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Original article (Orijinal araştırma)

First report of sugar beet nematode, *Heterodera schachtii* Schmidt, 1871 (Nemata: Heteroderidae) in sugar beet growing areas of Şanlıurfa, Turkey

Şanlıurfa ili Şeker pancarı üretim alanlarında yeni bir zararlı; Şeker pancarı kist nematodu, *Heterodera schachtii* Schmidt (Nemata: Heteroderidae)

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Summary

The sugar beet nematode, *Heterodera schachtii*, is the major pest of sugar beet and causes serious yield losses of about 10-70%. *Heterodera schachtii* occurs in more than 50 countries and regions, however, there has be limited investigation of *H. schachtii* in Turkey. Therefore, a survey of *H. schachtii* in the sugar beet producing regions of Şanlıurfa was conducted in 2014 and 2015 growing seasons. Using morphological and molecular methods, 12 samples collected from three districts, Bozova, Karaköprü and Siverek, in Şanlıurfa Province, Turkey, were identified as *H. schachtii*. In pathogenicity test, the seedling emergence was delayed and reduced, the seedlings were stunted and necrotic, and the white females of *H. schachtii* were evident 25 days after inoculation. Phylogenetic analyses were also conducted. The 12 *H. schachtii* populations from Şanlıurfa Province clustered together with populations from Europe and Morocco at the value of 99%. Sugar beet is the second largest crop in Turkey with the annual production of more 16 Mt. To our best knowledge, this is the first report of *H. schachtii* in Şanlıurfa Province of Turkey.

Keywords: Cyst nematode, identification, phylogenetic analyses, sugar beet

Özet

Şeker pancarı kist nematodu, *Heterodera schachtii* Schmidt (Nemata: Heteroderidae), şeker pancarı üretim alanlarında önemli bir zararlı olup, epidemi yaptığında %10-70 arasında bir ürün kaybına neden olabilmektedir. Günümüzde *H. schachtii* 50'den fazla ülkede yaygın olarak bulunmakla birlikte, Türkiye'de *H. schachtii* araştırmaları oldukça sınırlıdır. Bu çalışmada, 2014 ve 2015 üretim sezonunda Şanlıurfa ili şekerpancarı üretim alanlarında *H. schachtii*'nin surveyi ve teşhisi yapılmıştır. Araştırma sonucunda, Şanlıurfa ilinde üç farklı lokasyonda; Bozova, Karaköprü ve Siverek ilçelerinden alınan on iki örnek, morfolojik ve moleküler yöntemlerle *H. schachtii* olarak tanımlanmıştır. Koch postülat testinde, beyaz *H. schachtii* dişileri inokülasyondan sonra 25. günde kist oluşturmuş, çimlenmede gecikme ve azalma olup, filizlenmede nekroz ve bodurlaşma görülmüştür. Ayrıca, çalışmada, filogenetik analiz yapılmış, Şanlıurfa ilinden toplanan *H. schachtii* popülasyonlarının Avrupa ve Fas popülasyonları ile %99 oranında benzerlik gösterdiği saptanmıştır. Şekerpancarı, yıllık 16 milyon tondan fazla üretimi ile Türkiye'de ikinci en fazla üretilen ürün olma özelliğinde olup, bu çalışma, Türkiye'de Şanlıurfa ili şekerpancarı üretim alanlarında şekerpancarı kist nematodu, *H. schachtii* için ilk kayıt niteliğindedir.

Anahtar sözcükler: Kist nematodu, teşhis, filogenetik analiz, şekerpancarı

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Introduction

The sugar beet nematode, *Heterodera schachtii* Schmidt, 1871, is a major pathogen on sugar beet (*Beta vulgaris* L. ssp. *vulgaris* var. *altissima*), and is the most serious nematode pest recognized in sugar beet (Cooke, 1987). *Heterodera schachtii* is widely distributed throughout the world, especially in Europe, North America, Australia, the Middle East, Africa and South America (Evans & Rowe, 1998). *Heterodera schachtii* has a wide range of other host species, including over 200 plant species within 95 genera from 23 different plant families; most hosts are found in the Chenopodiaceae and Cruciferae (Steele, 1965). In Europe, the annual yield loss associated with the sugar beet nematode was estimated at 90 million EUR (Muller, 1999).

Heterodera schachtii, Schmidt, 1871, *Heterodera betae* Wouts, Rumpenhorst & Sturhan, 2001 and *Heterodera trifolii* Goffart, 1932 belong to the *H. schachtii sensu stricto* group and can be distinguished by their morphology (Subbotin et al., 2000). Identifications based on morphological characters of cysts and J2s is difficult and needs skillful nematologists, so it can more accurate by the use of molecular techniques (Subbotin et al., 1999). During the last fifteen years, researchers have collected and characterized more than 40 species of nematodes from the genus *Heterodera* (Waeyenberge et al., 2009). The SCAR-PCR (sequence characterized amplified region) amplification with species-specific primers has been used for the identification of several cyst nematodes such as *Heterodera avenae* Wollenweber, 1924, *Heterodera filipjevi* Madzhidov, 1981, *Heterodera latipons* Franklin, 1969, *Heterodera glycines* Goffart, 1936 and *H. schachtii* without requiring the subsequent RFLP step (Yan et al., 2013). *Heterodera schachtii* was reported for the first time from sugar beet growing areas in Kırklareli Province in Turkey by Diker (1959). Later on, it has been increasingly reported from other areas and has become recognized as a damaging pathogen of sugar beet producing areas in Eskişehir and Adapazarı Provinces in Central Anatolian Region of Turkey (Susurluk & Ökten, 1999; Osmanoğlu, 1999). The southeast region of Turkey has been the main area for the growing of crops from the Chenopodiaceae, Cruciferae and Leguminosae (Albayrak et al., 2010; Bagdatli & Acar, 2009). However, no previous survey has been conducted to detect *H. schachtii* in Şanlıurfa Province of Southeast Anatolian Region of Turkey.

Here we report the occurrence of the *H. schachtii* in Şanlıurfa Province of Southeast Anatolian Region of Turkey through morphological, molecular and phylogenetic identification. The objectives of this study were to 1) survey sugar beet growing areas in the Southeast Anatolian Region of Turkey to extend knowledge of the distribution of *H. schachtii*, 2) identify *H. schachtii* specimens to the species level using molecular and morphological tools, and 3) verify the pathogenicity of detected populations. Above all, this survey was designed to determine the current status of species distribution as a basis for developing SCN-management strategies.

Materials and Methods

Sampling and DNA extraction

In 2014 and 2015, a cyst nematode survey was conducted in sugar beet fields in southeast regions of Turkey. A total of 1 kg of soil was collected from each field by sampling five random points taken along a zigzag transect. In total, 130 points were sampled in the southeast region of Turkey (Table 1). From each soil sample, 100-ml subsamples were taken for extraction of cysts by wet sieving and sucrose flotation centrifugation procedures (Riggs et al., 2000). Cysts from each sample were hand-picked with a brush under a stereomicroscope. Cysts were randomly selected from each subsample, sterilized in 0.5% NaClO for 5 min and rinsed 3 times in sterile distilled water, and transferred to 24-well culture plates at 20°C for hatching (Griffin, 1981). The hatched invasive second-stage juveniles (J2s) were collected for pathogenicity testing, morphological observation and molecular identification.

Nematode genomic DNA was extracted as described by Qi et al. (2012). The cyst sample in the microtube was frozen in liquid nitrogen and melted in 37°C water bath, and this process was repeated four times. Successively, samples were frozen at -80°C for 1.5 h and then incubated at 65°C for 1.5 h followed by 10 min at 95°C in PCR thermo-cycler. Afterwards, the samples were centrifuged at 1000 revs/min at a temperature of 4°C for 1.5 min. Then the supernatant was transferred into a new microtube and stored at -20°C for later use. At least three replicates of each nematode population detected were tested.

Table 1. Origin and occurrence of populations of *Heterodera schachtii* in The South Anatolia Region, Turkey, and the identification of *Heterodera schachtii* species within specific SCAR primers

Province	Valid samples[a]	Cysts in 100 ml soil	Location	Results
Diyarbakir	6 (15)	5	Oğuzlar	-
Diyarbakir	5 (7)	6	Alabal	-
Gaziantep	6 (10)	11	Karkamış	-
Gaziantep	5 (13)	7	Oğuzeli	-
Kilis	3 (5)	11	Elbeyli	-
Kilis	2 (6)	6	Üçdamlar	-
Kilis	6 (9)	10	Yavuzlar	-
Kilis	3 (5)	4	Kızıltepe	-
Kilis	2 (5)	8	Havuzluçam	-
Mardin	7 (10)	15	Şanlı	-
Mardin	6 (8)	11	Şenyuva	-
Mardin	4 (5)	7	Toprakkale	-
Mardin	6 (9)	6	Akıncılar	-
Mardin	7 (9)	8	Ortaköy	-
Şanlıurfa	5 (5)	7	Siverek	+
Şanlıurfa	3 (3)	9	Bozova	+
Şanlıurfa	4 (6)	12	Karaköprü	+

[a] Numbers in brackets are the total number of samples. [b] "-" negative result, "+" positive result.

SCAR-PCR identification

Extracted DNA (2.5 µl) was added into an Eppendorf tube containing: 12.5 µl 2×Taq PCR StarMix buffer (GenStar, Beijing, China), 1µl of 10 µM of primers and ddH_2O was added to make a final volume of 25 µl. SCAR-PCR primers for *H. schachtii* (SHF6 and rDNA2) were used to PCR-amplify the specific fragments (Amiri et al., 2002). The PCR program consisted of 4 min at 94°C, 10 cycles of 30 s at 94°C, 40 s at 45°C and 1 min at 72°C for elongation; 20 cycles of 30 s at 94°C, 40 s at 55°C and 1 min at 72°C for elongation. The reaction was terminated by a final extension cycle at 72°C for 10 min. After PCR amplification, 5 µl of each PCR products was separated on a 1.5% agarose gel.

Morphological observation

For morphological observation and species identification, mature cysts containing eggs and fully developed juveniles were selected randomly. Vulva cone from mature cysts were mounted (Zhang, 1988). Slides of J2s were prepared as described by Lin (1991) and Duan & Chen (2006) with minor adaptations, fixed with 10% formalin overnight and transferred into the glass cavity block and filled with 3% glycerol (3

ml glycerol and 95 ml sterile water) and covered with a glass lid at room temperature. Three weeks later, the nematode specimens were transferred to a clean drop of anhydrous glycerol on a glass slide and the mounted specimens were covered with a cover slip and sealed using clear nail polish. These slides were viewed under a microscope (Leica DM2500) attached with a digital camera connected to a computer for processing and storing the images. The distance measurement function of the image analysis software was first calibrated using a stage micrometer for each of the objectives before taking measurements of the specimen according to the manufacturer's instructions. All measurements were recorded and for each of the samples, the data was summarized by calculating the averages, range and standard error.

Inoculation for pathogenicity test

To prove a causal relationship between the *H. schachtii* and the disease, hatched J2s were collected and used to infect sugar beet (*B. vulgaris* KWS2320) roots, three-day-old sugar beet roots were inoculated with 200 pre-parasitic J2s. The infected plants were planted in a sterile mixture of sand and loam (1:1, v/v) and kept in a glasshouse at 24°C for observation (Griffin, 1981). Plants were grown under normal plant growing conditions with irrigation, fertilization, and disease and insect control as needed.

rDNA-ITS amplification and phylogenetic analyses

The primers TW81 and AB28 described by Joyce et al. (1994) were used to amplify the ITS-rDNA region. PCR program consisted of an initial denaturation step at 95°C for 4 min followed by 35 cycles of 30 s at 94°C (denaturation), 45 s at 56°C, and at 72°C for 1 min for elongation. The reaction was terminated at a final extension cycle at 72°C for 10 min. ITS-PCR products of the populations were purified using the TIAN gel Midi Purification Kit (Tiangen Biotech, Beijing, China) per the manufacturer's instructions. The purified products were cloned into the pGEM®-T Easy Vector and transformed into DH5α Competent Cells (Tiangen Biotech). The clones of each population were isolated using blue and white selection, and subjected to PCR for confirmation.

The positive clones were then sequenced. All sequences of ITS-rDNA obtained were submitted to GenBank (GenBank Accession No. KT874516 - KT874527) and a database search performed using BLAST. Twelve new sequences, along with 16 sequences of *Heterodera* genera as the in-group taxa and one *Globodera* genera as outgroup taxa were download from the NCBI, where subjected to phylogenetic analysis (Table 2). The sequences were edited and analyzed with MEGA 5.05 (Center for Evolutionary Medicine and Informatics, Biodesign Institute, Tempe, AZ, USA) (Tamura et al., 2011). To determine statistical consistency of the classification, the phylogeny reconstructions statistical method using maximum likelihood and bootstrap analysis with 1000 bootstrapped data sets was used. Gaps were treated as a missing data. A tree clustering the populations at different levels based on genetic distance was constructed from the ITS sequence alignment with MEGA 5.05 (Tamura & Nei, 1993).

Results

SCAR-PCR identification and pathogenicity testing

In this survey, cysts were detected in 62% of the 130 samples (Table 1). Four to 15 cysts were checked from every 100-ml subsample. Twelve specimens, collected from three districts Siverek, Karaköprü and Bozova of Şanlıurfa Province were identified as *H. schachtii* by SCAR-PCR (Table 2). The size of SCAR-PCR products was 255 bp for single juveniles from these *H. schachtii* populations, same to the positive control (Figure 1). The density of *H. schachtii* in Siverek, Karaköprü and Bozova was 11.0, 18.1 and 37.5 eggs/g soil, respectively. The results of the pathogenicity test indicated that the growth of *B. vulgaris* KWS2320 seedlings was delayed by the infection of with *H. schachtii*. Ten days after inoculation, the inoculated plants were stunted, and by 20 days, yellowing and necrosis of the foliage and white females in the roots were evident. After 35 days brown cysts had been formed. The pathogen was confirmed to be *H. schachtii*.

Table 2. Nematode species and populations used in the phylogenetic analyses

Species	Population	Country	Accession number	Source of data
H. arenaria	Linconshire	UK	AF274396.1	Subbotin et al., 2001
H. aucklandica	Zaaren	Belgium	AY148379.1	Subbotin et al., 2003
H. australis	York	Australia	AY148395.1	Subbotin et al., 2003
H. avenae	Cukurova	Turkey	AY148364.1	Subbotin et al., 2003
H. filipjevi	Selcuklu	Turkey	AY148398.1	Subbotin et al., 2003
H. latipons	Kilis	Turkey	KM199826.1	Imren et al., 2014
H. mani	Hamminkeln	Germany	AY148377.1	Subbotin et al., 2003
H. pratensis	Otterndorf	Germany	AY148383.1	Subbotin et al., 2003
H. ripae	Ussurijskii	Russia	AF393840.1	Eroshenko et al., 2001
H. ustinovi	unknown	Belgium	AY148407.1	Subbotin et al., 2003
H. schachtii	Sanliurfa	Turkey	KT874516	Present study
H. schachtii	Sanliurfa	Turkey	KT874517	Present study
H. schachtii	Sanliurfa	Turkey	KT874518	Present study
H. schachtii	Sanliurfa	Turkey	KT874519	Present study
H. schachtii	Sanliurfa	Turkey	KT874520	Present study
H. schachtii	Sanliurfa	Turkey	KT874521	Present study
H. schachtii	Sanliurfa	Turkey	KT874522	Present study
H. schachtii	Sanliurfa	Turkey	KT874523	Present study
H. schachtii	Sanliurfa	Turkey	KT874524	Present study
H. schachtii	Sanliurfa	Turkey	KT874525	Present study
H. schachtii	Sanliurfa	Turkey	KT874526	Present study
H. schachtii	Sanliurfa	Turkey	KT874527	Present study
H. schachtii	Aisne	France	EF611103	Madani et al., 2007
H. schachtii	Herme	Belgium	EF611107	Madani et al., 2007
H. schachtii	Mouloya	Morocco	EF611118	Madani et al., 2007
H. schachtii	Munster	Australia	EF611123	Madani et al., 2007
H. betae	unknown	Germany	EF611122	Madani et al., 2007
H. trifolii	Hokkaido	Japan	LC030417	Kushida et al. unpub
G. rostochiensis	British	Canada	FJ212167.1	Madani et al., unpubl

Morphological identification

The cysts were lemon shaped, and light to dark brown in color. The vulval cone was ambifenestrate with dark brown, molar-shaped bullae positioned underneath the vulval bridge (Figure 2). The key morphometrics of cysts (n = 12) were: body length (excluding neck) from 610 to 783 µm; body

width from 395 to 530 µm; length/width ratio from 1.44 to 1.57 µm; underbridge from 98 to 120 µm; and vulval slit from 40.7 to 45 µm (Table 3). The J2s were cylindrical in shape, stylet moderately heavy with prominent, forward directed knobs, tail acutely conical with rounded tip and a hyaline region in the tail terminus. The key morphometrics of J2 (n = 20) were: mean body length from 426 to 510 µm; mean body width from 21 to 23 µm; mean stylet length from 24 to 25 µm, mean tail length from 55 to 62 µm; and mean length of the hyaline tail region tail from 31 to 38 µm (Table 3).

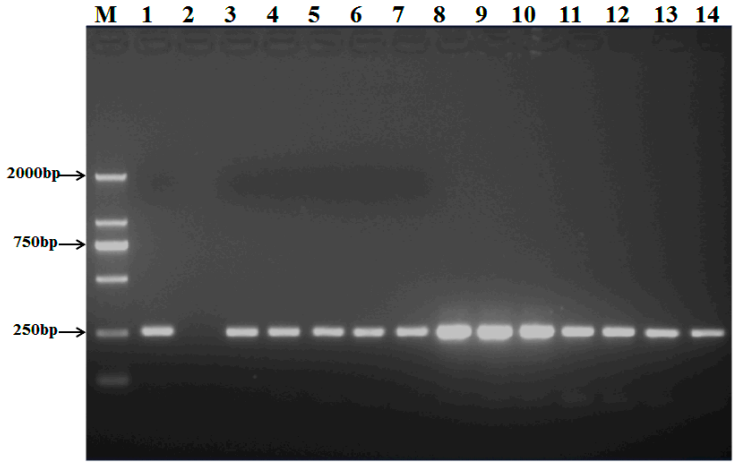

Figure 1. PCR patterns of *Heterodera schachtii* amplified using SCAR primers (Lane 1 is the positive control; Lane 2 is the negative control; Lanes 3-14 are *Heterodera schachtii* populations collected in Turkey; and M is a 2000 bp DNA ladder).

Table 3. Morphometrics of cysts and J2s of *Heterodera schachtii* population in Şanlıurfa, Turkey (all measurements in µm)

Traits	*Heterodera schachtii* population		
	Siverek	Bozova	Karaköprü
	Mean ± SD (Range)	Mean ± SD (Range)	Mean ± SD (Range)
Cysts (n=12)			
Body length excluding	692.0±57.0 (638-755)	700.3±80.1 (610-783)	703.2±61.5 (628-765)
Body width (W)	453.8±31.5 (425-490)	459.8±52.2 (395-505)	473.2±49.6 (410-530)
L/W	1.52±0.02 (1.50-1.54)	1.52±0.03 (1.48-1.56)	1.49±0.04 (1.44-1.53)
Underbridge length	117.0±2.9 (114-120)	113.3±10.4 (98-120)	115.5±4.79 (110-120)
Vulva slit length	44.8±0.1 (44.7-44.9)	43.9±1.9 (41-45)	44.6±0.2 (44.5-44.9)
Second-stage juveniles (J2s) (n=20)			
Body length	461.2±15.6 (442-488)	470.8±23.7 (426-490)	479.0±11.7 (455-489)
Midbody width	21.1±0.4 (21-22)	21.3±0.8 (21-23)	21.4±0.5 (21-22)
Stylet length (S)	24.2±0.4 (24-25)	24.3±0.5 (24-25)	24.7±0.4 (24-25)
Tail length (T)	46.7±1.0 (45-48)	47.0±1.4 (45-49)	47.6±1.0 (47-49)
Hyaline tail length (H)	24.3±0.51 (24-25)	24.0±0.6 (23-25)	24.6±1.1 (23-26)
H/S	1.01±0.03 (0.96-1.04)	0.99±0.02 (0.96-1.00)	0.99±0.04 (0.92-1.04)
T/H	1.92±0.06 (1.80-1.95)	1.96±0.07 (1.88-2.02)	1.94±0.08 (1.85-2.08)

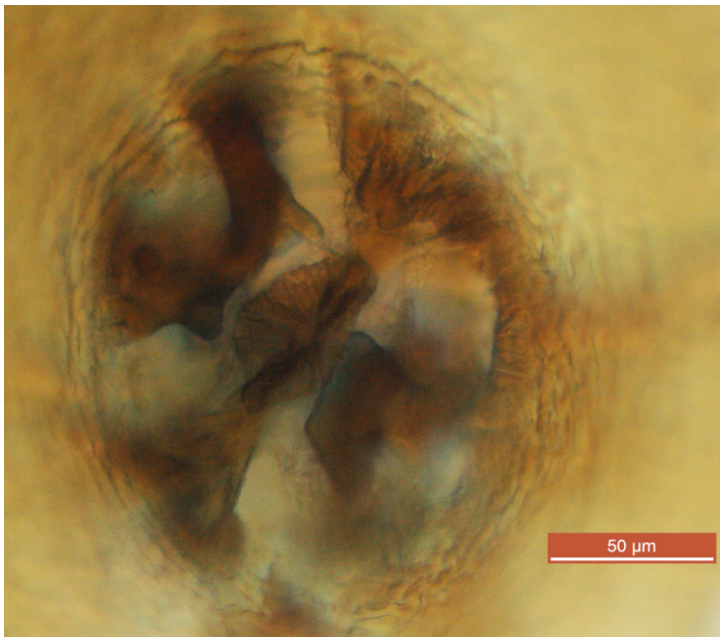

Figure 2. Vulval cone of *Heterodera schachtii* from Şanlıurfa, Turkey, showing the identity of the internal structures (fenestra, vulva slit, underbridge and bullae).

ITS-rDNA sequences and phylogenetic analyses

Ribosomal DNA (rDNA) from the ITS regions was PCR-amplified from each confirmed population of *H. schachtii* and then sequenced. Twenty-eight *Heterodera* populations were clustered, with a *Globodera rostochiensis* Wollenweber, 1923, population as an outgroup. A phylogenetic tree of the *Heterodera* populations was constructed based on the ITS1-5.8S-ITS2 rDNA sequences. All bootstrap values were larger than 60%. Species with bootstrap values of over 70% were placed in clades. The populations of the same species and same sites clustered together. All the groups formed were distinguished from each other in the *Heterodera* sequences, supported by a moderate to high bootstrap value. *Heterodera schachtii sensu stricto* clustered together as group A with a value of 100%, distinctly different from the group B, *H. avenae* complex populations and the outgroup *Globodera rostochiensis* population (Figure 3). *Heterodera schachtii* group included the 12 *H. schachtii* populations collected from Şanlıurfa, *H. schachtii* populations from Australia (EF611123.1), Morocco (EF611118.1), Belgium (EF611107.1), France (EF611103.1), *H. trifolii* from Japan (LC030417.1), and *H. betae* from Germany (EF611122.1). *Heterodera schachtii* populations from Belgium (EF611107.1) and France (EF611103.1) clustered as a sub-branch with a value of 95%. *Heterodera trifolii* from Japan (LC030417.1), *H. betae* from Germany (EF611122.1) and *H. schachtii* populations from Morocco (EF611118.1) clustered as sub-branch with a value of 87%. *Heterodera avenae* complex populations included *H. ripae* (AF393840.1), *H. ustinovi* (AY148407.1), *H. pratensis* (AY148383.1), *H. australis* (AY148395.), *H. mani* (AY148377.1), *H. arenaria* (AF274396.1), *H. aucklandica* (AY148379.1) and the populations of *H. avenae* (AY148364.1), *H. filipjevi* (AY148398.1), *H. latipons* (KM199826.1) from Turkey. *Heterodera avenae* complex populations were clustered in one branch at a value of 99%.

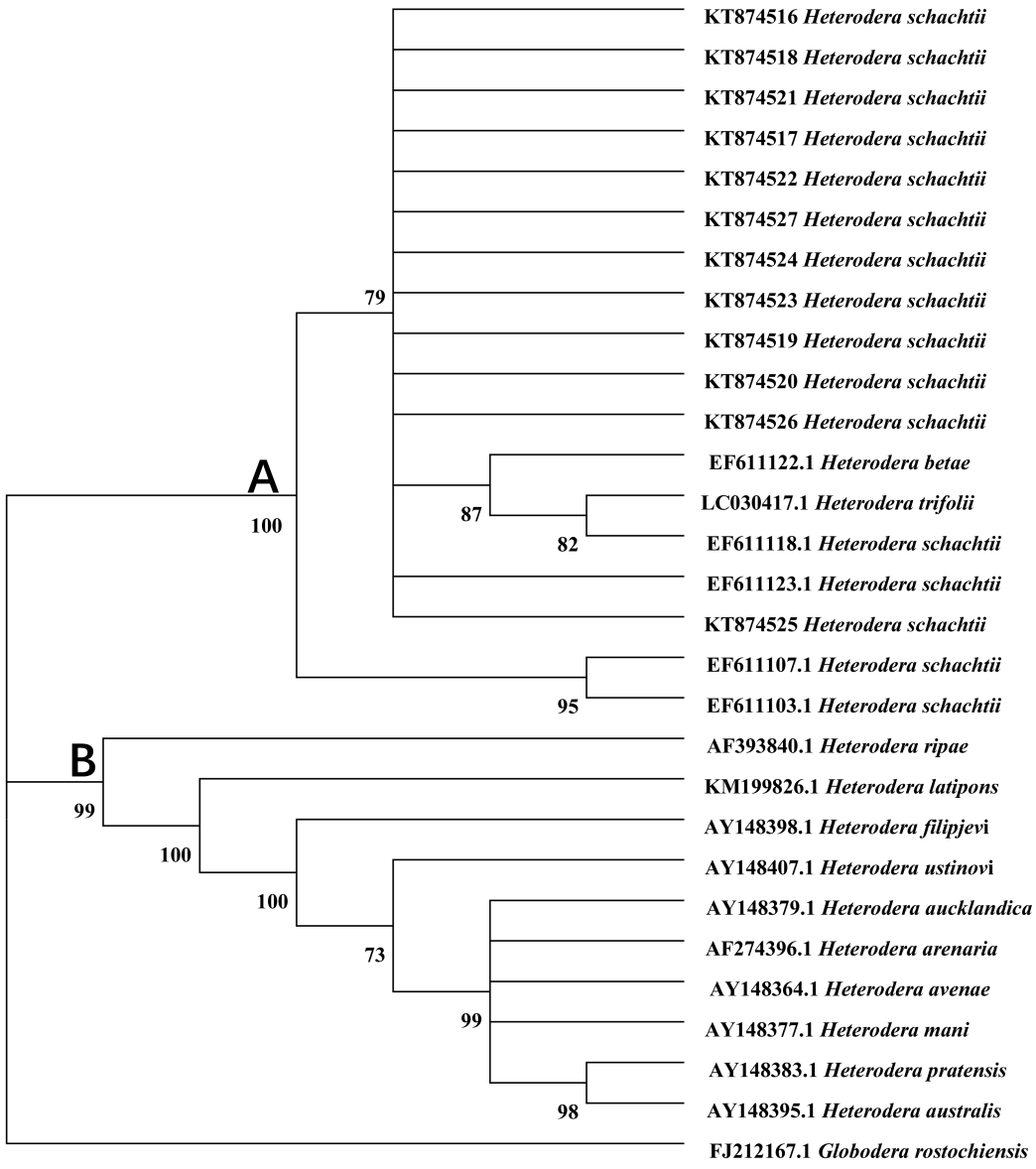

Figure 3. Molecular phylogenetic analysis (maximum likelihood) constructed through the ITS sequences alignment for 29 cyst-forming nematodes. Bootstrap values (more than 70%) are given in the appropriate clades.

Discussion

Yield loss in sugar beet caused by the plant parasitic nematodes is estimated to be about 10%, of which nearly 90% is associated with the cyst nematode, *H. schactii* (Rahmani et al., 2013). In Turkey, sugar beet is the crop with the second largest production, with the annual yield of more than 16 Mt (FAO, 2016). In Turkey, 80% of the agricultural holdings (about 500,000) that produce sugar beet are smaller than 1 ha (Albayrak et al., 2010). The fields surveyed in 2014 to 2015 were of limited size making it very to for growers to control sugar beet nematode systematically. The eggs are the survival stage and can stay viable for several years in soil. A small percentage of eggs within cysts have been reported to survive under fallow conditions for over 12 years (Hafez, 1997). Initial population (Pi) level at of 9, 18, 34 and 68 eggs and larvae per g soil resulted in deceased marketable yield of table beets of 23, 25, 42 and 54%, respectively. Marketable head weight of direct-seeded cabbage was also decreased by 21, 28, 46 and 54%, and that of transplanted cabbage by 25, 31, 34 and 42%, respectively (Abawi & Mai, 1980).

The density of *H. schachtii* in Siverek, Karaköprü, Bozova was 11.0, 18.1 and 37.5 eggs per g soil, respectively, which are potentially damaging based on the findings of Abawi & Mai (1980). However, there have been few studies focusing on *H. schachtii*. These results indicate the need to monitor *H. schachtii* population in Turkey to prevent significant economic loss in sugar beet.

Heterodera schachtii was reported for the first time from sugar beet fields in Kırklareli Province in Turkey (Diker, 1959). Later on, it was also detected many part of sugar beet areas in Kırklareli Province (Tokmakoğlu, 1974). More recently, Susurluk & Ökten (1999) and Osmanoğlu (1999) reported it from Eskişehir and Adapazarı Provinces in the Central Anatolian Region of Turkey, and it has been increasingly detected and has become recognized as a damaging pathogen of sugar beet producing areas in in those areas. The first symptom of *H. schachtii* is yellowing and stunting of plant growth (Polychronopoulos & Lownsbery, 1968). These symptoms are consistent with our pathogenicity test: after sugar beet seedlings were inoculated with *H. schachtii* J2s, the seedling emergence was delayed and reduced, plants were stunted and necrotic, and the infected roots had a whiskered appearance with more lateral roots. The differences between *H. schachtii* and the *H. avenae* group were: *H. avenae* had no obvious underbridge and more bullae, *H. filipjevi* had a slight underbridge with a few bullae, and *H. latipons* had a heavy underbridge with no bullae. Additionally, there was a heavy underbridge with bullae of *H. schachtii*. For the J2s, the main diagnostic features were the stylet and tail lengths followed by body length. *Heterodera schachtii*, *H. betae* and *H. trifolii* belongs to the Schachtii group, *H. betae* is a member of the *H. trifolii* species complex, and is distinguished from closely related species by a combination of morphological and morphometric characteristics. *Heterodera schachtii with a* terminal vulval slit about as long as vulval bridge differs from *H. trifolii* by a shorter average fenestral length and from *H. betae* by shorter average cyst body length. *Heterodera schachtii* J2s have the shortest average tail length and the shortest average length of the hyaline part of the tail. The data showed the morphology of the cysts and juveniles were consistent with those reports of *H. schachtii* (Subbotin et al., 2010).

ITS-rDNA within *H. schachtii* is complex and heterogeneous (Amiri et al., 2002). Amiri et al. (2002) designed primers, using the available ITS-rDNA sequence information, which is specific for species of the *H. schachtii sensu stricto* group. This method of identification of *H. schachtii* is highly sensitive, with amplification obtained even when a single J2 or a single cyst was mixed with other nematode species (Amiri et al., 2002). The different levels of intraspecific variation of the ITS-rDNA sequence have an effect on the evolution and cluster analysis (Madani et al., 2007). The analyses of phylogenetic relationships of the ITS sequences showed that the *H. schachtii* populations from Turkey were clustered together with populations from Europe, Australia and Morocco. The 12 new *H. schachtii* populations clustered with populations from Australia (EF611123.1) and Morocco (EF611118.1) in one sub-branch with a bootstrap value of 79%, the *H. schachtii* populations from Belgium (EF611107.1) and France (EF611103.1) clustered closer to another sub-branch with a bootstrap value of 95%. Relative genera and species of *H. schachtii*, the *H. trifolii* and *H. betae* were clustered together in one branch as the Schachtii group, and separate from the *H. avenae* complex group. ITS-rDNA sequences did not group isolates according to their geographical origin or taxonomic grouping. Concerted evolution has not homogenized all rDNA variants within individual populations. When two ITS clone variants were sequenced from the same population these sequences did not clustered together in Maximum Parsimony trees (Madani et al., 2007). Mostly, Maximum likelihood and Bayesian outperformed Neighbor Joining, Maximum Parsimony and Parsimony in terms of tree reconstruction accuracy (Hall, 2005; Ogden & Rosenberg, 2006). The results presented here are consistent with the report of Madani (2007) but differ from those of Tanha Maafi (2003) for *H. trifolii*. Ogden & Rosenberg (2006) indicated that as the length of the branch and of the neighboring branches increase, alignment accuracy decreases, and the length of the neighboring branches is the major factor in topological accuracy. Thus, multiple-sequence alignment can be an important factor in downstream effects on topological reconstruction (Ogden & Rosenberg, 2006). Additionally, we found the *H. schachtii* population from Morocco (EF611118.1) was more closely related to *H. trifolii* from Japan (LC030417.1) and clustered as a sub-branch with a value of 87%, and *H. betae* from Germany (EF611122.1) clustered together with *H. schachtii* (EF611118.1) and *H. trifolii* (LC030417.1) as a subgroup with a value of 87% within the Schachtii group. *Heterodera betae* may *have*

originated from interspecific hybridization of two diploid amphimictic species, with *H. schachtii* suggested as one of the parent species, or *H. betae* represents a polyploid parthenogenetic form that has evolved from autopolyploidization of diploid amphimictic *H. schachtii* or another very closely related species (Madani et al., 2007). This hypothesis could explain the origin and evolution relationship of *H. trifolii*, *H. betae* and other polyploid species from the Schachtii group.

Heterodera schachtii has been recognized as a serious problem for sugar beet production and is an important quarantine nematodes in many countries due to its devastating damage to sugar beet (Peng et al., 2015). Sugar beet nematode has spread extensively in many regions of sugar beet cultivation, and therefore efforts have been made to reduce its effects on sugar and root yield. The most appropriate control method has been the release of resistant cultivars (Rahmani et al., 2013) with resistance genes introduced from wild *Beta* species (Kleine et al., 1998). The annual rate of decline of viable eggs and larvae in fields after removal of sugar beet or another host crop can vary from 40 to 50% depending on the type of soil, soil temperature, soil moisture, history of pesticide use (including herbicides), susceptibility and availability of host plants (including weeds), and the presence of predators and parasites (Hafez et al., 1997). Furthermore, their combined effects should aim to decrease and maintain *H. schachtii* population densities below the damage threshold.

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References

Abawi, G. S. & W. F. Mai, 1980. Effects of initial population densities of *Heterodera schachtii* on yield of cabbage and table beets in New York State. Phytopathology, 70: 481-485.

Albayrak, M., E. Gunes & B. Gulcubuk, 2010. The effects of irrigation methods on input use and productivities of sugarbeet in Central Anatolia Turkey. African Journal of Agricultural Research, 5: 188-195.

Amiri, S., S. A. Subbotin & M. Moens, 2002. Identification of the beet cyst nematode *Heterodera schachtii* by PCR. European Journal Plant Pathology, 108: 497-506.

Bagdatli, M.C. & B. Acar, 2009. Evaluation of trickle irrigation systems for some vegetable crops in Konya-Turkey. Journal of International Environmental Application & Science, 4: 79-85.

Cooke, D A., 1987. Beet cyst nematode (*Heterodera schachtii* Schmidt) and its control on sugar beet[J]. Agricultural Zoology Reviews 2, 132-183.

Diker, T., 1959. Nebat Parazit Nematodları. Türkiye Şeker Fabrikaları Anonim Şirketi Neşriyatı, Yayın No. 70. Ankara, 100 s.

Duan, Y. X. & L. J. Chen, 2006. Processing methods of plant nematode slides. China Journal of Parasitology and Parasitic Diseases. 24(S1):65-68.

Evans, K. & J. A. Rowe, 1998. Distribution and economic importance. In: Sharma SB, editors. The Cyst Nematodes. London, UK: Kluwer Academic Publishers, pp 1-30.

FAO, 2016. (Web page: http://faostat3.fao.org/browse/area/223/E), (Date accessed: May 2016).

Griffin, G. D., 1981. The relationship of plant age, soil temperature, and population density of *Heterodera schachtii* on the growth of sugarbeet. Journal Nematology, 13: 184-190.

Hafez, S. L., 1997. Sugar Beet nematodes in Idaho and Eastern Oregon. University of Idaho, College of Agriculture, Cooperative Extension System, Agricultural Experiment Station.

Hall, B. G., 2005. Comparison of the accuracies of several phylogenetic methods using protein and DNA sequences. Molecular Biology and Evolution, 22(3), 792-802.

Joyce, S. A., A. Reid, F. Driver & J. Curran, 1994. “Application of polymerase chain reaction (PCR) methods to identification of entomopathogenic nematodes, 178-187”. In: COST 812 Biotechnology: Genetics of Entomopathogenic Nematode–Bacterium Complexes (Eds. A. M. Burnell, R. U. Ehlers & J. P. Masson). Proceedings of Symposium & Workshop, St. Patrick’s College, Maynooth, Co. Kildare, Ireland, Luxembourg, European Commission, DG XII.

Kleine, M., H. Voss, D. & C. C. Jung, 1998. Evaluation of nematode-resistant sugar beet (*Beta vulgaris* L.) lines by molecular analysis. Theoretical Applied Genetics, 97: 896-904.

Lin, M. S., 1991. Processing of plant nematode glass slides. Plant Quarantine, 02:90-91.

Maafi, Z. T., S. A. Subbotin & M. Moens, 2003. Molecular identification of cyst-forming nematodes (*Heteroderidae*) from Iran and a phylogeny based on ITS-rDNA sequences. Nematology, 5: 99-111.

Madani, M., S. A. Subbotin & M. Moens, 2005. Quantitative detection of the potato cyst nematode, *Globodera pallida*, and the beet cyst nematode, *Heterodera schachtii*, using real-time PCR with SYBR green I dye. Molecular Cellular Probes,19: 81-86.

Madani, M., T. Kyndt, N. Colpaert, S. A. Subbotin, G. Gheysen & M. Moens, 2007. Polymorphism among sugar beet cyst nematode *Heterodera schachtii* populations as inferred from AFLP and ITS-rDNA gene analyses. Russian Journal of Nematology, 15:117-128.

Muller, J., 1999. The economic importance of *Heterodera schachtii* in Europe. Helminthologia, 36: 205-213.

Ogden, T. H. & M. S. Rosenberg, 2006. Multiple sequence alignment accuracy and phylogenetic inference. Systematic Biology, 55(2), 314-328.

Osmanoğlu, A., 1999. Adapazarı ili ve Çevresi Şeker Pancarı Ekim Alanlarında *Heterodera schachtii* Schmidt, 1871" in Yayılışı Üzerine Araştırmalar, Yüksek Lisans Tezi A. Ü. Fen Bilimleri Enstitüsü, Ankara, 76s.

Peng, D. L., H. Peng & H. Liu, 2015. Research progress in occurrence, biology and management of the sugar beet cyst nematode *Heterodera Schachtii* Schmidt. Plant Protection, 41:1-7.

Polychronopoulos, A. G. & B. F. Lownsbery, 1968. Effect of *Heterodera schachtii* on sugar beet seedlings under monoxenic conditions. Nematologica, 14: 526-534.

Qi, X. L., D. L. Peng, H. Peng, H. B. Long, W. K. Huang & W. T. He, 2012. Rapid molecular diagnosis based on SCAR marker system for cereal cyst nematode. Scientia agricultura Sinica, 45:4388-4395.

Rahmani, N., M. Mesbah & P. Norouzi, 2013. Identification of molecular markers linked to sugar beet cyst nematode resistance gene(s). Journal of Sugar Beet Research, 28: 81-85.

Riggs, R. D., J. A. Wrather, A. Mauromoustakos & L. Rakes, 2000. Planting date and soybean cultivar maturity group affect population dynamics of *Heterodera glycines*, and all affect yield of soybean. Journal of Nematolology, 32: 334-342.

Steele, A. E., 1965. The host range of the sugar beet nematode, *Heterodera schachtii* Schmidt. Sugar Beet Technology, 13: 573-603.

Subbotin, S. A., L. Waeyenberge, I. A. Molokanova & M. Moens, 1999. Identification of species from the *Heterodera avenae* group by morphomometrics and ribosomal DNA RFLPs. Nematology, 1: 195-207.

Subbotin, S. A., L. Waeyenberge & M. Moens, 2000. Identification of cyst forming nematodes of the genus *Heterodera* (Nematoda: *Heteroderidae*) based on the ribosomal DNA-RFLP. Nematology, 2: 153-164.

Susurluk, A. & M. E. Ökten, 1999. Eskişehir ili ve çevresi şekerpancarı ekim alanlarında *Heterodera schachtii*, Schmidt, 1871 (Tylenchida: *Heteroderidae*)"nın yayılışı üzerine araştırmalar, Türkiye Entomoloji Dergisi, 23 (2), 143-147.

Tamura, K. & M. Nei, 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution 10:512-526.

Tamura, K., D. Peterson, N. Peterson, G. Stecher, M. Nei & S. Kumar, 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution, 28: 2731-2739.

Tokmakoğlu, O., 1974. Şekerpancarı Hastalık ve Zararlıları Atlası, Türkiye Şeker Fabrikalrı Anonim Şirketi Yayını. No: 190, Ankara.

Waeyenberge, L., N. Viaene, S. A. Subbotin & M. Moens, 2009. Molecular identification of *Heterodera* spp., an overview of fifteen years of research. In: Cereal cyst nematodes: status, research and Outlook (Eds. I. T. Riley, J. M. Nicol & A. A. Dababat). Ankara: CIMMYT, pp. 109-114.

Yan, G. P., R. W. Smiley, P. A. Okubara & A. M. Skantar, 2013. Species-specific PCR assays for differentiating *Heterodera filipjevi* and *H. avenae*. Plant Disease, 97: 1611-1619.

Zhang, S. L., 1988. Technology of cyst nematode vulval cone slides. Chinese Journal of Zoology, 23: 33.