based on the ITS-rDNA gene and random amplified polymorphic DNA (RAPD) analysis (Castillo 2012). The first ribosomal transcribed spacer (ITS1) is a part of the eukaryotic cistron of ribosomal DNA located between the genes coding for 18S and 5.8S rRNA. Due to its noncoding structure, ITS1 shows a high evolutionary rate and has been used for phylogenetic studies of closely related species of animals, plants, and fungi at the population and species levels (Cherry et al. 1997; Khrisanfova et al. 2008). The existing evidence suggests that populations of nematodes can be analysed through PCR–RFLP digestion of the ITS1 region (Powers et al. 1997). Variations in the ITS1 region in *Rotylenchulus reniformis* populations from Alabama show two main clusters with individual branches, confirming the presence of variability in the ITS1 rDNA region (Tilahun et al. 2008). Szalanski et al. (1997) successfully identified a variety of cyst nematodes with PCR–RFLP (restriction fragment length polymorphism) of ITS1. ITS1 was used to classify *Ditylenchus destructor* based on haplotype analysis (Hou et al. 2011; Subbotin et al. 2011).

There has been increasing interest in studying the genetic diversity and spread of *H. avenae* populations worldwide. *H. avenae* in China is distinct from those in other countries (except Australia) based on RFLP and the rDNA-ITS gene found by Fu et al. (2011). High genetic diversity within the *H. avenae* population in four provinces of China was revealed using ISSR markers and ITS-rDNA sequence analysis (Huang et al. 2012). Based on the phylogeographical analysis and age estimation of clades of mitochondrial DNA (mt DNA) genes with a molecular clock approach, Subbotin et al. (2018) speculated that several species of the *H. Avenae* group primarily originated and diversified in the Irano-Anatolian hotspot during the Pleistocene and Holocene periods and then dispersed from this region across the world. Shao et al. (2022a) studied the origin of *H. avenae* in China and suggested that the Chinese and Australian populations have the same ancestry, while populations in other countries have different origins from those in China. Qing et al. (2022) reported that Chinese *H. avenae* populations originated from the Pleistocene northwest and originally parasitized grasses. The mt DNA is maternally inherited with a high mutation rate, and there is already some basis for using mitochondrial genes to study the genetic diversity of *H. avenae* (Subbotin et al. 2018; Qing et al. 2022; Shao et al. 2022a). Also, nuclear genes are the most common target for our genetic analysis, and ITS-rDNA of *H. avenae* has more available data in GenBank. Understanding the differences in genetic analysis of ITS-rDNA and mt DNA of *H. avenae* will provide a reference for future studies on the genetic diversity of plant parasitic nematodes.

To date, the dispersal routes of *H. avenae* populations worldwide, their origins, and the influences that hosts have on them have not been reported. Therefore, there is an urgent need to study the dispersal pathways and genetic diversity of *H. avenae* populations from different hosts and countries. The objectives of this study are to survey the distribution of CCNs in the wheat-producing regions of China, to analyse phylogenetic relationships among *H. avenae* populations based on ITS sequences, and to clarify the relationship between the haplotype and genetic structure of *H. avenae* populations from different hosts in China and other countries. The data on the distribution and genetic diversity of *H. avenae* presented in this study will help elucidate the evolutionary processes of the populations, including the development of haplotypes

and adaptation to selection pressure. This work will provide a theoretical basis for future research on the origin, spread, and evolution of *H. avenae* in China and worldwide.

## Results

### Geographic distribution and degree of damage caused by CCN in China

A total of 821 cereal and weed field samples were collected from 16 major cereal crop-producing provinces (84 from Anhui, 47 from Jiangsu, 83 from Shandong, 50 from Gansu, 135 from Henan, 104 from Hebei, 122 from Qinghai, 45 from Ningxia, 23 from Tianjin, 15 from Hubei, 20 from Shanxi, 15 from Shaanxi, 8 from Beijing, 2 from Chongqing, 64 from Inner Mongolia, and 4 from Xinjiang) (Fig. 1a; Table 1). Cyst nematodes were detected in 463 samples in 65 cities in 15 provinces, and the infection rate of cyst nematodes was 56.39% (Fig. 1b; Table 1). The highest infection rate of cyst nematodes was 100%, which was observed in samples from Tianjin (23/23). A cyst nematode infestation rate of approximately 80% was found in the samples from Hebei (80.86%), Shanxi (80.00%), and Henan provinces (79.04%). A cyst nematode infection rate of more than 50% was detected in the samples from Beijing (66.67%), Inner Mongolia (64.06%), Hubei (61.54%), and Xinjiang (50%). The rates for the samples from other provinces were < 50%. The highest mean cyst densities were detected in the samples from Henan (50.94 cysts/100 $cm^3$ of soil), followed by Hebei, Tianjin, Qinghai, Shanxi, and Shaanxi (33.69, 25.21, 22.42, 21.2, and 20.27 cysts/100 $cm^3$ of soil, respectively). The mean counts of cyst nematodes in other provinces were < 20 cysts/100 $cm^3$ of soil. Cyst nematodes were not found in the samples from Chongqing (Table 1). In addition, a small amount of *H. filipjevi* was found in the samples from Henan, Anhui, Jiangsu, Shandong, Shanxi, and Qinghai provinces. The cyst density of *H. filipjevi* was the highest in Henan Province, with 5 cysts/100 $cm^3$ of soil. The cyst densities of *H. filipjevi* in the samples from Anhui, Jiangsu, Shandong, Shanxi, and Qinghai provinces were 3.0, 2.5, 2.0, 2.0, and 1.0 cysts/100 $cm^3$ of soil, respectively (Table 2; Fig. 1c). The 21 samples from Henan, Jiangsu, Anhui, Shandong, Shanxi, and Qinghai provinces were infected by a mixed population of *H. filipjevi* and *H. avenae*. The results of previous studies (Chen et al. 1992; Peng et al. 1996, 2008, 2012; Zheng et al. 1996; Liu et al. 2005; Li et al. 2016; Li et al. 2018) and the present study indicate that the *H. avenae* population was first discovered in Hubei (Fig. 1d) and subsequently spread across other provinces in China (Fig. 1e, f).

### Phylogenetic and sequence analysis with the rDNA-ITS gene

The lengths of the rDNA-ITS sequences obtained from 111 samples in China and 14 samples in Europe and the Middle East were 1041–1048 bp (Additional file 1: Table S1). No differences in sequences were detected within each population. The dendrogram obtained by phylogenetic analysis indicated that the specimens from the present study were in one of two main clades (Fig. 2). All Chinese and Australian populations were clustered in the same branch, while the other samples were clustered in a separate branch (PP = 92). Among the large branches of the Australian and Chinese populations, the Australian population and two samples from Henan Province (HNZZ1 and HNZZ2) were grouped on a small branch. Except for the three populations (QHHY1, QHYET, and QHHB2) in Qinghai, populations from the remaining provinces in Northwest China, including Tibet, Gansu, Qinghai, Ningxia, and Shaanxi, were clustered, while populations in North China, including Hebei, Henan, Anhui, Jiangsu, Shandong, Shanxi, Hubei, and Beijing, were clustered on another branch.

### Variation analysis and phylogenetic analysis of ITS1 and ITS2

For variable sites in the ITS sequences, there were more variable sites in *H. avenae* populations, with a total of 76 variable sites and 24 parsim-informative sites, i.e., 46 variable sites and 18 parsim-informative sites in ITS1 sequences, 16 variable sites and 2 parsim-informative sites in 5.8S rDNA sequences, 14 variable sites and 4 parsim-informative sites in ITS2 sequences, which constituted 7.23% and 2.28% of the total sites, respectively (Table 3). Phylogenetic trees were separately constructed based on the ITS1 and ITS2 region sequences. The results of the phylogenetic analysis of ITS1 sequences were consistent with those of rDNA-ITS sequences (Fig. 3a). This revealed that ITS1 could be used as a target for the identification of *H. avenae* populations. In contrast, the phylogenetic analysis of ITS2 sequences suggested that Chinese populations clustered with populations from other countries, and only the HUBXY1 population in Hube and the SXJC population in Shaanxi were on a separate branch (Fig. 3b). Hence, ITS2 cannot be used to distinguish among different geographical populations of *H. avenae*.

### Population structure of *H. avenae* in different countries

Seventy populations were included in the haplotype analysis to reveal the origins of *H. avenae* populations worldwide. The haplotype results of the phylogenetic network show that 15 Chinese populations and 55 populations

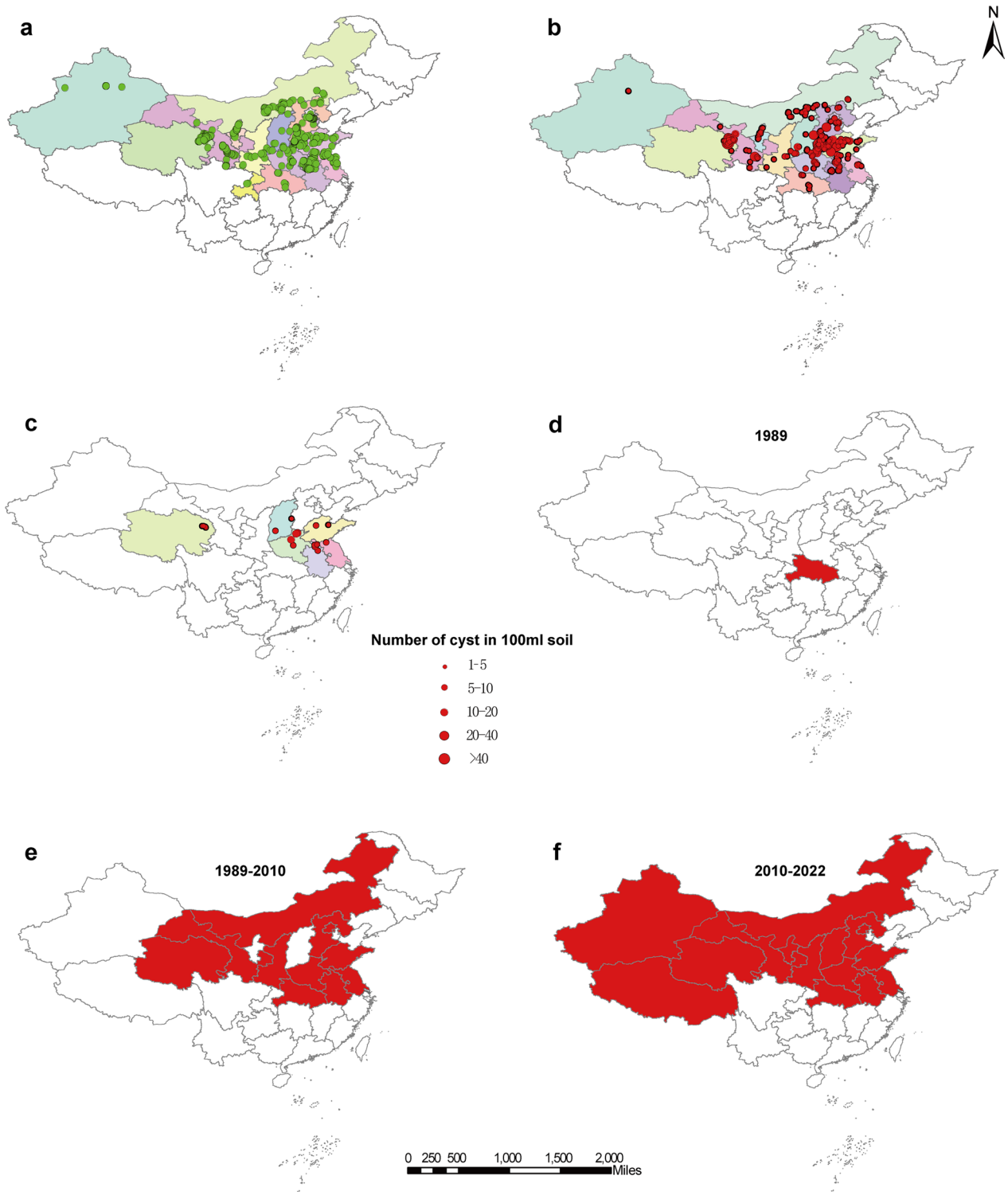


**Fig. 1** Distribution of cereal cyst nematodes (*Heterodera* spp.). **a** Distribution of all samples collected. **b** Distribution of *H. avenae*. **c** Distribution of *H. filipjevi*. **d** Provinces in which *H. avenae* occurred in 1989. **e** Provinces in which *H. avenae* occurred in 1989–2010. **f** Provinces in which *H. avenae* occurred in 2010–2022

**Table 1** Occurrence and distribution of *Heterodera avenae* in the main cereal crop production areas of China

| Order | Province (Autonomous region) | Samples | Number of samples containing *H. avenae* | *H. avenae* detection rate (%) | Populations density (Cysts per 100 ml soil) | |
|---|---|---|---|---|---|---|
| | | | | | Maximum | Average |
| 1 | Anhui | 84 | 36 | 42.67 | 137 | 4.88 |
| 2 | Jiangsu | 47 | 4 | 7.84 | 13 | 0.25 |
| 3 | Shandong | 83 | 38 | 45.79 | 58 | 7.27 |
| 4 | Gansu | 50 | 16 | 32.00 | 98 | 13.71 |
| 5 | Henan | 135 | 108 | 79.04 | 292 | 50.94 |
| 6 | Hebei | 104 | 84 | 80.86 | 569 | 33.69 |
| 7 | Qinghai | 122 | 53 | 43.50 | 387 | 22.42 |
| 8 | Ningxia | 45 | 21 | 46.15 | 42 | 2.96 |
| 9 | Tianjin | 23 | 23 | 100 | 138 | 25.21 |
| 10 | Hubei | 15 | 9 | 61.54 | 33 | 11.02 |
| 11 | Shanxi | 20 | 16 | 80.00 | 184 | 21.2 |
| 12 | Shaanxi | 15 | 7 | 45.45 | 65 | 20.27 |
| 13 | Beijing | 8 | 5 | 66.67 | 38 | 14.33 |
| 14 | Chongqing | 2 | 0 | 0 | 0 | 0 |
| 15 | Inner Mongolia | 64 | 41 | 64.06 | 59 | 4.34 |
| 16 | Xinjiang | 4 | 2 | 50.00 | 10 | 3 |
| | Total | 821 | 463 | 56.39 | 569 | 19.08 |

**Table 2** Occurrence and distribution of *Heterodera filipjevi* in the main cereal crop production areas of China

| Order | Province | Samples | Number of samples containing *H. filipjevi* | Detection rate (%) | Populations density (Cysts per 100 $cm^3$ soil) | |
|---|---|---|---|---|---|---|
| | | | | | Maximum | Average |
| 1 | Henan | 135 | 5 | 3.70 | 8 | 5 |
| 2 | Anhui | 84 | 3 | 3.57 | 4 | 3 |
| 3 | Jiangsu | 47 | 3 | 6.38 | 4 | 2.5 |
| 4 | Shandong | 83 | 2 | 2.40 | 3 | 2 |
| 5 | Shanxi | 15 | 2 | 13.33 | 3 | 2 |
| 6 | Qinghai | 122 | 6 | 4.91 | 1 | 1 |

from other countries were obviously clustered into three different groups (Fig. 4a). Group 1 includes haplotypes (Hap11, Hap12, Hap13, Hap14, Hap15, Hap16, Hap17, Hap18, Hap19, and Hap20) of all *H. avenae* populations in China and Australia. Haplotypes (Hap2, Hap3, Hap4, Hap9, and Hap10) of *H. avenae* populations in India, France, Egypt, Israel, Turkey, Iran, and Syria in the Middle East were grouped into Group 2. Among them, Hap2 was shared across seven countries and was prevalent in the Middle East. Group 3 comprised haplotypes from ten countries: Turkey, France, the USA, Morocco, the UK, Syria, Serbia, Spain, Germany, and Saudi Arabia. Hap1 was widespread in 10 countries in Europe. PCoA clustering results showed that the populations of *H. avenae* from China and Australia were clustered, while there were a few connections among the populations of *H. avenae* from the Middle East and Europe (Fig. 4b).

### Haplotype analysis of different hosts

A total of 24 *H. avenae* ITS sequences were collected from different hosts, including *Triticum aestivum*, *Avena sativa*, *Hordeum vulgare,* and weeds, in China. Based on the phylogenetic results of *H. avenae* populations of diverse hosts (Fig. 5a), populations of *H. avenae* from the same hosts were clustered on their respective branchlets. *H. avenae* populations that had weeds as their hosts were clearly distributed independently of other hosts. Populations from *H. vulgare* were closely related to those in *A. sativa*. Similarly, the population that parasitized *T. aestivum* was more related to the population that parasitized

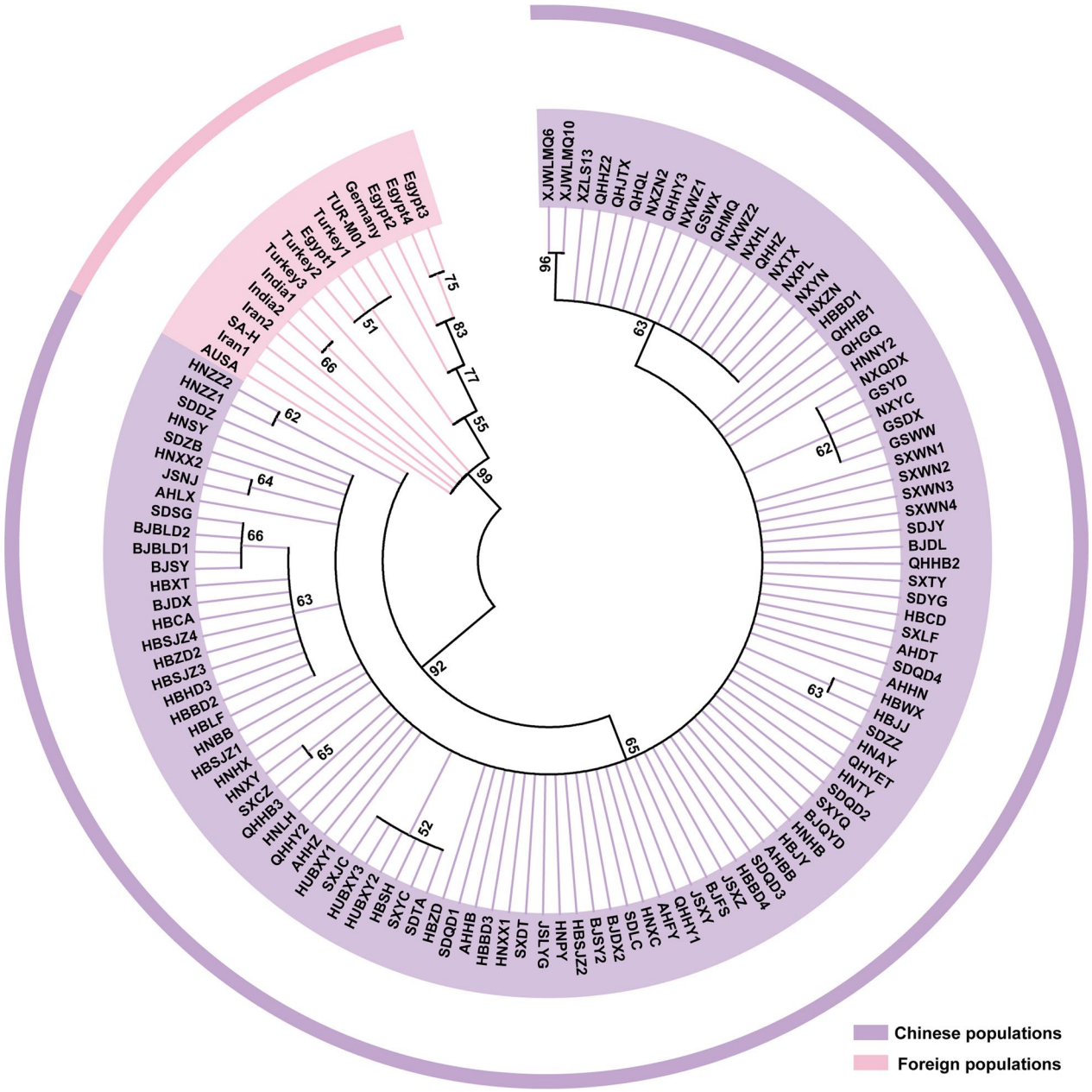


**Fig. 2** Phylogenetic analysis of *Heterodera avenae* populations based on ITS genes using PhyloSuite. Bootstrap support values for analysis are provided for appropriate clades. Values less than 50% are not shown

**Table 3** Analysis of loci variation in different regions of ITS genes

| Sequence region | Variable sites | Parsim-informative sites | Percentage of variable sites (%) | Percentage of variable sites in a parsim-informative site (%) |
|---|---|---|---|---|
| ITS1 | 46 | 18 | 4.38 | 1.71 |
| 5.8S rRNA | 16 | 2 | 1.52 | 0.19 |
| ITS2 | 14 | 4 | 1.33 | 0.38 |
| ITS1-5.8S rRNA- ITS2 | 76 | 24 | 7.23 | 2.28 |

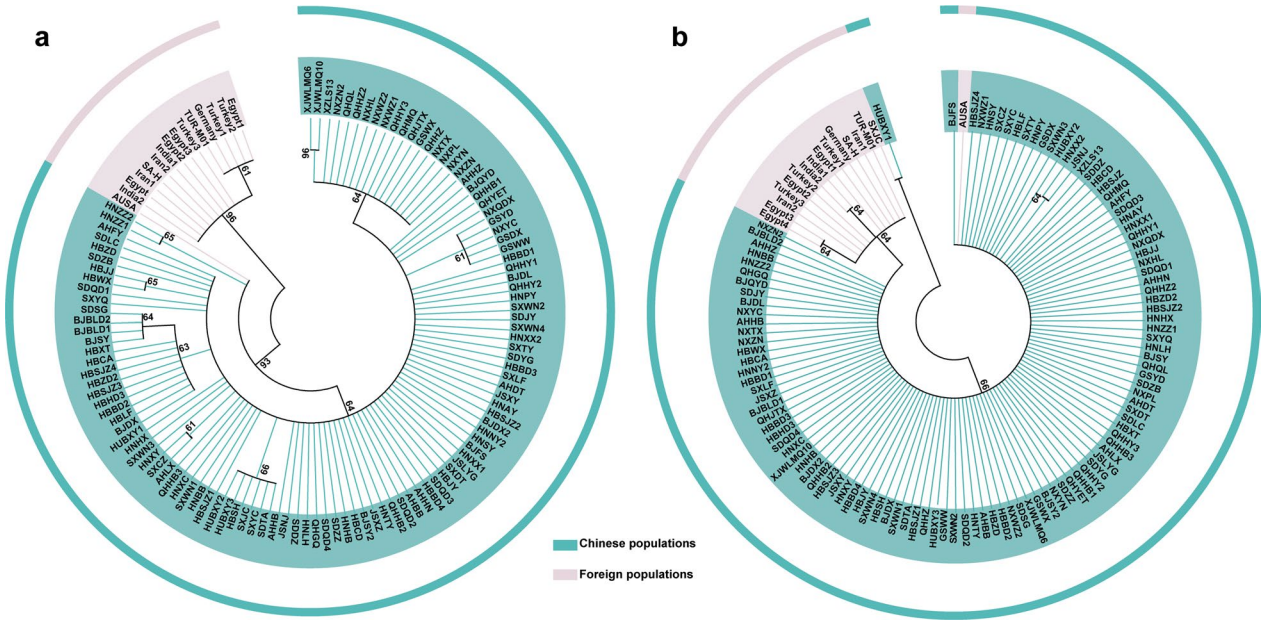


**Fig. 3** Phylogenetic analysis of *Heterodera avenae* populations based on ITS1 (**a**) and ITS 2 (**b**) sequences. Bootstrap support values for analysis are provided for appropriate clades. Values less than 50% are not shown

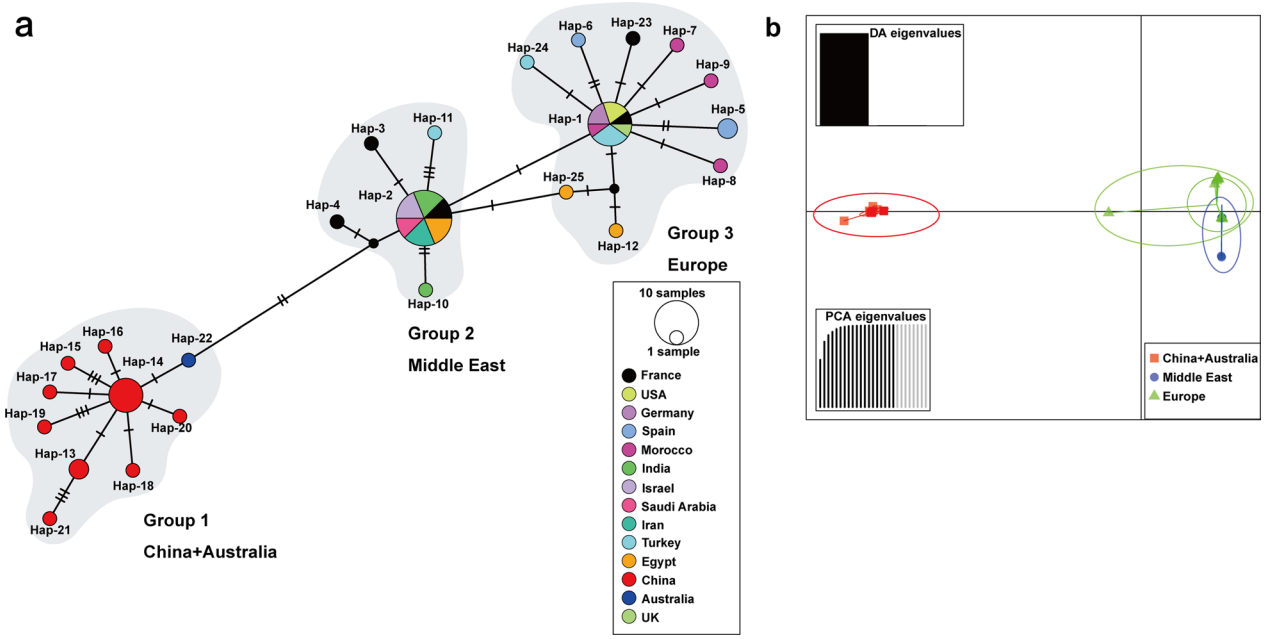


**Fig. 4** Analysis of haplotype networks (**a**) and PCoA clustering (**b**) for *Heterodera avenae* populations from 14 countries

*A. chinensis*, which reveals a strong host bias in the genetic evolution of *H. avenae* populations.

A total of 14 haplotypes were identified (Table 4). Among them, Hap5, Hap10, and Hap12 occurred at the highest frequency of 16.67% (4/24). The *H. avenae* population of *T. aestivum* dominated Hap5 and Hap10. Except for Hap11, shared by two populations, the other 10 haplotypes occurred only in a single population and were unique in China. The network of haplotypes suggested that Hap12 was centrally located and could spread among weeds and other hosts (Fig. 5b). Haplotypes of *H. avenae* from the same host were clustered

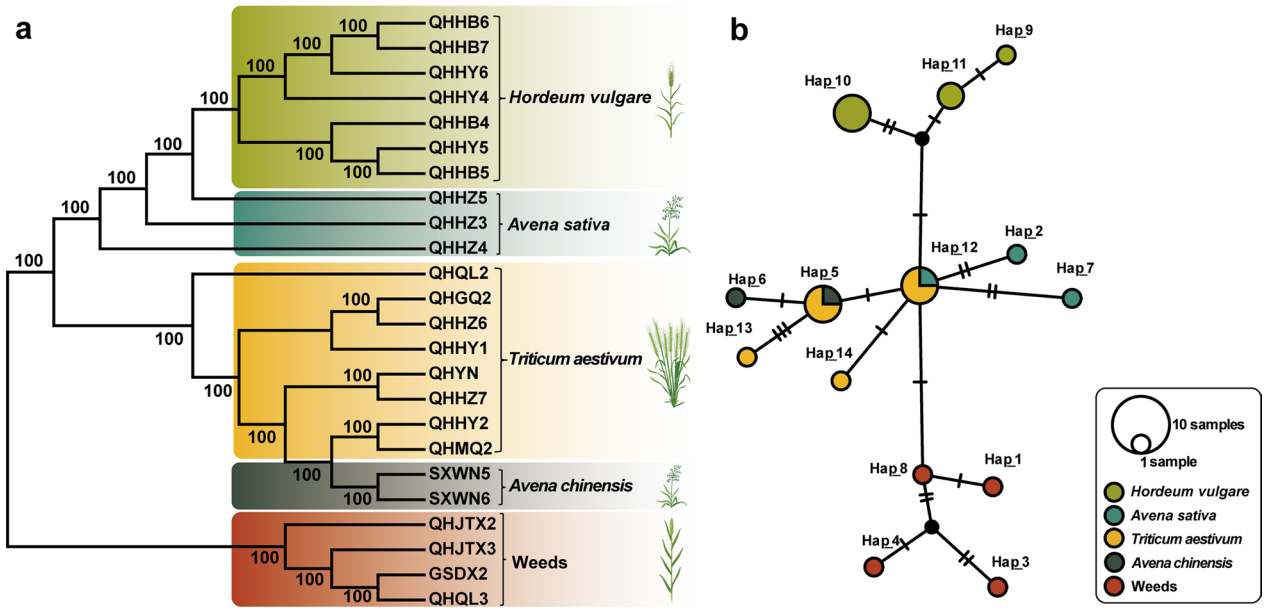


**Fig. 5** Phylogenetic analysis (**a**) and haplotype analysis (**b**) of *Heterodera avenae* populations isolated from different hosts. Bootstrap support values for analysis are provided for appropriate clades. Values less than 50% are not shown

**Table 4** Haplotypes identified in *Heterodera avenae* populations based on ITS1

| Haplotype | N* | Sample codes | GenBank accession number | Haplotype frequency (%) |
|---|---|---|---|---|
| Hap1 | 1 | QHJTX2 | OP217022 | 4.17 |
| Hap2 | 1 | QHHZ3 | OP217028 | 4.17 |
| Hap3 | 1 | GSPL | OP217024 | 4.17 |
| Hap4 | 1 | QHQL3 | OP217025 | 4.17 |
| Hap5 | 4 | QHHY1, QHMQ2, SXWN5 and QHHZ7 | OP217043, OP217042, OP217027, OP217041 | 16.67 |
| Hap6 | 1 | SXWN6 | OP217043 | 4.17 |
| Hap7 | 1 | QHHZ4 | OP217023 | 4.17 |
| Hap8 | 1 | QHJTX3 | OP217029 | 4.17 |
| Hap9 | 1 | QHHY5 | OP217030 | 4.17 |
| Hap10 | 4 | QHHY4, QHHY6, QHHB6, and QHHB7 | OP217031, OP217032, OP217035, OP217036 | 16.67 |
| Hap11 | 2 | QHHB4 and QHHB5 | OP217033, OP217034 | 8.33 |
| Hap12 | 4 | QHHY2, QHGQ2, QHHZ6 and QHHZ5 | OP217037, OP217039, OP217045, OP217044 | 16.67 |
| Hap13 | 1 | QHYN2 | OP217026 | 4.17 |
| Hap14 | 1 | QHQL2 | OP217040 | 4.17 |

N* represents the number of sequences in each haplotype

separately. *H. avenae* populations with *T. aestivum* as the host shared Hap12 and Hap5 with *H. avenae* populations on *A. sativa* and *A. chinensis*, respectively. The populations parasitizing weeds and *H. vulgare* did not share haplotypes with other hosts.

## Discussion

CCN, a globally distributed plant-parasitic nematode, is one of the most significant pests in cereal crops. At present, no comprehensive studies are available on their occurrence and distribution in China. In this study, 821

soil samples were collected from 16 provinces in China, and CCN occurrence and distribution were described in detail. Our surveys showed that CCN was present in 463 samples in 65 cities in 15 provinces. Based on our data and literature reports, we constructed a detailed occurrence and distribution map for cereal cyst nematodes in China. *H. avenae* was first discovered in 1989 in Tianmen in Hubei (Chen et al. 1992) (Fig. 1d) and was subsequently reported in Henan (1992), Hebei, Beijing, Inner Mongolia, Qinghai (Peng et al. 1996), Anhui (Zheng et al. 1996), and Shandong (Liu et al. 2005), Shaanxi and Gansu (Peng et al. 2008), and Jiangsu (2008) (Fig. 1e). In recent years, *H. avenae* has been found in Tianjin (Peng et al. 2012), Shanxi (Li et al. 2018), Xinjiang and Tibet in China (Li et al. 2016) (Fig. 1f). In our study, the population density of *H. avenae* in Henan Province (50.94 cysts per 100 $cm^3$ soil) was the highest, and the occurrence rate was 79.04%. The population density of *H. avenae* in Hebei Province (33.69 cysts per 100 $cm^3$ soil) was second only to that in Henan Province, while the infection rate in Hebei Province was the highest (80.86%). In contrast, different *H. avenae* occurrence rates have previously been reported in Hebei (52.7%) (Li et al. 2013), Anhui (52%) (Tao et al. 2012), Shanxi (29.7%) (Li et al. 2018), and Qinghai (7.14–15.38%) (Hou et al. 2011). Studies indicated that *H. avenae* populations have tended to increase and cause more damage each year in these provinces. The cyst density at almost all survey sites exceeded the threshold level for *H. avenae*, which raises concerns. Densities of five *H. avenae* or *H. filipjevi* J2s per gram of soil were capable of causing economic damage to irrigated wheat crops in India (Singh et al. 2009). Notably, the occurrence of *H. filipjevi* has been increasing rapidly in China. *H. filipjevi* was first reported in wheat in Henan Province by Peng et al. (2010). Our results show that *H. filipjevi* is found in Henan, Anhui, Hebei, Shandong, Shanxi, and Qinghai provinces (Table 2 and Fig. 1c). In particular, the occurrence of *H. filipjevi* in Xuchang city, Henan Province, has seriously affected the yield of wheat in the area. Its coverage area has expanded and shown a tendency to gradually replace *H. avenae* (Shao et al. 2022b). In summary, we found that CCN populations have rapidly increased in China. CCN has become a vital pest affecting wheat crops and is a severe threat to the productivity of Chinese cereal crops.

To date, many target genes have been used for the identification and phylogenetic analysis of plant parasitic nematodes, including rDNA-ITS, the intergenic spacer region (IGS), 28S D2–D3, satellite DNA (satDNA), and mt DNA (Piotte 1995; Stanton et al. 1997; Wishart et al. 2002; Ye et al. 2015; Vallejo et al. 2021). Mitochondrial DNA is characterized by maternal inheritance and a fast rate of evolution; based on phylogenetic analysis of the mt COI gene, *H. avenae* populations in China differ from those in other countries (Subbotin et al. 2019; Qing et al. 2022; Shao et al. 2022a). The internal transcribed spacer regions ITSl and ITS2 do not participate in ribosome formation, are subject to low selection pressure and evolve rapidly, so they can also be used to study the genetic structure of species, subspecies, and even individual populations (Tilahun et al. 2008). Currently, rDNA-ITS sequences of *H. avenae* from many countries are catalogued in NCBI libraries, and the lengths of ITS sequences vary widely due to the inconsistent primers used in previous studies (Additional file 1: Table S2). There are much more gene sequence numbers of ribosomal DNA in *H. avenae* than those of mitochondrial DNA. To maximize the use of sequences in GenBank and analyse the global genetic diversity of *H. avenae*, we performed phylogenetic analyses of ITS1 and ITS2, in which there were significant variations in these two regions. The results indicate that the ITS1 used in this study can distinguish between the *H. avenae* populations in China and those in other countries, while ITS2 cannot. These results are related to the fact that more variable sites are present in the ITS1 region (Table 3). Thus, ITS1 can be used as a target to identify and study the relationships among *H. avenae* populations based on genetic evolution, and the results were consistent with the studies of the mt COI gene (Subbotin et al. 2019; Qing et al. 2022; Shao et al. 2022a). The ITS1 region could potentially be used as a molecular target for high-throughput sequencing to analyse further the genetic relationships among CCN populations.

In our haplotype results, 70 tested populations were obviously clustered into three different groups, including Group 1 (China and Australia), Group 2 (Middle East), and Group 3 (Europe and Americas) (Fig. 4a). In this study, the Australian populations were clustered with the Chinese populations based on the results of the phylogenetic tree and haplotype structure, which is consistent with the results of Shao et al. (2022a), once again showing that the *H. avenae* populations in China and Australia may have a common origin. Several authors believe that the Middle East and Irano-Anatolian hotspots, which overlap with the distribution area of Group 2, were likely the primary locations in which the *H. avenae* group originated and diversified (Bekal and Gauthier 1997; Subbotin et al. 2018). Based on previous reports and our current results, we speculated that *H. avenae* populations spread eastwards from the Middle East to China and Australia and westwards to Europe and the United States. Qing et al. (2022) speculated that *H. avenae* populations spread worldwide mainly through the wheat trade. Furthermore, the principal component analysis indicated that the Chinese and Australian populations were significantly

different from the European and Middle Eastern groups (Fig. 4b). Previous results confirm that geographic isolation may be the main cause of the genetic differentiation in *H. avenae* populations (Shao et al. 2022a). Interestingly, significant genetic differences exist between Asian populations and European populations, which may occur because the Himalayas and the Kunlun Mountains in Asia formed a barrier that resulted in the formation of a separate evolutionary group in China; in contrast, no obvious geographical isolation of *H. avenae* populations took place between the Middle East and the European countries. Similarly, effective isolation from oceans and mountains has affected the spread of wheat powdery mildew, allowing for significant genetic differences between populations in different regions (Sotiropoulos et al. 2022).

Significant differences were observed in *H. avenae* populations isolated from weeds and wheat hosts (Subbotin et al. 2018; Qing et al. 2022). Host plants of *H. avenae* include wheat, barley, oat, rye, and many other cereal plants (Smiley et al. 2017). The phylogenetic and haplotype network results of ITS rRNA genes in our study indicated genetic differences among *H. avenae* populations isolated from different hosts, including *H. vulgare*, *A. sativa*, *T. aestivum*, *A. chinensis,* and weeds. *H. avenae* populations isolated from weeds are distinct from *H. avenae* populations isolated from other hosts, and this result is consistent with that from a previous study (Qing et al. 2022). Notably, our results indicated that *H. avenae* populations from different hosts are clustered on their respective branches, suggesting a strong host bias in the genetic evolution of *H. avenae* populations. Interestingly, the haplotype (Hap12) for the wheat-hosted *H. avenae* population in this study was located at the centre of all haplotypes, sharing Hap5 and Hap12 with *A. sativa* and *A. chinensis,* and *H. avenae* populations from *H. vulgare* were clustered separately. We confirmed that the high host diversity of *H. avenae* in China is a major factor contributing to the rich genetic diversity of *H. avenae* populations.

## Conclusions

The results of the present study show that cereal cyst nematodes are found in the main wheat-producing areas of China, their occurrence regions are expanding, and the degree of damage they cause is increasing rapidly, and these issues require urgent attention. In addition, based on the ITS1 results, it is speculated that the Chinese *H. avenae* populations may have originated in the Middle East, and the significant genetic differences observed between the Middle Eastern and Chinese populations were caused by geographic isolation. Moreover, the identity of the plant species that host cereal cyst nematodes is also a major factor leading to genetic differentiation in these pests. This work provides a theoretical basis for future research on the origin, spread, and evolution of *H. avenae* in China and worldwide.

## Methods

### Sample collection and nematode isolation

From 2019 to 2022, 821 samples were collected in 78 cities in 16 provinces in China. Soil samples were collected from spring wheat fields in late June or early July and from winter wheat fields in late October or early November. Each sample consisted of a composite of 3 shovel slices of soil approximately 20 cm deep obtained from a location close to the roots of cereal hosts (Wen et al. 2019). Ten samples were collected from each wheat field and mixed evenly, approximately 2 kg in total. The soil samples were placed in plastic sample bags and air-dried at room temperature until use. The locations of the sampling sites were recorded using a global positioning system (GPS) receiver.

A cyst isolation method optimized based on a traditional sieving-decanting method described by Persmark et al. (1992) was used. Tap water was added to approximately 100 $cm^3$ of soil from each sample, and the soil particles were allowed to settle for 2 min. The supernatant containing floating cysts was decanted into another container. The decanting process was repeated three times, and the final solution was passed through two sieves (250-μm-pore sieve at the bottom and 1-mm-pore sieve on the top) by a high-pressure water spray. The cysts and other debris that collected on the bottom sieve were filtered onto a 90-mm-diameter Whatman filter paper disk (GE Healthcare, Chicago, IL) and transferred to a 100 mm × 15 mm petri dish (Fisher Scientific, Hampton, NH) (Wen et al. 2019). The cysts were picked under a dissecting microscope for DNA extraction.

The population density was calculated as the average number of cysts per 100 $cm^3$ of soil; population range was determined as the minimum and maximum densities of cysts per 100 $cm^3$ of soil; and prevalence (%) was estimated by the formula: (number of samples with cysts/total number of samples collected) × 100%. ArcGIS software (10.8.1) (Hayes et al. 2004) was used to assign values to all sampling sites and the number of CCNs in survey sites in each province. A distribution map of all samples and a map of CCNs were constructed.

### DNA extraction, PCR amplification, and sequencing

A population was defined as five cysts obtained from one site. DNA was extracted from a single cyst using a method described by Ou et al. (2008). The universal primers TW81 (5′- GTTTCCGTAGGTGAACCTGC-3′) and AB28 (5′- A TATGCTTAAGTTCAGCGGGT -3′) (Joyce et al. 1994) were used to amplify the rDNA-ITS

region of all samples, which contained ITS1, 5.8S, and ITS2 regions. The PCR amplification mixture contained 5 µL 10×PCR buffer, 10 µL 2.5 mM dNTPs, 1 µL ExTaq DNA polymerase (5 U/µL) (Takara-Bio, Shiga, Japan), 2 µL 10 µM each primer, 2 µL template DNA, and double-distilled water ($ddH_2O$) to a total volume of 50 µL. The thermocycler was operated under the following conditions: 94°C for 4 min, 35 cycles at 94°C for 1 min, 53°C for 1 min, and 72°C for 2 min, followed by 72°C for 10 min, and 4°C at the end. The PCR products were purified with a gel extraction kit (Tiangen Biotech, Beijing, China), cloned into the pMD19-T vector (Takara Bio, Beijing, China), and transformed into DH5a competent cells. Three strains were selected for cloning from each cyst and were sequenced by BGI Tech Solutions Co., Ltd. (Beijing, China).

### Phylogenetic analysis

First, the ITS sequences of 111 Chinese populations and 6 populations from other countries obtained in this study were combined with 9 published ITS sequences of *H. avenae* from GenBank for phylogenetic analysis (Additional file 1: Table S1). Second, to understand the conserved regions of the ITS1 and ITS2 sequences in the rDNA ITS of *H. avenae*, phylogenetic trees were constructed for the ITS1 and ITS2 sequences of the above populations. This study analyzed the genetic relationships among *H. avenae* populations from different hosts, including seven populations from *H. vulgare*, three from *A. sativa*, eight from *T. aestivum*, two from *A. chinensis*, and four from weeds (Additional file 1: Table S1). Phylogenetic analysis was performed by extracting sequences using PhyloSuite v1.2.2 (Zhang et al. 2020) and then aligning the sequences in batches using MAFFT v7.313 (Katoh and Standley 2013). The optimized data were used for optimal model selection by Model Finder (Kalyaanamoorthy et al. 2017). Bayesian inference with a GTR model for ITS sequences was performed in the MrBayes plugin in PhyloSuite. MrBayes was run with default settings and $5\times10^6$ metropolis-coupled MCMC generations. The topologies were used to generate a 50% majority-rule consensus tree. Trees and posterior probabilities were visualized with FigTree v1.4.2 (Rambaut 2018).

### Analysis of haplotype

The ITS sequences from different hosts were determined using DnaSP V5 software (http://www.ub.edu/dnasp/). The ITS1 sequences of 15 Chinese populations and 55 populations from 14 other countries downloaded from GenBank were also analyzed by DnaSP V5 (Additional file 1: Table S2). The phylogenetic relationships among all haplotype sequences of *H. avenae* were estimated from a TCS network using PopART v1.7 (Leigh and Bryant 2015). The SNPs of the sequences were calculated for PCoA clustering using the biostrings package in R (v. 4.1.3) (Jombart et al. 2010).

#### Abbreviations

| | |
|---|---|
| CCNs | Cereal cyst nematodes |
| IGS | Intergenic spacer region |
| ITS | Internal transcribed spacer |
| mt DNA | Mitochondrial DNA |
| RAPD | Random amplified polymorphic DNA |
| RFLP | Restriction fragment length polymorphism |
| SatDNA | Satellite DNA |

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s42483-023-00185-x.

**Additional file 1**: **Table S1**. Heterodera avenae samples from wheat-growing areas used in this study. **Table S2**. GenBank accession number information for ITS sequences from Heterodera avenae from different countries.

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#### Authors' contributions

HS and LZ wrote the original draft. ZL collected samples, HS and RJ performed the experiments, and HS, ZL, LZ, HP, WH, and LK analysed the data. HP, SL, DP, and CL wrote, reviewed, and edited the manuscript. All authors read and approved the final manuscript.

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#### Availability of data and materials

The rDNA-ITS gene sequences of *Heterodera avenae* are available in GenBank (OP216909-P217045).

## Declarations

#### Ethics approval and consent to participate

Not applicable.

#### Consent for publication

Not applicable.

#### Competing interests

The authors declare that they have no competing interests.

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