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Heterodera schachtii (Nematoda: Heteroderidae) Associated with Cabbage-Cultivation Systems in Anatolia Region of Türkiye

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Abstract: *Heterodera schachtii* (Schmidt, 1871) (Nematoda: Heteroderidae) is one of the most widespread plant-parasitic nematodes (PPNs) associated with cabbages, which cause severe yield losses in cruciferous vegetables. This study aimed to improve the current understanding of the prevalence and detection of *H. schachtii* in the cabbage-growing areas of Niğde Province, Türkiye. Field surveys were conducted between November and December 2021, and 100 soil samples were collected immediately after cabbage harvesting. *Heterodera schachtii* populations were identified by morphological and morphometric methods along with Internal Transcribed Spacer of the ribosomal region (ITS-rDNA) and Cytochrome Oxidase Subunit (COI-mtDNA) sequencing. The mean body length of *H. schachtii* was 463 ± 7 µm, while stylet and hyaline length ranged between 20.7–27.8 µm and 20.1–32.1 µm, respectively. Nearly half of the surveyed areas were infested with *H. schachtii* with a 41% incidence rate. However, the Merkez District had the highest proportion of infested fields with an over 51% incidence rate. The population density was determined in 41 samples with a mean of 79.5 cysts per 250 g of soil. These results will help to determine the control and management strategies of *H. schachtii*.

Keywords: COI; sugar beet cyst nematode; crucifers

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1. Introduction

Cabbage, *Brassica oleracea* var. *capitata* (L.) (Brassicales: Brassicaceae) is one of the most important vegetable crops widely produced and consumed worldwide. Türkiye, with an 860 kt annual production capacity, is one of the leading cabbage producers and exporters in the world [1]. Among the cabbage production centers of Türkiye, Niğde province is the second-largest producer of cabbages due to favorable climatic conditions [2]. However, numerous pests and pathogens reduce the yield and marketable value of cabbages, including sugar beet cyst nematodes, *Heterodera schachtii* (Schmidt 1871) (Nematoda: Heteroderidae). *Heterodera schachtii* is a common PPN worldwide [3]. However, *H. schachtii* tends to occur more frequently in temperate climates, and less commonly in subtropical–tropical climates [4]. The nematode has been recorded in Africa, Australia, Europe, the Middle East, China, Türkiye, and America [4–8]. Even though *H. schachtii* is primarily a sugar beet pathogen and called the beet cyst nematode (BCN), it can reproduce on more than 200 plant species including brassica crops (e.g., *Brassica napus*, *Brassica oleracea*, *Brassica rapa*, *Raphanus sativus* and *Sinapis alba* (Brassicales: Brassicaceae), chenopod crops (e.g., *Spinacia*

oleracea), and amaranth crops (e.g., *Beta vulgaris*) (Caryophyllales: Amaranthaceae) [9–11]. *Heterodera schachtii* can inflict considerable damage to Brassica crops, causing yield losses of up to 50% [4,9].

The symptoms of *H. schachtii* on crops are generally stunted, yellow-colored leaves, a reduction in size, and the more intense formation of fibrous root systems [3]. While the impact of *H. schachtii* on cabbage production has not been fully defined, Abawi and Mai [9] reported that the nematode can reduce the marketable head weight of cabbage from 42 to 54%. For efficient management of *H. schachtii*, knowing the phylogenetic relationships of populations has potential importance in management. The availability of molecular analyses, such as Internal Transcribed Spacer of the ribosomal region (ITS-rDNA) and Cytochrome Oxidase Subunit (COI-mtDNA) sequencing, has enabled the determination of phylogenetic links and reconstruction cyst nematode phylogenetic trees [12,13]. In addition, *H. schachtii*, which is under the *schachtii* group, can be separated from the closely related species [*Heterodera trifolii,* (Goffart, 1932), *Heterodera glycines* (Ichinohe, 1952), *Heterodera betae* (Wouts Rumpenhorst and Sturhani, 2001) (Nematoda: Heteroderidae) and others] by morphological and morphometric characteristics. This study is aimed at determining the distribution, prevalence and population density of *H. schachtii* and to identify and investigate the phylogenetic connections of the *H. schachtii* populations in the Niğde province, the second-largest cabbage-growing area in Türkiye.

2. Materials and Methods

2.1. Nematode Population Collection

In total, 100 soil samples were collected from the cabbage fields in Merkez, Bor, and Ulukışla districts of Niğde Province after the harvest of the crops in 2021. Sampling consisted of a randomized collection of soil (soil weight of 250 g) from each field (Figure 1). Five 50 g subsamples were taken in a zigzag pattern across the field, with about 5–8 m between the sampling points. Cysts were extracted from 250 g soil samples using a modified sieving–decanting method [14]. Cysts were collected under a stereomicroscope (Zeiss, Munich, Germany) for further observations and measurements.



Figure 1. The map of the sampling areas.

2.2. Morphological Studies

For morphological characterization, the under-bridge shape of the fenestra area and bullae were used as a reference. The fenestra length, semi-fenestra width, vulval bridge width, and vulval slit length were used for morphometric identification of the cysts. The morphometric ratios (a, b, and c) published by Handoo [15] and Subbotin et al. [16] were used as a standard reference. *Heterodera schachtii* J2s and cysts were compared with descriptions and identification keys of the previously published ones by Handoo [15], Franklin [17], and Mulvey and Golden [18]. A Leica light microscope model DM5500 B (Leica, Wetzlar, Germany) equipped with a Leica DFC295 optic camera was used to examine and photograph the samples. Leica software version 4.1.0 was used to record and analyze all measurements.

2.3. Molecular Identification

For DNA extraction, a single full cyst was randomly selected from each of 12 *H. schachtii* representative samples from three districts (Merkez, Bor, and Ulukışla), and the DNA of each population was extracted according to Holterman et al. [19]. The ITS1-5.8S-ITS2 of rRNA was amplified using the forward primer TW81 (5′-GTT TCC GTA GGT GAA CCT GC-3′) and the reverse primer AB28 (5′-ATA TGC TTA AGT TCA GCG GGT-3′) [20]. For amplification of the partial mtDNA-COI gene, the forward JB3 (5′-TTT GGG CAT CCT GAG GTT TAT-3′) and reverse JB5 (5′-AGC ACC TAA ACT TAA AAC ATA ATG AAA ATG-3′) primers were used [21,22]. The polymerase chain reaction (PCR) program was set for the ITS primers as follows: 95 °C for 5 min; 35 cycles at 94 °C for 60 s, 55 °C for 45 s, and 72 °C for 120 s; and a final extension at 72 °C for 10 min. For COI primers, the program was set at 95 °C for 10 min; 40 cycles at 95 °C for 60 s, 41 °C for 45 s, and 72 °C for 120 s; and a final extension step at 72 °C for 10 min.

Products of the PCR were visualized through agarose gel electrophoresis, following a typical protocol, and sequenced by a commercial sequencing company (Macrogen Inc., Seoul, South Korea). The obtained sequences of the COI and ITS were blasted in NCBI GeneBank to identify the nematodes (www.ncbi.com) (accessed on 12 March 2024). MEGA software (version 7) was used to perform phylogenetic analysis on the obtained sequences, as well as the reference sequences and nucleotides from various countries available in GenBank [23]. The sequences of OP028952, OP028953, OP028954, OP028955, OP028956, OP028957, OP028958, OP028959, and OP028960 were included in the ITS analysis, whereas sequences of OP036613, OP036614, OP036615, OP036616, OP036617, OP036618, OP036619, OP036620, OP036621, OP036622, OP036623, and OP036624 were used in the COI locus analysis, respectively. Tamura and Nei [24]'s model was used to construct a tree by the neighbor-joining method with 1000 bootstrap replicates.

3. Results

The *H. schachtii* cysts were detected in 41 out of the 100 soil samples collected from the surveyed cabbage fields (Table 1). The prevalence in Merkez, Bor, and Ulukışla districts were 51%, 33%, and 42%, respectively. The determined infested samples with *H. schachtii* showed an average of 79.5 cysts per 250 g of soil (Table S1).

Table 1. The prevalence and density of *Heterodera schachtii* in cabbage fields sampled in three districts of Niğde Province, Türkiye in 2021.

Province/District	Total Samples	Infested Fields	Non-Infested Sample	Incidence (%)	Total Cysts (Cysts/250 g of Soil)	Average Density (Mean ± S.E.) *
Niğde/Bor	45	15	30	33	2799	90 ± 24
Niğde/Merkez	29	15	14	51	1802	80 ± 28
Niğde/Ulukışla	26	11	15	42	1387	54 ± 14
Total	100	41	59	41	5988	80 ± 15

* Average density was given as cysts/250 g of soil; S.E.: The standard error of the mean. All samples collected from each district were analyzed in 250 g of soil.

3.1. Morphological Identification

All the cysts of *H. schachtii* that were examined and identified were lemon-shaped, light to dark brown, and had zigzag ridges (Figure 2). The morphological characteristics and morphometric measurements of the J2s and cysts of *H. schachtii* are shown in (Figures 2 and 3). Thirty cysts and J2s were used in morphological studies. The cyst length and width of *H. schachtii* populations varied from 603–987 µm to 405–689 µm, respectively. The fenestra length and width were 31–56 µm and 21–25 µm, respectively. The cysts vulval bridge width varied from 4.3 to 8.8 µm. The morphometric measurements of the cysts were consistent with the previous studies and are given in Table 2.

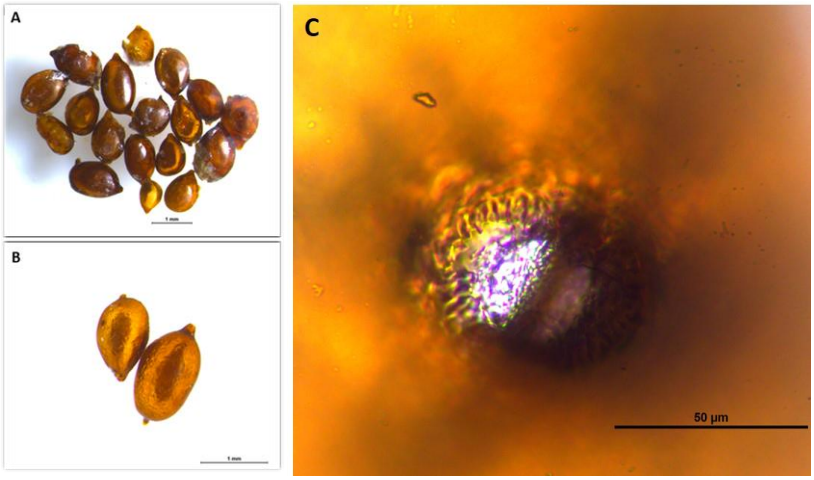


Figure 2. Microscope images of *Heterodera schachtii* cysts obtained from Niğde Province: (**A**,**B**): cysts of *H. schachtii*; (**C**): fenestra region cysts of *H. schachtii*.



Figure 3. Microscope images of *Heterodera schachtii* second-stage juveniles obtained from Niğde Province. (**A**): second-stage juveniles; (**B**): tail region.

Table 2. Morphometric characteristics (Mean ± S.E.) of *Heterodera schachtii* cyst populations extracted from cabbage fields that were sampled in Niğde Province, Türkiye.

Character/Cysts *	Current Study (n = 30)	Xijiang, China Huan et al. [25]	Jeongseon, Korea Mwamula et al. [26,27]	Bozova, Türkiye Dababat et al. [28]
Length	785 ± 20 (603–987)	752 ± 87 (622–867)	796 ± 97 (640–883)	700 ± 80 (610–783)
Width	554 ± 17 (405–689)	485 ± 55 (391–594)	511 ± 75 (378–600)	460 ± 52 (395–505)
Fenestra Length	43 ± 1 (31–56)	37 ± 6 (33–44)	42 ± 3 (36–45)	-
Fenestra Width	23 ± 1 (21–25)	-	-	-
Vulval Slit Length	-	40 ± 4 (37–49)	37 ± 5 (30–42)	44 ± 2 (41–45)
Vulval Bridge Width	7 ± 1 (4–8)	-	-	-

* Measurements are given in mean µm ± standard error of the mean.

The body length and body width of *H. schachtii* J2s ranged between 391–517 and 18–27, respectively. Body length (mean = 463.22 ± 7.41 and range = 390.8–516.6 µm); stylet knob width (mean = 3.63 ± 0.28 and range = 1.2–5.8 µm); stylet length (mean = 24.45 ± 0.37 and range = 20.7–27.8 µm), median bulb length (mean = 15.26 ± 0.74 and range = 9.3–21.1 µm); dorsal gland orifice to stylet (DGO) (mean = 4.29 ± 0.17 and range = 2.8–5.7 µm); tail (mean = 47.14 ± 0.8 and range = 40.2–53.3 µm); body diameter at the anus (mean = 13.02 ± 0.46 and range = 9.8–17.1 µm); hyaline (mean = 26.2 ± 0.66 and range = 20.1–32.1 µm). The morphometric measurements of the cysts were consistent with previous studies and were given in Table 3.

Table 3. Selected morphometric characters (Mean ± S.E.) of second-stage juveniles of *Heterodera schachtii* populations recovered from cabbage fields in Niğde Province, Türkiye.

Characters/J2s *	Current Study (n = 30)	Xijiang, China Huan et al. [25]	Jeongseon, Korea Mwamula et al. [26]	Bozova, Türkiye Dababat et al. [28]
Body length	463.2 ± 7.4 (390.8–516.6)	442.1 ± 13.7 (429.2–512.3)	484.2 ± 11.1 (473.0–504.2)	–
Body width	21.9 ± 0.5 (18.1–26.5)	–	–	–
Stylet knob width	3.6 ± 0.3 (1.2–5.8)	–	–	–
Stylet length	24.5 ± 0.4 (20.7–27.8)	24.2 ± 0.5 (22.3–26.5)	22.1 ± 0.9 (21.3–24.3)	24.3 ± 0.5 (24–25)
Median bulb length	15.3 ± 0.7 (9.3–21.1)	–	–	–
Lip height	4.5 ± 0.1 (3.6–5.2)	–	–	–
Lip diameter	9.4 ± 0.1 (8.6–10.2)	–	–	–
DGO	4.3 ± 0.2 (2.8–5.7)	4.4 ± 0.5 (3.5–5.1)	4.0 ± 0.3 (3.5–4.4)	–
Tail	47.1 ± 0.8 (40.2–53.3)	50.8 ± 2.1 (47.5–52.7)	49.9 ± 1.8 (48.2–53.2)	47.0 ± 1.4 (45–49)
Body diameter at the anus	13.1 ± 0.5 (9.8–17.1)	–	–	–
Hyalin	26.2 ± 0.7 (20.1–32.1)	27.8 ± 2.7 (24.7–30.9)	29.2 ± 2.1 (26.7–34.3)	24.0 ± 0.6 (23–25)
Anterior end to excretory pore	93.2 ± 1.7 (79–108)	73.1 ± 0.4 (65.1–78.4)	76.4 ± 0.4 (75.8–77)	–

Table 3. *Cont.*

Characters/J2s *	Current Study (n = 30)	Xijiang, China Huan et al. [25]	Jeongseon, Korea Mwamula et al. [26]	Bozova, Türkiye Dababat et al. [28]
a	23.5 ± 0.4 (19.5–26.9)	23.7 ± 1.1 (22.2–24.8)	23.8 ± 0.9 (22.9–25.1)	–
b	4.7 ± 0.1 (4.0–6.1)	4.5 ± 0.3 (4.1–4.7)	4.2 ± 0.1 (3.7–4.6)	–
c	9.8 ± 0.2 (8.4–11.2)	9.8 ± 0.6 (8.9 –10.1)	9.6 ± 0.4 (9.4–10.0)	–

* Measurements are given in mean µm ± standard error of the mean. a: total body length divided by maximum body diameter; b: total body length divided by distance from anterior end to base of pharynx; c: body length divided by tail length.

3.2. Nucleotide Sequence and Phylogenetic Analysis

Twelve representative *H. schachtii* populations were selected from the samples collected from each district and identified using the COI region. All the species were also confirmed by the rDNA-ITS region, except for three populations (Table 4). The DNA of all the randomly selected cysts were successfully extracted and amplified using ITS and COI primer sets according to Holterman et al. [19]. PCR products were detected via a standard gel electrophoresis protocol and the observed band sizes were 1028 and 470 bp for the ITS and the COI regions, respectively.

Table 4. Accession numbers for populations of *Heterodera schachtii* obtained from the cabbage fields of Niğde, Türkiye.

Sample No	District/Town	Species	Gene	Accession	Species	Gene	Accession
38	Bor/Kaynarca	*H. schachtii*	ITS	OP028956	*H. schachtii*	COI	OP036617
47	Bor/Kaynarca	-	-		*H. schachtii*	COI	OP036624
7	Bor/Bahçeli	*H. schachtii*	ITS	OP028952	*H. schachtii*	COI	OP036613
49	Bor/Kaynarca	*H. schachtii*	ITS	OP028954	*H. schachtii*	COI	OP036622
10	Bor/Sazlıca	*H. schachtii*	ITS	OP028960	*H. schachtii*	COI	OP036614
23	Bor/Kemerhisar	*H. schachtii*	ITS	OP028957	*H. schachtii*	COI	OP036616
19	Bor/Kaynarca	*H. schachtii*	ITS	OP028958	*H. schachtii*	COI	OP036621
42	Bor/Kaynarca	*H. schachtii*	ITS	OP028955	*H. schachtii*	COI	OP036618
11	Bor/Kemerhisar	*H. schachtii*	ITS	OP028959	*H. schachtii*	COI	OP036615
5	Bor/Kaynarca	-	-	-	*H. schachtii*	COI	OP036619
50	Bor/Kaynarca	-	-	-	*H. schachtii*	COI	OP036623
71	Merkez/Sazlıca	*H. schachtii*	ITS	OP028953	*H. schachtii*	COI	OP036620

After sequencing, the BLAST analysis performed on 12 samples showed a 99.81–100% similarity to the *H. schachtii* sequences from Algeria (MG800690), China (MW856650), France (EF611103), Germany (EF611115.1), Morocco (EF611108), South Africa (MF754150.1), South Korea (MF043911), and the USA (AY590282), whereas the COI nucleotide results showed a 99.81–100% similarity index to the *H. schachtii* sequences from Iran (MG598807), Japan 135 (LC421661), Mexico (MK134702), Poland (KC172918), South Korea (KY775597), and the USA (KY884308). Phylogenetic trees constructed from Turkish populations of *H. schachtii* nucleotides, including GenBank samples from various countries, had Turkish populations grouped under the same branches and the other *Heterodera* species separated from each group, as expected. Genetic variation in the COI sequences obtained from the Turkish population was very low compared with the ITS sequences. The high bootstrap values support these findings (Figure 4).

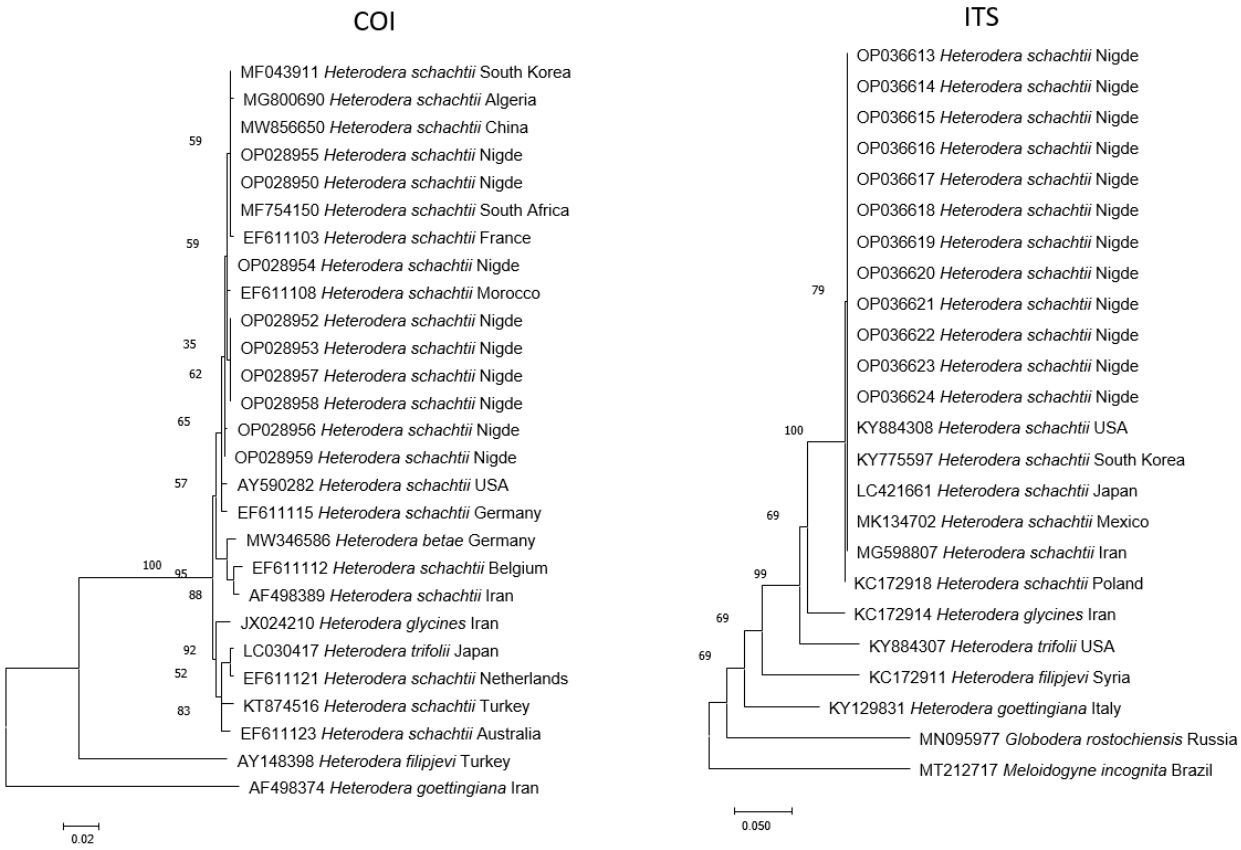


Figure 4. The phylogenetic analysis of *Heterodera schachtii* populations based on ITS rDNA and cytochrome oxidase II gene sequences.

4. Discussion

Various researchers have described the structure and morphological characteristics of cyst nematodes. The earliest description of the top-cone structures was by Oostenbrink and Den Ouden [29]. Despite Cooper [30]'s disagreement with Oostenbrink and Den Ouden [29] that *H. schachtii* and *H. trifolii* can be distinguished by the measurement of the fenestra length, Oostenbrink and Den Ouden's [29] method of distinguishing for cyst nematodes was followed. Both researchers stated that *H. schachtii* and *H. trifolii* had an average fenestra length of between 32.1 and 45.6 µm. In addition, Mulvey [31] noted that there was no significant relationship between the cyst size and fenestra length of *H. schachtii*. Also, the anal bullae present in several cysts are important for distinguishing *H. schachtii* [31]. *Heterodera schachtii,* for example, has a noticeably high cone, whereas *Heterodera cruciferae* [18], (Rhabditia: Hoplolaimidae) has a considerably lower cone [31]. *Heterodera schachtii* has been reported in the previous studies in the sugar beet fields of the Kırklareli [32–34], Eskişehir and Adapazarı [35,36], Konya [37], and Şanlıurfa provinces in Türkiye [28]. Similar to the aforementioned studies, in the present study, the morphological and morphometric characteristics of cysts from Niğde populations were measured and recorded. In the current study, low magnification separation of specimens was made based on bullae and the presence of an under-bridge. However, when identifying cyst nematode species, J2s are also convenient [38].

Differentiating between various *Heterodera* species may be possible through the study of the morphological characteristics of the juvenile stylet. For *H. schachtii,* the most reliable juvenile characteristics are the stylet length and the hyaline portion of the tail [26]. Based on the morphological characteristics of cysts and J2s, including the morphometric measurements, the present study identified 41 *H. schachtii* populations. Previous studies have also used morphological and morphometric identification methods of cysts and J2s to identify *H. schachtii* populations in the Southeast Anatolian Region of Türkiye [28]. The cysts and

J2s morphological and morphometric characteristics from Niğde *H. schachtii* populations were highly identical to those of South Korea [26,27], Türkiye [28], and Xijiang, China populations [25].

According to morphological and morphometric characteristics, *H. schachtii* which is a member of the *schachtii* group, may be distinguished from closely similar species (*H. trifolii*, *H. glycines*, *H. betae*) [38–40]. Molecularly, a species may be distinguished from other members of the *schachtii* group by the sequences of the PCR ITS-RFLP, ITS-rRNA, and COI regions [41,42]. Sequences of the ITS ribosomal DNA can be used to assume the phylogeny of *Heterodera* species [41]. The mtDNA gene COI would alternatively provide a strong candidate for more reliable species definition and ultimately, successful DNA barcoding for cyst nematode species [42].

Accordingly, 9 of the specimens collected from two districts, Kaynarca and Sazlıca, of the province of Niğde were identified as *H. schachtii* by ITS primers, while 12 of the specimens collected from the same two districts of Niğde Province were identified as *H. schachtii* by COI primers. After that, populations of *H. schachtii* from other countries were compared to the studies samples, and the characteristics of the J2s and cysts populations of Niğde were found to be quite similar. The population of Niğde in the same branch with other populations of *H. schachtii*, and the sequence showed high similarity of 99.81–100%, according to the molecular analyses of the rDNA-ITS and mtDNA COI gene sequences.

During this study, the following symptoms were observed in the infested cabbage crops: yellowing of leaves on plants, plants that are dwarfed, plants with signs of wilting and young plants that are dead. The roots had an unusual quantity of highly branched roots; cysts that were white within the early stage later became brown on the root. The study's findings are in line with the expected symptoms of field infestation with *H. schachtii* [16]. *Heterodera schachtii* has a major impact on cabbage production and the previous reports showed that the marketable head weight of transplanted cabbage was reduced by 25, 31, 34, and 42%, and that of seed-sown cabbage by 21, 28, 46, and 54%, respectively, at the initial population densities of 9, 18, 34, and 68 eggs and larvae per g of soil, respectively [9]. Alternatively, according to the reports of Abawi and Mai [9] and Dababat et al. [28], the present study has determined the prevalence of cyst nematodes in the surveyed area and found it to be 51, 33, and 42% in Merkez, Bor, and Ulukşla, respectively, with an average cyst density of 79.5 cysts per 250 g of soil.

5. Conclusions

These findings suggest that it is necessary to determine *H. schachtii* prevalence in cabbage production areas to avoid large economic losses in production. With the high prevalence and yield-limiting population densities reported in cabbage fields in Niğde, farmers will need to have the status of their fields analyzed to determine the presence and density of *H. schachtii* to apply suitable control and management strategies. The present study managed to establish a phylogenetic connection among *H. schachtii* populations collected from the cabbage-growing areas of Niğde Province, Türkiye. Also, this study identified the cyst populations in the cabbage fields in Niğde Province, Türkiye, based on morphological and molecular characteristics. This is the first definitive finding of *H. schachtii* in the Niğde cabbage fields, which can be scaled up to a national level to have a more efficient management strategy.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae10060635/s1, Table S1. Basic information about the study area.

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and editing, E.Y., E.E., A.D., M.İ., R.B. and H.T.; project administration, A.D., M.İ., R.B. and H.T. All authors have read and agreed to the published version of the manuscript.

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