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Molecular characterization of internal transcribed spacer (ITS) of ribosomal RNA gene, haplotypes and pathogenicity of potato rot nematode *Ditylenchus destructor* in China

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Abstract

Potato rot nematode *Ditylenchus destructor* is one of the most damaging pests in potato-producing regions and causes severe yield losses worldwide. However, *D. destructor* has been rarely reported in potato crops in China. We collected 542 samples from 17 Chinese provinces during 2016–2020 for the detection of *D. destructor* using species-specific primers and universal primers targeting ribosomal internal transcribed spacer (ITS) or 28S ribosomal RNA (rRNA) sequences. *D. destructor* was detected in 14.94% of total samples, primarily in Inner Mongolia Autonomous Region (43 sites), Jilin Province (12 sites), and Shaanxi Province (9 sites). The nematode was for the first time detected in Guizhou Province and Ningxia Hui Autonomous Region. Phylogenetic analysis of 33 ITS sequences along with the prediction of the secondary structure of the helix H9 of ITS1 sequences revealed that haplotypes A, B, and C accounted for 6.06%, 9.09%, and 69.70% of our detections, respectively, while 15.15% were comprised of haplotypes H–L, and extensive genetic diversity of ITS sequences was detected in samples from Gansu Province. Using RNAfold software, we analyzed the haplotypes of 124 Chinese *D. destructor* populations based on their ITS sequences, and the results showed that haplotypes A, B, C, E, F, and new haplotypes M–P accounted for 49.19%, 12.10%, 15.32%, 3.23%, 1.61%, and 18.55% of the total sequences, respectively. Infection tests on the potato cultivar 'Helan 15' showed significant difference in infection capacity among different *D. destructor* populations. The information obtained in this study on spatial patterns of *D. destructor* haplotypes in China provides valuable insights into the development of an integrated approach for the management of this plant-parasitic nematode.

Keywords: *Ditylenchus destructor*, Distribution, Haplotype, Infection

Background

Potato (*Solanum tuberosum* L.) is the third major food crop in the world after rice and wheat and plays a crucial role in global food security (Mackay 2009; Hancock et al. 2014). The total annual potato production in China will be increased to 220 million tons by 2025 (www.chinabgao.com). During their growth and development, potato plants are often attacked by many plant-parasitic nematodes, which feed on tubers or stems to cause direct or indirect yield losses (Mugniéry and Phillips 2007). Potato rot nematode *Ditylenchus destructor* Thorne (Thorne 1945), commonly known as a stem nematode, is the second-ranking nematode pest of potato after the potato cyst nematode and usually causes significant yield losses,

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especially in temperate climate zones (Plowright et al. 2002). In addition, *D. destructor* can infect sweet potato, sugar beet, carrot, *Angelica sinensis, Codonopsis pilosula*, and several other crops (EPPO 2013; FAO 2015). Potato and sweet potato plants are usually discolored after infected by *D. destructor*, and the underground tubers become rotten (Thorne 1945). *D. destructor* is a quarantine pest in many countries (Kruus 2012).

In 1931, *D. destructor* was first found attacking potatoes in Prince Edward Island, Canada (Baker 1946). Then, the damage caused by *D. destructor* to potato plants was found in Europe, South Africa, Central Asia, and North America (EPPO, https://gd.eppo.int/taxon/DITYDE/distribution). In China, *D. destructor* was first detected in sweet potatoes in Hebei Province in 1937 (Fan et al. 1999) and was subsequently reported in other sweet potato-producing areas of Beijing, Henan, and Anhui provinces (Yu et al. 2009). Infestation of potato plants by *D. destructor* was first found in Hebei Province in 2006 (Liu et al. 2006) and now has been reported in many regions in China. Potato yield losses caused by *D. destructor* generally reach 20% to 50%, and even 100% in endemic regions. In China, the planting area of potato has exceeded 4200 hectares only in Chongqing, Gansu, Guizhou, Shaanxi, Shanxi, Sichuan, and Yunnan provinces (National Bureau of Statistics of the People's Republic of China 2017). Despite the economic importance of potato in China, there has been no comprehensive survey of *D. destructor* on this crop.

Accurate identification of a pathogen is critical for understanding the occurrence of the disease caused by this pathogen and to develop effective control measures accordingly. *Ditylenchus* spp. are very similar in morphology but differ in molecular characteristics (Brodie et al. 1993; Wendt et al. 1993; Vovlas et al. 2011), thus it is essential to develop more molecular detection methods to differentiate between species of the genus. Diverse molecular tools provide better support for accurate and fast identification of the species under investigation. Ribosomal RNA (rRNA) genes have been widely used for species identification, including *Ditylenchus* spp.. *D. destructor* populations exhibit a high level of intraspecific variations in ITS regions and 28S rRNA gene sequences. In the previous studies, 22 *D. destructor* populations were divided into two different types, type A (940 bp) and type B (1100 bp), based on their ITS sequences (Wan et al. 2008); through phylogenetic analysis based on the sequences of D2/D3 regions of 28S rRNA, those 22 *D. destructor* populations were also classified into types A and B (Yu et al. 2009). Following the proposal by Wan et al. (2008), Subbotin et al. (2011) kept the haplotypes A and B and identified 5 additional haplotypes, i.e. haplotypes C, D, E, F, and G, based on the secondary structure of ITS1-H9. Meanwhile, they clustered 78 ITS rRNA gene sequences of *D. destructor* into two main clades based on the presence or absence of the repetitive elements in the ITS1. Additionally, the species-specific primer pair D1/D2 was developed, which can detect all haplotypes of *D. destructor* (Liu et al. 2007); two primer pairs: DdS1/DdS2 and DdL1/DdL2 for the detection of haplotype A and all other haplotypes, respectively, were developed by Wan et al. (2008); another pair of primers (Des2-F/Des1-R) that can also detect all haplotypes of *D. destructor* except haplotype A was designed by Marek et al. (2010). In this study, we used the species-specific primer set D1/D2 (Liu et al. 2007) and ITS-rRNA sequences to identify and study the genetic diversity of *D. destructor*.

The objectives of this study were to survey the occurrence and distribution of *D. destructor* in potato-producing regions in China, to analyze the genetic diversity of the nematode populations in ITS sequences, and to evaluate population pathogenicity on potato plants. The data on the occurrence and genetic diversity of *D. destructor* in potato fields obtained in this study will help understand the evolutionary processes of the populations, including the development of haplotypes and adaptation to selection pressure. The results provide insights into the effective use of resistant potato cultivars as a strategy for management of this plant-parasitic nematode.

Results

Geographic distribution of *D. destructor* in China

In 2016–2017, we collected a total of 158 soil samples from different potato-producing regions of 7 provinces/autonomous regions in China (72 from Jilin, 2 from Liaoning, 55 from Inner Mongolia, 22 from Qinghai, 2 from Xinjiang, 4 from Yunnan, and 1 from Guizhou). *D. destructor*-positive samples were identified based on the morphological characteristics and molecular detection (specific amplicons) of the nematode. *D. destructor* was detected in a total of 45 samples (28%), all containing more than 30 individuals per 100 g soil (Additional file 1: Table S1). Of the 33 positive samples collected from Inner Mongolia Autonomous Region, three contained 30 to 100 individuals per 100 g soil, while the remainder contained more than 100 individuals per 100 g soil. Of the 9 positive samples collected from Jilin Province, three contained 30 to 100 individuals per 100 g soil, with more than 100 individuals per 100 g soil in the remainders. All three positive samples collected from Qinghai Province contained over 100 individuals per 100 g soil.

In 2018–2020, a total of 384 samples (one sample from each site) were collected from potato-producing regions, and it was shown that 36 sampling sites were infested by *D. destructor* through morphological identification and PCR detection. No nematodes were detected in Anhui,

Beijing, Henan, Qinghai, Shanxi, and Yunnan, with 4, 3, 16, 14, 4, and 6 sampling sites, respectively. However, one potato tuber sample in 12 sampling sites of Gansu, 3 samples in 38 sites of Guizhou, one sample in 17 sites of Hebei, 2 samples (one was potato tuber) in 7 sites of Heilongjiang, 10 samples (4 were potato tubers) in 148 sites of Inner Mongolia, 3 samples in 39 sites of Jilin, 2 samples (one was potato tuber) in 13 sites of Liaoning, 4 samples in 47 sites of Ningxia Hui Autonomous Region, one potato tuber sample in Shandong, and 9 samples (2 were potato tubers) in 15 sites of Shaanxi, were infested by nematodes. Of 36 positive samples, on the average, 15 contained 2 to 6 individuals per 100 g soil, 11 had 20–70 individuals per 100 g soil, and 10 had over 100 individuals per 1 g potato (Additional file 1: Table S1). Morphological characteristics of *D. destructor* recovered from infected potato tubers are shown in Fig. 1.

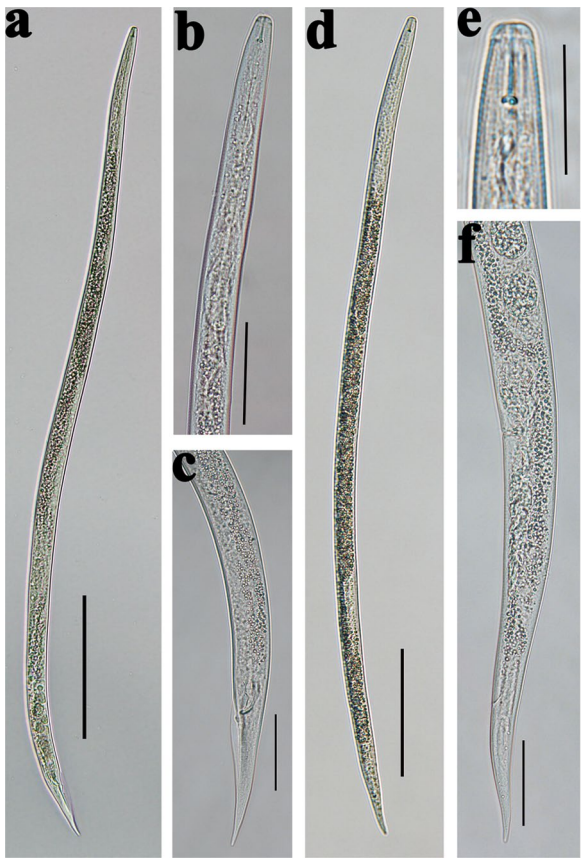

Fig. 1 Microscopic observation of *Ditylenchus destructor*. **a** Entire male. **b** Male anterior region. **c** Spicule and partial tail of male. **d** Body of juvenile. **e** Female anterior region. **f** Vulva and partial tail of female. Scale bars in **a**, **b**, **c**, **d**, **e**, and **f** are 200, 50, 50, 100, 20, and 50 µm, respectively

Phylogenetic analysis of *D. destructor*

D. destructor-positive DNA samples, which had been successfully detected by species-specific primer pair, were amplified with universal primers targeting ITS region or 28S rRNA gene of the nematode. Thirty-three ITS sequences and one 28S-rRNA sequence (GenBank accession number OK330255) were obtained from 29 samples collected during 2018–2020. All amplicons were sequenced and blasted against the sequences of *D. destructor* that were already present in the GenBank database.

To distinguish different haplotypes of *D. destructor*, phylogeny analysis was performed based on the ITS rRNA gene sequences. All 33 ITS sequences obtained in this study were nested within the clade of the *D. destructor* complex, which was highly supported by the Bayesian posterior probability values (Fig. 2). In addition to the previously described three haplotypes (haplotypes A, B, and C), five new haplotypes (haplotypes H, I, G, K, and L) were also included in this clade. Among them, two were haplotype A (6.06%), three were haplotype B (9.09%), twenty-three were haplotype C (69.70%), and the five new haplotypes (haplotypes H–L) comprise 15.15% of the total. Thus, haplotype C is a dominate type among the *D. destructor* populations isolated from the potato. Representative *D. destructor* isolates with different haplotypes detected in this study were deposited in the National Center for Biotechnology Information (NCBI) GenBank database under the accession numbers shown in Table 1. Four samples (JL18-35, GS18-11, NM19-15, and ShaanX18-12) showed mixed infection by two or three haplotypes (Table 1).

We analyzed the ITS rRNA gene sequences of 156 *D. destructor* isolates obtained from the NCBI database, after removing those sequences that have no information on host plants or locations. We first analyzed the 124 ITS sequences of *D. destructor* from China, among which 61 (49.19%) belonged to haplotype A and were isolated from sweet potato (43), potato (1), *Angelica sinensis* (10), *Codonopsis pilosula* (2) and an unknown host (5); fifteen (12.10%) belonging to haplotype B were isolated from sweet potato (6), potato (7), and *Codonopsis pilosula* (2); nineteen (15.32%) belonging to haplotype C were isolated

(See figure on next page.)

Fig. 2 Phylogenetic tree of *Ditylenchus destructor* based on 33 ITS-rRNA sequences obtained in this study and 27 new haplotype sequences in China from NCBI. The 50% majority-rule consensus tree from Bayesian analysis was generated from the ITS-rRNA gene sequence dataset for *D. destructor* using the GTR+I+G model. *D. myceliophagus* and *D. dipsaci* were used as outgroups. The scale bar indicates the number of expected changes per site. The haplotypes are color-marked. The sequences obtained in this study are in bold font

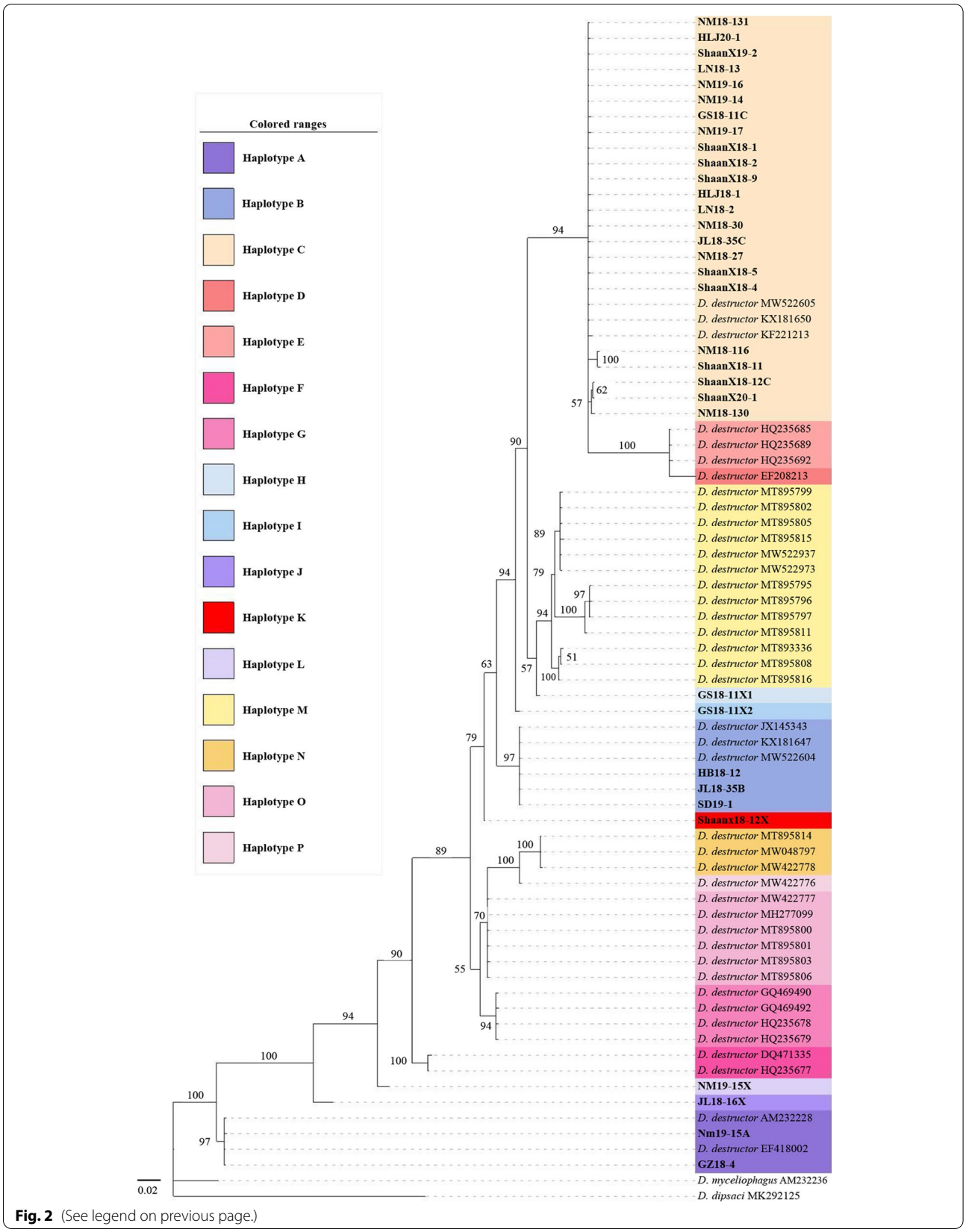

Fig. 2 (See legend on previous page.)

Table 1 The haplotypes of *Ditylenchus destructor* isolates collected from potato fields in this study

Isolate code	Geographic location	GenBank accession No.	Haplotype
HB18-12	Zhangjiakou City, Hebei Province	OK348890	B
LN18-2	Beizhen City, Liaoning Province	OK348884	C
LN18-13	Chaoyang City, Liaoning Province	OK348872	C
JL18-16X	Yanbian Prefecture, Jilin Province	OK348866	J
JL18-35B	Changchun City, Jilin Province	OK348891	B
JL18-35C	Changchun City, Jilin Province	OK348886	C
GS18-11C	Zhangye City, Gansu Province	OK348875	C
GS18-11X1	Zhangye City, Gansu Province	OK348863	I
GS18-11X2	Zhangye City, Gansu Province	OK348864	H
NM18-27	Chifeng City, Inner Mongolia	OK348887	C
NM18-30	Ulanqab City, Inner Mongolia	OK348885	C
NM18-116	Tongliao City, Inner Mongolia	OK348881	C
NM18-130	Ordos City, Inner Mongolia	OK348868	C
NM18-131	Ordos City, Inner Mongolia	OK348867	C
NM19-14	Xilinhot, Inner Mongolia	OK348874	C
NM19-15A	Chifeng City, Inner Mongolia	OK348893	A
NM19-15X	Chifeng City, Inner Mongolia	OK348895	L
NM19-16	Ulanqab City, Inner Mongolia	OK348873	C
NM19-17	Ordos City, Inner Mongolia	OK348876	C
ShaanX18-1	Yulin City, Shaanxi Province	OK348878	C
ShaanX18-2	Yulin City, Shaanxi Province	OK348879	C
ShaanX18-4	Yulin City, Shaanxi Province	OK348889	C
ShaanX18-5	Yulin City, Shaanxi Province	OK348888	C
ShaanX18-9	Yulin City, Shaanxi Province	OK348882	C
ShaanX18-11	Yulin City, Shaanxi Province	OK348880	C
ShaanX18-12C	Yulin City, Shaanxi Province	OK348877	C
ShaanX18-12X	Yulin City, Shaanxi Province	OK348865	K
ShaanX19-2	Yulin City, Shaanxi Province	OK348871	C
ShaanX20-1	Yulin City, Shaanxi Province	OK348869	C
GZ18-4	Liupanshui City, Guizhou Province	OK348894	A
SD19-1	Linyi City, Shandong Province	OK348892	B
HLJ20-1	Suihua City, Heilongjiang Province	OK348870	C
HLJ18-1	Qiqihar City, Heilongjiang Province	OK348883	C

from sweet potato (3), potato (15), and carrot (1); four (3.22%) belonging to haplotype E were isolated from sweet potato (1) and carrot (3); two (1.61%) belonging to haplotype F were isolated from sweet potato (1) and *Astragalus mongholicus* (1); Twenty-three sequences were identified as four new haplotypes M, N, O, and P, and the host plants were *Angelica sinensis* and *Codonopsis pilosula* (Figs. 2, 3 and Additional file 2: Figure S1). Subsequently, we analyzed the rest 32 sequences from other countries, among which one (AY987007) isolated from potato belonged to Haplotype B; seven (JX162205, DQ151459, JN376068, KC923223, KC923224, HQ235675, and HQ235676) belonged to haplotype C, with one from garlic and the other six from potato; thirteen (JN166693, HQ235692, HQ235694, HQ235690, HQ235696, HQ235687, HQ235688, HQ235689, HQ235695, HQ235685, HQ235686, HQ235691, and HQ235693) from potato belonged to haplotype E; ten (GQ469491, GQ469490, GQ469492, HQ235678, HQ235679, HQ235680, HQ235683, HQ235681, HQ235682, and HQ235684) from potato belonged to haplotype G; and one from sweet potato (EF208213) belonged to haplotype D. The results showed that haplotypes A (61, 49.19%), B (15, 12.10%), and C (19, 15.32%) were distributed most widely in China; however, haplotypes C (7, 21.88%), E (13, 40.63%), and G (10, 31.25%) were observed most frequently in other countries. In China, the main haplotypes of *D. destructor* infecting potato were haplotypes B (7) and C (15), whereas haplotype A (43) was the major haplotype of *D. destructor* infecting sweet potato (Fig. 4).

Haplotypes of *D. destructor* in China

In this study, the populations of *D. destructor* in Anhui, Beijing, Henan, Guizhou, and Xinjiang Uygur Autonomous Region were identified as haplotype A, while those in Heilongjiang and Liaoning were identified as haplotype C. Four haplotypes A, B, C, and L were detected in Inner Mongolia, with haplotype C being the most frequent haplotypes. Haplotypes A and B were detected in Hebei. Gansu had nine haplotypes (A, B, C, F, H, I, M, N, and O), with haplotype B comprising 66.7% of the isolates. Jilin, Jiangsu, Shandong, Shaanxi, Qinghai, and Shanxi had four (A, B, C, and J), three (A, B, and C), five (A, B, C, E, and F), three (A, C, and K), three (N, O, and P), and two (A and N) haplotypes, respectively (Figs. 2, 4). The new haplotypes identified in Jilin, Inner Mongolia, Gansu, Qinghai, Shanxi, and Shaanxi differed from those reported previously (Subbotin et al. 2011). Gansu had a highly diverse collection of haplotypes including five new haplotypes, indicating that the *D. destructor* populations in this province were genetically diverse.

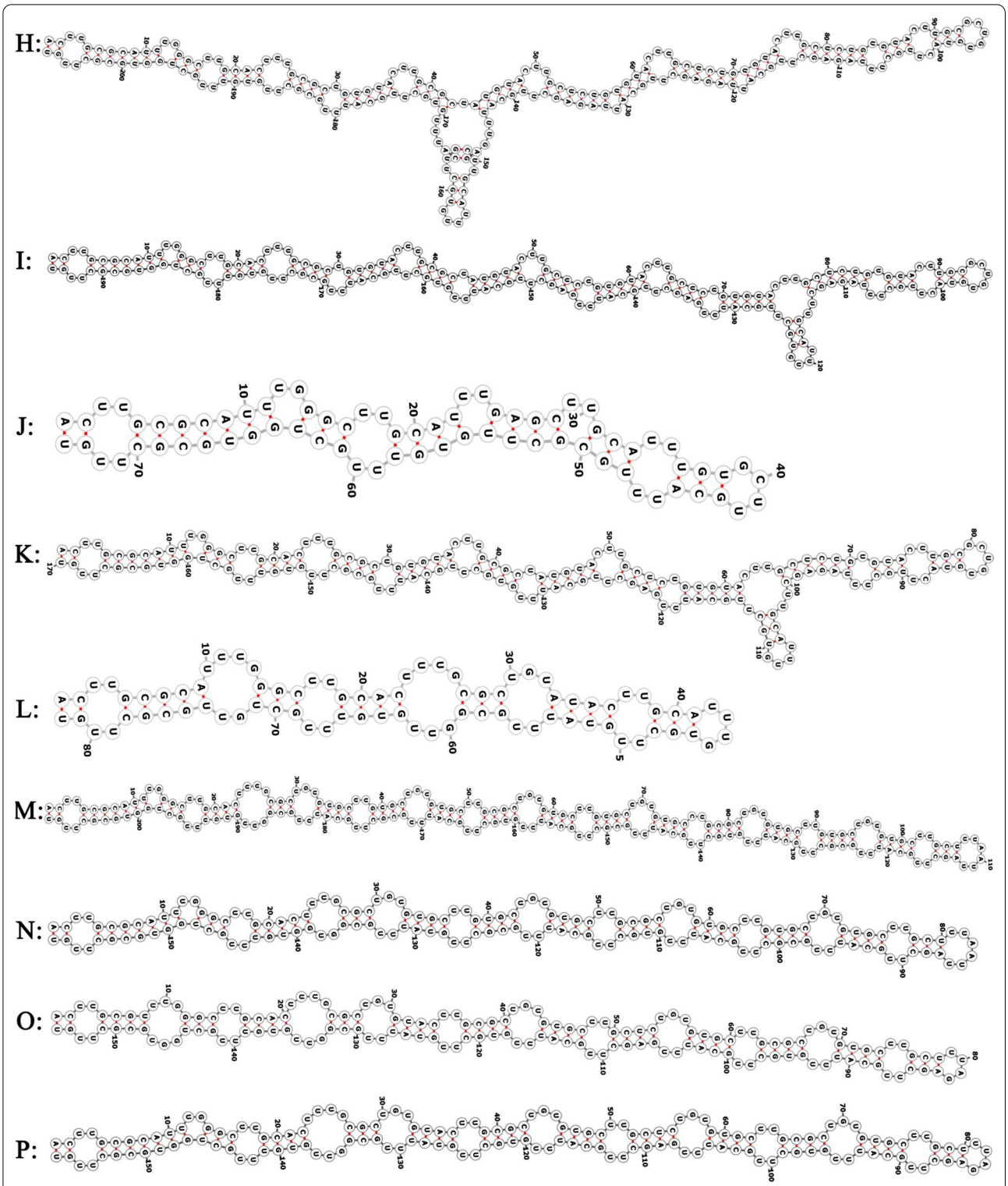

Fig. 3 New types of helix H9 of ITS1 of *Ditylenchus destructor*

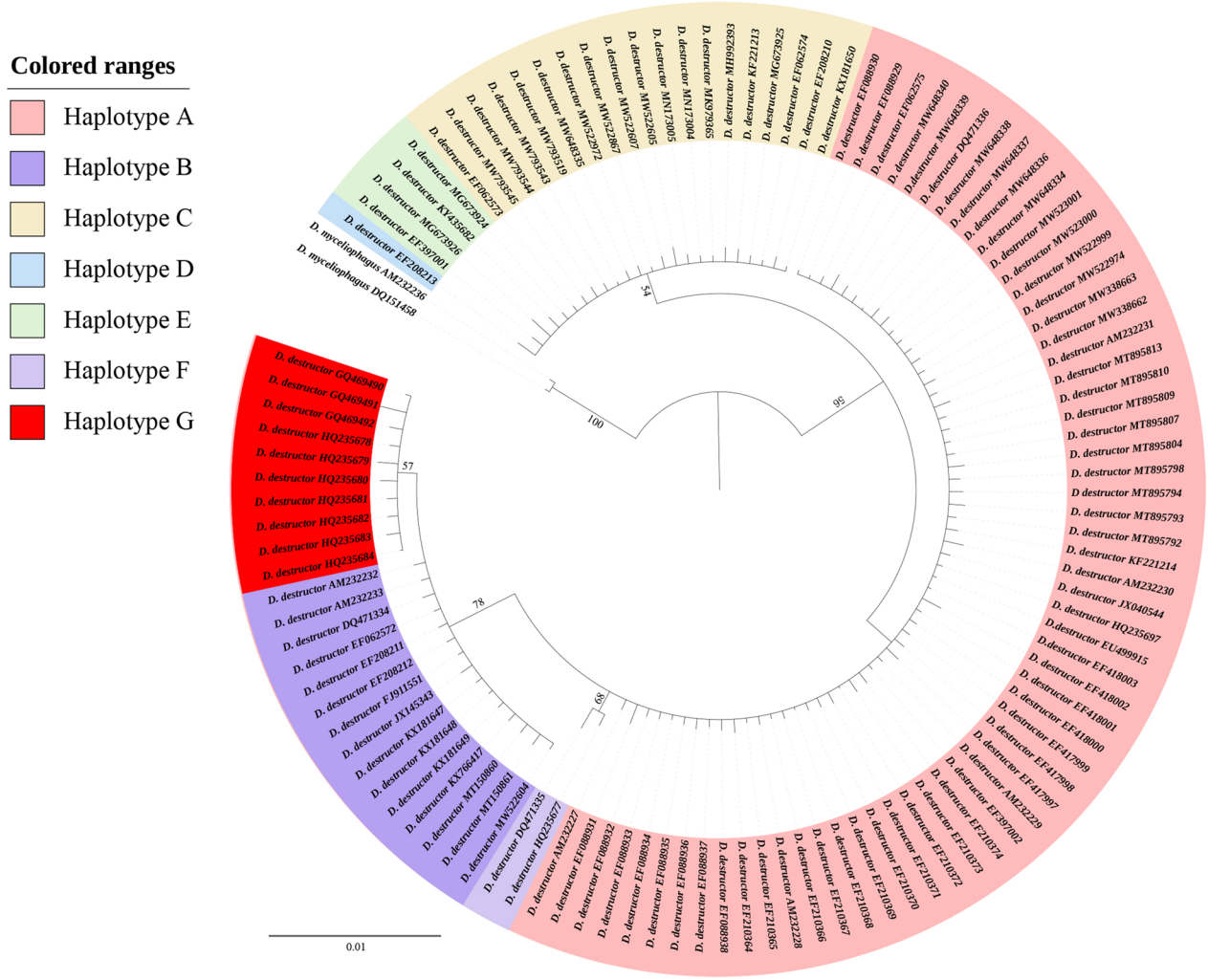

Fig. 4 Phylogenetic tree based on 101 ITS-rRNA sequences of *Ditylenchus destructor* from China. The sequence data were obtained from NCBI (https://www.ncbi.nlm.nih.gov). The 50% majority-rule consensus tree from Bayesian analysis was generated from the ITS-rRNA gene sequence dataset for *D. destructor* using the HKY+I model. *D. myceliophagus* was used as an outgroup. The scale bar indicates the number of expected changes per site. The haplotypes are color-marked as given in the legend

Infection test of *D. destructor* on potato

Not all the *D. destructor* populations we obtained in this study can infect the potato cultivar 'Helan 15'. Pathogenic *D. destructor* populations induced typical soft white spots, dry rot, and papery, cracked skin on potato tubers (Fig. 5), and ITS rRNA sequences of nematodes isolated from all the symptomatic potatoes were identical to those of the populations used as inoculum.

Significant difference ($P < 0.05$) in disease index was produced by the different *D. destructor* populations tested in this study. The results showed that 'Lulong' and 'BX' were the most virulent populations, with an average disease index of 75% and 68.75%, and RF of 23.52 and 5.61, respectively. By contrast, 'PH19-07', 'CX', and 'GS18-11' were significantly less virulent than other populations (Table 2).

Discussion

D. destructor, an economically important plant-parasitic nematode, affects potato production mostly in temperate climates. In China, the gradual increase in potato-producing areas has resulted in outbreaks of *D. destructor* in several provinces/autonomous regions such as Inner Mongolia and Liaoning in the past decade (Ou et al. 2017; Mao et al. 2020). In this study, during a survey of potato nematodes in 2016–2020, a total of 542 samples were collected from 17 main potato-producing provinces

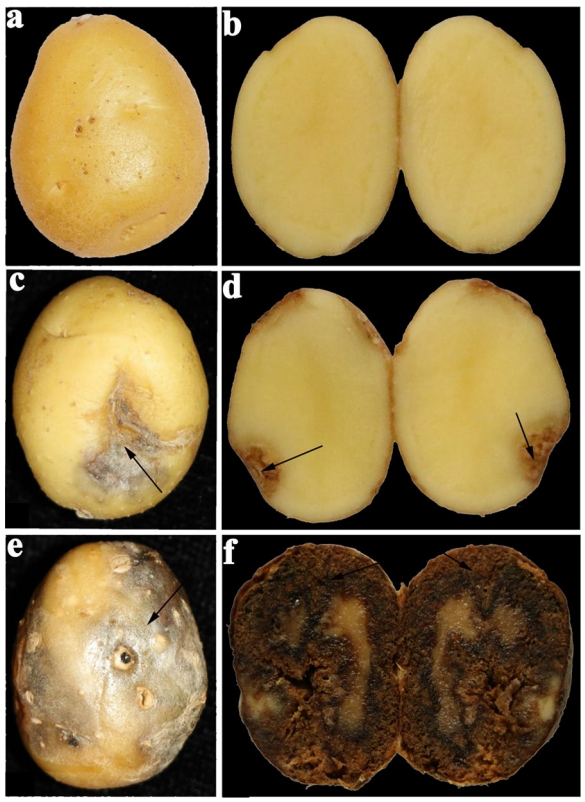

Fig. 5 External and internal symptoms in potato tuber caused by *Ditylenchus destructor*. **a**, **b** Healthy potato tubers. **c**, **d** Potato tubers lightly infected by nematodes, with light-brown and partially rotten interior tissues. **e**, **f** Severely infected potato tubers, with dark-brown and spongy interior tissues. Arrows indicate the typical external and internal symptoms in infected potato tubers

including Jilin and Shaanxi, and Inner Mongolia Autonomous Region, of which 81 samples (14.94%) were *D. destructor*-positive (Additional file 1: Table S2). Meanwhile, *D. destructor* was for the first time detected in Guizhou Province and Ningxia Hui Autonomous Region, China. Previously, the potential distribution areas of *D. destructor* in China were predicted using MAXENT model, and risk levels for occurrence of the nematode in these areas, including Inner Mongolia Autonomous Region, Jilin and Shaanxi provinces, were assessed (Li 2008). *D. destructor* can adapt to low temperature, northern China is therefore suitable for its propagation (Huang et al. 2012). Additionally, Yulin City of Shaanxi Province was also predicted to be a suitable habitat for *D. destructor* (Hong et al. 2017). Gansu Province is a special area with complicated geomorphology and diverse climate regimes where the genetic diversity of *D. destructor* was the highest among all the *D. destructor* populations investigated in this study, and we detected nine haplotypes of ITS sequences in the samples collected from Gansu (Figs. 2, 4 and Table 1). Serious damage would be caused by *D. destructor* when its population density reaches 20–50 individuals per 1000 g soil (Butorina et al. 2006). Our data showed that the density of *D. destructor* during 2016–2020 was no less than 20 individuals per 1000 g soil in China (Additional file 1: Table S1). Accordingly, *D. destructor* has posed a serious threat to potato production in China.

D. destructor produces conical pits along with skin splitting and rotting on potato tubers (Fig. 5, Jenkins and Taylor 1967). In this study, we evaluated the virulence of *D. destructor* isolates collected from 8 provinces

Table 2 Infection test of *Ditylenchus destructor* populations on the potato cultivar 'Helan 15'

Population name	Mean percentage of potatoes with lesions (%)	Disease index*	Final nematode population (Pf)	Reproduction factor (RF)
PH19-07	0	0.00 ± 0.00d	0.00 ± 0.00	0.00 ± 0.00
PH19-30	25	3.13 ± 3.13d	80.00 ± 60.00	0.04 ± 0.03
Lulong	100	75.00 ± 14.43a	47,032.50 ± 19,478.87	23.52 ± 9.74
JT	100	25.00 ± 0.00bc	1187.00 ± 32.30	0.59 ± 0.02
BX	100	68.75 ± 11.97a	11,213.25 ± 5768.64	5.61 ± 2.88
SD19-1	75	12.50 ± 7.22 cd	1295.00 ± 719.72	0.65 ± 0.36
CX	0	0.00 ± 0.00d	0.00 ± 0.00	0.00 ± 0.00
ShaanX18-12	25	6.25 ± 6.25 cd	168.75 ± 153.75	0.08 ± 0.08
NM19-14	75	18.75 ± 6.25bcd	7206.25 ± 3225.80	3.60 ± 1.61
GS18-11	0	0.00 ± 0.00d	0.00 ± 0.00	0.00 ± 0.00
LN18-13	100	33.33 ± 8.33b	2481.67 ± 851.69	1.24 ± 0.18
ShaanX19-2	100	25.00 ± 0.00bc	1090.75 ± 2263.67	0.56 ± 0.43
CK	0	0.00 ± 0.00d	0.00 ± 0.00	0.00 ± 0.00

* Data are shown with mean and standard error among samples. Values in a column followed by the same letter are not significantly different from the control treatment at $P < 0.05$

of China on potato and sweet potato and biologically characterized 12 geographic *D. destructor* populations, which exhibited significant difference in genotypes and pathogenicity (Table 2; Additional file 1: Table S3). Previously, it was revealed that the *D. destructor* populations from Hebei Province were more virulent than those from Henan and Jiangsu provinces (Ji et al. 2006), and the population 'DeHB' was more virulent than populations 'DeJJ' and 'DeLY' on sweet potato (Wang 2009). In addition, the same *D. destructor* population was found to produce multiple symptoms on potato plants depending on potato variety, initial population density of the nematode, temperature, and soil moisture, as such factors can influence the distribution and behavior of *D. destructor* and eventually the incidence and severity of potato rot caused by this nematode (Fiers et al. 2012; Ivanyuk and Ilyashenko 2008). Therefore, the reported results may not reflect the true potential virulence of these populations. Potato tubers infested by *D. destructor* are generally the primary source of potato rot, thus transportation of *D. destructor*-infested seed potatoes can promote the spread of the disease especially when light infestation occurred. In this case, the infested potatoes present healthy appearance and are easily neglected by a visual check (Kikas 1969), the nematode populations are thus transmitted to other potato-producing areas. *D. destructor* may be spread not only by planting materials but also by farm operations, such as ploughing and irrigating (PLH 2014). The results of our survey in this study clearly indicate a broad distribution of *D. destructor* in China (Additional file 1: Tables S1, S2). Therefore, as described by EFSA Panel on Plant Health (PLH) (2014), farmland management and plant quarantine practices such as the utilization of certified planting material and disinfested farm equipment and machinery, and avoidance of flood irrigation should be encouraged to prevent introduction and further spread of this nematode into new potato-producing regions.

High genetic diversity of *D. destructor* populations has been detected in China according to the restriction map of ITS and D2/D3 regions or using an inter-simple sequence repeat (ISSR) molecular marker (Yang and Peng 2005; Huang et al. 2012). In this study, as it showed based on the haplotypes of ITS sequences, an abundant genetic diversity existed in *D. destructor* populations in China (Table 1). We identified several different haplotypes of ITS sequences of *D. destructor* including 5 new haplotypes (H–L) that can feed on potato, and some samples were even infected by a mix of two or three haplotypes (Table 1), which may be related to genetic exchange among different haplotypes of *D. destructor* populations and subsequent migrations of this nematode.

Conclusions

D. destructor is a migratory plant-parasitic nematode widely distributed in temperate regions. This nematode can parasitize more than 100 species of plants and weeds, such as potato, sweet potato, carrot, *Codonopsis pilosula,* and *Angelica sinensis*. This study is the first to uncover the occurrence and geographical distribution of *D. destructor* in China. The genetic diversity of *D. destructor* populations on different host plants was examined and the variation in pathogenicity was detected. To the best of our knowledge, this is the first report of *D. destructor* infesting potatoes in Guizhou Province and Ningxia Hui Autonomous Region in China. Surveys of the occurrence and distribution of this crop-parasitic nematode by precise detection and an understanding of its population diversity are crucial for making phytosanitary decisions as well as for breeding resistant cultivars.

Methods

Sample collection and nematode isolation

An approximately 0.5 kg of soil was obtained from each sampling site using the five-spot sampling method. The locations of the sampling sites were recorded using a Global Positioning System (GPS) receiver. Rhizosphere soil or potato tuber samples were collected during the entire growing season. In 2016–2020, a total of 542 soil samples and potato tuber samples were collected from different potato fields in China (Additional file 1: Table S1). We isolated nematodes from 100 g (dry weight) soil samples in an Oostenbrink dish (Oostenbrink 1960). The nematodes were settled in a glass beaker and the supernatant was removed. Nematodes was counted under an inverted microscope and individual specimens were transferred to a microscope slide with a needle for extraction of DNA. *D. destructor* was identified by general morphology and molecular identification (FAO 2015). Males and females are similar in general appearance: cuticular and head annulation fine, head often narrower than adjacent body; stylet length 10–12 μm, stylet cone round and sloping backwards, median bulb muscular; excretory pore opposite oesophageal glands; lips of vulva thick, elevated; tail of both sexes conical, usually ventrally curved, terminus rounded (FAO 2015). Additionally, the density of *D. destructor* was estimated by counting the number of individuals in 100 g soil (dry weight) or in 1 g potato tuber samples.

DNA extraction, PCR amplification and sequencing

Nematode DNA was extracted from single individuals as described by Subbotin et al. (2011). The number of individuals analyzed per population varied depending on the nematode populations found in each sample. If the number of the target nematode was over 5 per 100 g soil,

five nematodes were used for DNA extraction. In brief, a single nematode was washed twice and transferred into a 0.2 mL Eppendorf tube containing 6 µL of double-distilled H_2O, 2 µL of 10 × PCR buffer (TaKaRa, Japan), and 2 µL of proteinase K (600 µg/mL) (Promega, USA). The nematode was chopped into pieces under a stereomicroscope. The tubes were incubated at 65 °C for 90 min and then kept at 90 °C for 10 min.

Species-specific primer pair D1/D2 (Additional file 1: Table S4) was used to identify *D. destructor* following a preliminary morphological identification (Liu et al. 2007). The PCR reaction volume was 25 µL containing 4.0 µL of DNA, 2.5 µL of 10 × PCR buffer (Mg^{2+} was added), 2 µL dNTP (10 mmol/L), 1 µL each of species-specific primers D1 (12.5 µmol/L) and D2 (12.5 µmol/L), 0.5 U *Taq* DNA polymerase (5 U/µL), and 14.4 µL of sterilized ddH_2O. PCR amplification was performed at 94 °C for 4 min; 35 cycles of 1 min at 94 °C, 45 s at 55 °C and 45 s at 72 °C; and then a final extension step of 10 min at 72 °C. PCR products were separated by agarose gel electrophoresis, and the expected product size was 346 bp. The primer pair D3A/D3B (Additional file 1: Table S4) was used as an internal reference with an expected product size of 345 bp under the same amplification conditions.

In 2018–2020, *D. destructor*-positive DNA samples were further amplified with universal primers targeting ITS region or 28S rRNA gene of nematode. The D2A/D3B primer pair (Yu et al. 2009) was used to amplify the D2-D3 sequence of 28s rRNA, and the rDNA1/rDNA2 and AB28/TW81 primer pairs (Liu et al. 2007; Wan et al. 2008) were used to amplify the ITS region (Additional file 1: Table S4). The PCR was performed in a 50 µL reaction volume containing 4.0 µL of DNA, 5 µL of 10 × PCR buffer (Mg^{2+} was added), 4 µL dNTP (10 mmol/L), 1 µL of each forward (12.5 µmol/L) and reverse primer (12.5 µmol/L), 1 U *Taq* DNA polymerase (5 U/µL), and 34.8 µL of sterilized ddH_2O. The PCR products were purified using a TIANgel Midi Purification Kit (TIANGEN, DP209) and cloned into a PMD19-T Vector (TAKARA, 6013), then transformed into *E.coli* DH5α (TIANGEN, CB101). Purified plasmid DNA was then sequenced at BGI (China, Beijing). DNA sequences were edited by Edit Seq software package (Lasergene, Madison, WI, USA) and blasted in NCBI. The newly obtained consensus sequences were deposited in the NCBI GenBank database under the accession numbers indicated in Table 1.

Molecular characterization and phylogenetic analysis

All ITS sequences obtained in this study were used for haplotype analysis, and the secondary structures of helix H9 of ITS1 sequences were predicted by the energy minimization approach using the RNAfold web-server software (http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi). We extracted the sequences from the GenBank files using PhyloSuite v1.2.2 (Zhang et al. 2020), then aligned the sequences in batches using MAFFT v7.313 (Katoh and Standley 2013). ModelFinder (Kalyaanamoorthy et al. 2017) was used to select the optimal evolutionary model. MrBayes 3.2 was used for the Bayesian inference analysis (Ronquist et al. 2012). We used the BI for phylogenetic reconstruction 1,000,000 generations, four Markov chain Monte Carlo chains, and the trees were sampled every 100 generations; an initial 25% of trees were discarded as burn-in. The topologies were used to generate a 50% majority-rule consensus tree. Trees from all analyses were visualized using iTOLv6 (http://itol.embl.de).

Infection test of *D. destructor* on potato

In this study, *D. destructor* populations consisting of three ITS haplotypes were collected from different host plants in different provinces/autonomous regions (Additional file 1: Table S3). Sweet potato 'Yanshu 25' (susceptible cultivar) was used to maintain and multiply these populations, and Baermann-funnel was used to isolate nematodes. Nematode suspensions were adjusted to 500 nematodes/mL of water.

Infection tests were carried out on the potato cultivar 'Helan 15' (*Solanum tuberosum* L.), whose seeds were planted in 1 L pots filled with growth medium (slow-release fertilizer was added to the growth medium at a ratio of 1.5 g/kg). Two weeks after planting, growing medium was infected with *D. destructor* suspension (2000 mixed nematodes of all stages). Each nematode population had four replicates, while plants treated with deionized water was used as a negative control. The potato plants were grown with sterile substrate in a greenhouse at 17–23 °C under a 13-h photoperiod and watered regularly. At 12-week post inoculation, the virulence of 12 nematode populations was assessed by measuring the lesion area on the potato and the RF of 12 nematode populations. The index rating and its corresponding lesion area in parentheses were: 0 (0%), 1 (0–25.0%), 2 (25–50.0%), 3 (50.1–75.0%), and 4 (75.1–100%). Differences in virulence caused by different *D. destructor* populations was determined by a one-way analysis of variance, and the least significant difference test at the 5% significance level was compared using IBM SPSS Statistics software.

Disease index = ∑(Number of diseased plants in that rating × Index rating)/(Total number of plants investigated × Highest rating) × 100.

Disease rate (%) = ∑(Number of diseased plants)/(Total number of plants investigated) × 100.

$RF = P_f/P_i$ (P_f is the final population and P_i is the initial population).

Abbreviations
ISSR: Inter-simple sequence repeat; ITS: Internal transcribed spacer; NCBI: National Center for Biotechnology Information; rRNA: ribosomal RNA.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s42483-022-00127-z.

Additional file 1: Table S1. Location and density of *Ditylenchus destructor* collected in China during 2016–2020. **Table S2.** Incidence of *Ditylenchus destructor* in samples collected in China during 2016–2020. **Table S3.** *Ditylenchus destructor* populations used for infection test. **Table S4.** Primers used in this study.

Additional file 2: Figure S1. Alignment of helix H9 sequences of ITS1.

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Author contributions
DLP and HP designed the research; YQL, LQH, RJ, SMH, JMG, and HXL collected samples and conducted the experiments; YQL and SML wrote the manuscript; SML, YML, QC, YML, WKH, and ZJC revised the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
Not applicable.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
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Competing interests
The authors declare that they have no competing interests.

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