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Original article (Orijinal araştırma)

Identification, distribution and genetic diversity of *Globodera rostochiensis* (Wollenweber, 1923) Skarbilovich, 1959 (Tylenchida: Heteroderidae) populations in Turkey

Globodera rostochiensis (Wollenweber, 1923) Skarbilovich, 1959 (Tylenchida: Heteroderidae)'in Türkiye popülasyonlarının tanımlanması, yaygınlık ve genetik çeşitliliği

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Abstract

The golden nematode, *Globodera rostochiensis* (Wollenweber, 1923) Skarbilovich, 1959 (Tylenchida: Heteroderidae) is one of the most economically important pests of potato in the world. Tests for this nematode are routinely performed for outbreaks and densities in potato growing areas. The morphological and molecular analyses for precise determination of the nematode are employed to establish appropriate management strategies. This study showed that 96% of soil samples obtained from Bozdağ and Ödemiş Districts of İzmir Province, during 2017 and 2018 potato growing seasons, were positive for *G. rostochiensis*. The mean number of cysts ranged from 0.01 to 3.70 cysts g^{-1} soil in the fields examined. The examination of the morphological and morphometric features of the second-stage juveniles and cysts of the *G. rostochiensis* revealed slight differences among the populations obtained from Bozdağ and Ödemis. To assess the accuracy of the identification, partial sequences of ribosomal DNA for all populations were amplified, sequenced, and deposited in GenBank. The comparisons of the sequences with those of corresponding *G. rostochiensis* populations available in GenBank showed 99.89-100% nucleotide similarity. The results of this study will help to better understand the physiology, ecology and biology of the nematode to quarantine this pest more effectively.

Keywords: *Globodera rostochiensis*, ITS, İzmir, morphology, phylogeny

Öz

Altın nematod, *Globodera rostochiensis* (Wollenweber, 1923) Skarbilovich, 1959 (Tylenchida: Heteroderidae) dünyada ekonomik açıdan en önemli patates zararlılarından birisidir. Genellikle patates yetiştirilen alanlarındaki dağılımları ve yoğunluklarını belirlemek amacıyla bu nematod için rutin olarak testler gerçekleştirilmektedir. Morfolojik ve moleküler analizler bu nematodun kesin tanısını yapmak ve mücadele stratejilerini oluşturmak amacıyla kullanılmaktadır. Bu çalışma, İzmir İli'nin Bozdağ ve Ödemiş ilçelerinden 2017 ve 2018 yılları patates yetiştiriciliği sezonlarında alınan toprak örneklerinin %96'sının *G. rostochiensis* ile enfekte olduğunu göstermiştir. Bulaşık alanlardaki ortalama kist sayısı, 0.01 ila 3.70 kist g^{-1} toprak arasında değişmiştir. *Globodera rostochiensis*'in ikinci dönem larva ve kistlerinin morfolojik ve morfometrik karakterlerinin incelenmesi, Bozdağ ve Ödemiş'ten elde edilen popülasyonlarda arasındaki hafif farklılıkları ortaya koymuştur. Tanımlamanın doğruluğunu değerlendirmek için, tüm popülasyonlar için kısmi ribozomal DNA sekansları amplifiye edilmiş, sekanslanmış ve GenBank veritabanına kaydedilmiştir. Morfolojik ölçümler ve filogenetik analizler sonucunda Bozdağ ve Ödemiş'ten elde edilen popülasyonlar arasında küçük farklılıkların olduğu belirlenmiştir. Sekansların GenBank'ta mevcut karşılık gelen *G. rostochiensis* popülasyonlarıyla karşılaştırılması sonucunda nükleotid benzerliği %99.89-100 oranında görülmüştür. Bu çalışmanın sonuçları, bu zararlıyı daha etkili bir şekilde karantinaya almak için nematodun fizyolojisini, ekolojisini ve biyolojisini daha iyi anlamaya yardımcı olacaktır.

Anahtar sözcükler: *Globodera rostochiensis*, ITS, İzmir, morfoloji, filogenetik

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Introduction

Potato (*Solanum tuberosum* L. subsp*. tuberosum*) is the most cultivated tuber crop and an important staple food for over one billion people in the world. It ranks as the fourth for its importance among food crops worldwide; following rice, wheat, and maize (FAOSTAT, 2020). Turkey is in the top ten potato producer countries in Europe with a production area of about 136 000 ha. Turkey annually produces about 34 t ha^{-1}, which is still below the average yield potential and the rest (about 40 t ha^{-1}) is being imported from developed countries (FAOSTAT, 2020). Potato yield reduction is attributed to several biotic and abiotic factors, including pests and pathogens (Subbotin et al., 2010). Plant-parasitic nematodes cause an annual loss of 12% in potato production worldwide (Chitwood, 2003). In tropical and subtropical climates, losses associated with nematodes are estimated at 14.6% compared to 8.8% in temperate countries (Sasser & Freckman, 1987). Among the top 10 plant-parasitic nematodes causing severe economic damage to crops around the world, six genera are resulting yield reduction in potato (Jones et al., 2013). Also, eight species from the seventeen quarantine nematodes declared by the European and Mediterranean Plant Protection Organization (EPPO, 2013) for the Euro-Mediterranean region, are major parasites of potato. Despite their considerable importance, nematodes of potato are not well studied in Turkey (Kepenekci, 2012).

Potato cyst nematodes (PCN), *Globodera* spp. are obligate parasites resulting in economic damage to potato around the world (Subbotin et al., 2010). These nematodes are quarantined internationally and subjected to strict regulatory measures (Fleming & Powers, 1998). These nematodes have already been found in 75 countries from Africa, Asia, Europe, North America, South America and Oceania (Ibrahim et al., 2000; Indarti et al., 2004; Andres et al., 2006; Gitty & Tanha Maafi, 2010). Species of PCN include golden potato cyst nematode, *Globodera rostochiensis* (Wollenweber, 1923) Skarbilovich, 1959, and pale potato cyst nematode, *Globodera pallida* Stone, 1973 (Tylenchida: Heteroderidae) are considered harmful quarantine organisms, described in European Union Directives 2000/29/EC and 2009/7/EC and are also part of EPPO A2 List (quarantine species already present in the EPPO region, A2/125 and A2/124, respectively) (EPPO, 2013). These species are regulated by the European Directive 2007/33/EC on the control of PCNs and are subject to stringent regulatory measures when detected singly or in combination (EPPO, 2013). Specific identification of these species is just possible by observation of the female color at the appropriate stage of development, either a change from white to yellow in *G. rostochiensis* or prolonged white (slightly cream but no yellow phase) in *G. pallida*.

The golden nematode, *G. rostochiensis*, is a regulated pathogen of potato and a threat to the potato industry in several countries (Scurrah et al., 2005). EPPO has recognized the nematode as plant health quarantine species in the A2 list, which shows the local presence of the pathogen within the Euro-Mediterranean region. The losses caused by the nematode mainly occurs in temperate regions, in Mediterranean countries, where the host plants are grown from mid-autumn to spring (Mugniéry, 1989). In Turkey, *G. rostochiensis* was recorded for the first time in 1985 (Enneli & Öztürk, 1996), following the importing of seed potato from European countries (Baldwin & Mundo-Ocampo, 1991). The number of infested potato-producing areas in Turkey has significantly increased in the last few years (Kepenekci, 2012). Therefore, the crop protection services observed and reported these nematodes in many potato growing areas in the country (Ulutaş et al., 2012; Imren, 2018; Özarslandan et al., 2019; Toktay et al., 2020).

The pathogenic variability within potato cyst nematode, *G. rostochiensis* populations is determined by a set of differential host genotypes, and populations are classified into five pathotypes designated as Ro1 to Ro5 (Subbotin et al., 2010). The generation rates of nematode populations on solanaceous plants containing resistance genes can be used to differentiate pathotypes (Kort et al., 1977). The identification of nematode species as well as their pathotype provides crucial information to select appropriate and efficient management strategies (Ganguly & Rao, 2003). The morphological discrimination of *Globodera* spp. is conducted via microscopic examination of the structures of cysts and infective juveniles (Golden,

1990; Siddiqi, 2000; Subbotin et al., 2010). The increasing number of species in the genus *Globodera* caused difficulties in obtaining sufficient criteria for the differentiation of species and requires highly specialized taxonomists due to the minor morphological and morphometric differences within its species (Subbotin et al., 1999, 2003). Molecular diagnostic techniques based on polymorphism of certain DNA fragments provide fundamental clues to overcome these taxonomic bottlenecks about morphological identification (Szalanski et al., 1997; Al-Banna et al., 2004; Subbotin et al., 2010). The sequences of the ribosomal DNA region including the ITS1, ITS2 and ribosomal genes facilitate reliable and rapid identification of *Globodera* spp. and differentiate them from other closely related cyst nematode species (Ferris et al., 1995; Subbotin et al., 2000; Madani et al., 2005, 2008; Skantar et al., 2007). The ribosomal DNA region can also be used as excellent genetic markers for diagnostics and the evaluation of phylogenetic relationships due to a large number of copies in individual cells, the lack of recombination, and strict maternal inheritance.

We conducted analyses to understand genetic structures of *G. rostochiensis* populations from two districts (Bozdağ and Ödemiş) of İzmir Province, Turkey. The objectives of the current study were (1) determine the distribution of the *G. rostochiensis* in Izmir Province, (2) to describe and evaluate the morphology and taxonomic features of local *G. rostochiensis* populations, and (3) to assess phylogenetic relationships of the populations based on the partial of ribosomal DNA sequences.

Materials and Methods

Nematode populations

A comprehensive survey was conducted during 2017 and 2018 potato growing seasons to collect soil samples from fields located in Bozdağ and Ödemiş Districts of Izmir Province, Turkey. Samples were taken prior to the potato harvest, between the end of September and the beginning of November (Table 1). Cyst extraction from soil samples was performed using a standard flotation and sieving technique (Southey, 1986). Extracted cysts were firstly categorized to genus level under a V20 model stereo-binocular microscope (Zeiss, Jena, Germany). At least 20 full cysts were selected and handpicked with a needle from each sample and stored at 4°C for further use in the morphological and molecular analysis. Additionally, the density of nematodes g^{-1} of soil was estimated.

Table 1. Location and density of *Globodera rostochiensis* sampled in this study

No	District	Location	Latitude	Longitude	Density (Cyst)	Accession Numbers
1	Bozdağ	Koca çayır	38º32’87 N	28º05’82 E	1.60	MT193688
2	Bozdağ	Yukarıçayır	38º32’90 N	28º06’12 E	0.50	
3	Bozdağ	Yukarıçayır	38º33’03 N	28º06’08 E	0.50	
4	Bozdağ	Tekke	38º33’42 N	28º06’22 E	1.10	MT193689
5	Bozdağ	Nazır	38º34’68 N	28º06’16 E	0.70	
6	Bozdağ	Burunucu	38º35’35 N	28º06’43 E	1.20	MT193690
7	Bozdağ	Kocaçayırlık	38º36’22 N	28º07’21 E	1.30	MT193691
8	Bozdağ	Çavdar	38º33’95 N	28º11’48 E	2.00	MT193692
9	Bozdağ	Çavdar	38º34’15 N	28º11’27 E	0.70	
10	Bozdağ	Büyük çavdar	38º34’55 N	28º11’71 E	0.50	
11	Bozdağ	Gündalan	38º35’53 N	28º10’95 E	1.20	MT193693
12	Bozdağ	Gündalan	38º34’84 N	28º10’47 E	3.70	MT193694
13	Bozdağ	Gündalan	38º35’87 N	28º10’01 E	2.60	
14	Bozdağ	Taşlıharım	38º32’52 N	28º06’07 E	1.50	MT193695

Table 1. Continued

No	District	Location	Latitude	Longitude	Density (Cyst)	Accession Numbers
14	Bozdağ	Taşlıharım	38º32’52 N	28º06’07 E	1.50	MT193695
15	Bozdağ	Taşlıharım	38º32’02 N	28º05’82 E	3.10	MT193696
16	Bozdağ	Ovacık	38º31’09 N	28º05’43 E	1.10	MT193697
17	Bozdağ	Ovacık	38º30’64 N	28º05’17 E	1.70	MT193698
18	Bozdağ	Gölcük Adabaşı	38º30’50 N	28º02’42 E	0.50	
19	Bozdağ	Karşıyaka	38º32’11 N	28º03’53 E	0.20	
20	Bozdağ	Gölcük	38º33’38 N	28º03’35 E	1.40	MT193699
21	Bozdağ	Boğaz	38º34’09 N	28º03’58 E	0.40	
22	Bozdağ	Çayırağzı	38º34’00 N	28º03’75 E	2.30	MT193700
23	Bozdağ	Aşağı Boğaz	38º35’13 N	28º03’47 E	1.20	MT193701
24	Bozdağ	Örselli yolu	38º31’41 N	28º02’11 E	0.40	
25	Bozdağ	Subatan	38º31’80 N	27º98’78 E	2.60	MT193702
26	Bozdağ	Subatan	38º32’33 N	27º99’22 E	0.80	
27	Bozdağ	Subatan	38º33’74 N	27º99’07 E	0.10	
28	Bozdağ	Kireçocağı	38º46’53 N	27º98’52 E	1.10	MT193703
29	Ödemiş	Küçükavulcuk	38º24’14 N	28º03’47 E	0.40	
30	Ödemiş	Küçükavulcuk	38º23’76 N	28º03’72 E	1.50	MT193704
31	Ödemiş	Küçükavulcuk	38º22’86 N	28º02’49 E	0.00	
32	Ödemiş	Küçükavulcuk	38º24’32 N	28º02’96 E	0.70	
33	Ödemiş	Büyükavulcuk	38º23’62 N	28º23’16 E	2.50	MT193705
34	Ödemiş	Gerçekli-Topçukuyu	38º22’39 N	28º06’29 E	2.10	MT193706
35	Ödemiş	Ocaklı-Türbe	38º22’88 N	28º01’36 E	1.50	MT193707
36	Ödemiş	Yolüstü-Beylikkırı	38º20’60 N	28º03’37 E	0.60	
37	Ödemiş	Gerekli-Petrol	38º19’86 N	28º04’26 E	0.20	
38	Ödemiş	Gerekli-Canigırı	38º21’81 N	28º05’60 E	2.10	MT193708
39	Ödemiş	Alidereli	38º21’46 N	27º99’91 E	0.20	
40	Ödemiş	Konaklı-Millik	38º12’95 N	27º99’74 E	1.30	MT193709
41	Ödemiş	Konaklı-Köy civarı	38º40’60 N	27º36’45 E	2.10	MT193710
42	Ödemiş	Yeniköy-Karadoğan	38º23’00 N	27º91’40 E	0.06	
43	Ödemiş	Karadoğan-Kırarası	38º23’85 N	27º91’52 E	0.40	
44	Ödemiş	Karadoğan Değirmen	38º24’48 N	27º92’22 E	1.20	MT193711
45	Ödemiş	Ortaköy-Yumurtacı	38º24’54 N	27º93’35 E	1.40	MT193712
46	Ödemiş	Ortaköy-Yumurtacı	38º24’57 N	27º93’17 E	0.08	
47	Ödemiş	Yeniköy-Köygirişi	38º23’91 N	27º95’06 E	0.00	
48	Ödemiş	Günlüce Altı	38º24’92 N	27º97’14 E	0.08	
49	Ödemiş	Günlüce at çiftliği	38º26’21 N	27º96’80 E	1.30	MT193713
50	Ödemiş	Günlüce Riga Çiftlik	38º25’38 N	27º97’05 E	0.04	
51	Ödemiş	Yeniköy-Karapınar	38º25’13 N	27º94’43 E	0.01	
52	Ödemiş	Seviköy Küsküt Mh.	38º20’21 N	27º86’86 E	1.10	MT193714
53	Ödemiş	Seviköy Akçagün	38º21’43 N	27º85’13 E	0.04	
54	Ödemiş	Yusufdere-Yolkıyı	38º22’11 N	27º84’18 E	0.02	
55	Ödemiş	Kayaköy-Alabaşı	38º18’51 N	27º84’47 E	2.10	MT193715
56	Ödemiş	Kayaköy-Alabaşı	38º19’06 N	27º83’99 E	2.10	MT193716
57	Ödemiş	Kayaköy köyaltı	38º20’04 N	27º82’93 E	3.40	MT193717

Morphological identification

The identity of a newly discovered population of potato cyst nematodes, *Globodera* spp., was first performed based on morphological characteristics of second-stage juvenile (J2) (length of body, stylet and tail, and hyaline portion) and cyst (distance fenestra to anus, fenestra diameter, Granek's ratio, and numbers of cuticular ridges between anus and vulva). Vulva cones were excised from cysts and mounted in Canada balsam (Hooper, 1970). Juveniles were treated by gentle heating (60°C) and fixed in a TAF solution (triethanolamine, formalin and ultrapure water at a ratio of 2:7:91) and processed to glycerin. TAF-fixed specimens were examined under an Axio Lab. A1 model light microscope (Carl Zeiss AG, Oberkochen, Germany). Measurements were estimated using ZEN Lite software with the support of an Axiocam ERc5s digital camera (Carl Zeiss AG, Oberkochen, Germany). The observed features of the cysts and second-stage juveniles were compared with those of reference materials and the description of the neotypes in the literature (Golden & Ellington, 1972; Manduric et al., 2004).

The data were normalized using the Shapiro-Wilk normality test before they were analyzed using analysis of variance (ANOVA) (Shapiro & Wilk, 1965). Significant differences among characters were determined using protected least significant differences using SPSS statistical software V 17.0 (IBM Corp., Armonk, NY, USA) at $P < 0.001$. The standard test of means was conducted to detect the significant variance between populations ($P \leq 0.05$).

Molecular identification

DNA was extracted from each population using the Worm Lysis Buffer Method (WLB) (Waeyenberge et al., 2000). A single J2 from a single cyst was handpicked and transferred into a 0.2 ml tube containing 10 µl sterile ultrapure water. Then 8 µl of lysis buffer (100 mM Tris-Cl, 500 mM KCl; 15 mM $MgCl_2$, 10 mM dithiothreitol, 0.1% gelatin, and 4.5% Tween 20) and 2 µl of proteinase K (Cat No./ID: 19131 Qiagen GmbH, Hilden, Germany) at 600 µg/ml were added to the mix in the tube. The microtube was heated to 65°C in a DB-100 dry-block heater (Techne, Cambridge, UK) for 1 h, and consecutively to 95°C for 5 min to inactivate the proteinase K (Holterman et al., 2006). The lysate was centrifuged at 16,000 g for 5 min and the supernatant was transferred into a new 0.2 ml tube and stored at -20°C for further use. F194 (5′-CGTAACAAGGTAGCTGTAG-3′) and F195 (5′-TCCTCCGCTAAATGATATG-3′) primers developed by Ferris et al. (1993) were used for amplification of the ITS for all samples. PCR reactions were carried out in a total volume of 50 µl containing 1 µl of nematode lysate, 1 × Ammonium Buffer, 1 µM each of primers, 0.2 mM dNTPs and 1-unit Ampliqon TEMPase Hot Start DNA polymerase (Berntsen, Rødovre, Denmark). Three PCR reactions lacking DNA (no template control) were also performed. PCR amplification was conducted in a T100 thermal cycler (Bio-Rad Laboratories, CA, USA) programmed as follows: initial denaturation at 94°C for 15 min followed by 30 cycles of 30 s at 94°C, 30 s at 58°C, 45 s at 72°C and a final extension at 72°C for 5 min. PCR products were detected by electrophoresis on 1.2% agarose gels and visualized by staining with ethidium bromide. The DNA banding patterns were visualized and documented by a G:Box F3 gel documentation system (Syngene, UK). PCR products were subjected to bidirectional sequencing by a commercial company (Macrogen Inc., Seoul, Korea) after purification with Wizard SV Gel and PCR Clean-Up System (Promega, Madison, WI, USA) according to the manufacturer instructions.

Phylogenetic analysis

A BLAST search for ITS sequences was performed in the GenBank database to determine the closest available reference sequences in the complete nucleotide collection of the National Center for Biotechnological Information (NCBI; http://blast.ncbi.nlm.nih.gov). All sequences of the populations obtained in the present study were deposited in the GenBank database under accession numbers from MT193688 to MT193717 (Table 1). The sequence with those of corresponding *G. rostochiensis* population from different countries available in the GenBank nucleotide database (EF622522 from Australia,

FJ212167 from Canada; KR057953 from Serbia, DQ847117 from the USA, JF907550 from Poland and MT193701 from Japan) were aligned with Clustal W (Thompson et al., 1994) and edited manually using MEGAX (Kumar et al., 2018). The maximum likelihood method was performed to construct a phylogenetic tree from the sequence data using MEGAX software based on the General Time Reversible model (Tamura & Nei, 1993). The confidence of phylogenetic tree topologies was confirmed by bootstrap analysis from 1,000 replicates (Felsenstein, 1985). The sequence of *G. pallida* population from the USA (EF153837) was included as an outgroup to root the phylogenetic tree.

Results and Discussion

The morphological and molecular identification confirmed the presence of the golden nematode, *G. rostochiensis*, in the surveyed areas. *Globodera rostochiensis* was detected in potato fields located both in Bozdag, with the mountain slopes at an altitude of about 1,000 m, and in flat areas in Ödemiş. Overall, 55 of 57 soil samples (96%) obtained from Bozdağ and Ödemiş Districts were found being infected with *G. rostochiensis*. Two locations in Ödemiş were found free of potato cyst nematode (Table 1). In Turkey, first report of the presence of *G. rostochiensis* was declared in 1996 from a field of seed potatoes in Dörtdivan District of Bolu Province (Enneli & Öztürk, 1996). Although, strict regulatory measures have been enforced to prevent further spread of nematode; it was not possible to completely prevent it from contaminating important potato producing areas in Turkey (Kepenekci, 2012). Ulutaş et al. (2012) reported that *G. rostochiensis* was found in 17 and 62% of the fields investigated in the Aegean Region and Ödemis District, respectively. Since then, a remarkable increase has occurred in the incidence of infestation of potato cultivation areas in İzmir Province. The presence of the nematode has recently been confirmed by Demirbaş Pehlivan et al. (2020). This phenomenon requires the extension services and quarantine departments to establish a control method to minimize the population of *G. rostochiensis* in the infested areas.

In this study, the mean number of cysts in all the infested fields was 0.14 cysts g^{-1} of soil; however, the infestation levels of *G. rostochiensis* varied between the surveyed locations. The fields with less than 0.20 cysts g^{-1} of soil represented about 65% of the total number of infested potato fields. The mean number of cysts of 0.21-0.50 represented 19% of infested fields, and the average of more than 0.5 cysts represented only 4.2% of the surveyed fields (Table 1). The highest density of cysts (3.70 g^{-1} of soil) was found in Gündalan in Bozdağ (sample 57) and the other higher densities were 3.40 and 3.10 cysts g^{-1} of soil in Kayaköy-Köyaltı location of Ödemiş (sample 15) and in Taşlıharım location Bozdağ (sample 12), respectively. The lowest cyst density (0.01 g^{-1} of soil) was found in Yeniköy-Karapınar (sample 51) and the other lower densities were 0.02 and 0.04 cysts g^{-1} of soil found in Yusufdere Yolkıyı (sample 54) and Günlüce-Riga Çiftlik (sample 50) location of Ödemiş, respectively. Similarly, Özarslandan et al. (2019) reported that the mean cysts number of *G. rostochiensis* in the soil was found 0.24 cysts g^{-1} of soil in Nevşehir Province in the Central Anatolia Region, Turkey. The occurrence and density of *G. rostochiensis* in potato growing areas in Izmir Province would ultimately reach the economic threshold levels. Greco et al. (1982) reported that the loss threshold for *G. rostochiensis* and *G. pallida* in Italian potato growing areas was between 1.4 and 2.1 eggs g^{-1} of soil, respectively. Also, the economic threshold for *G. rostochiensis* in the UK is calculated as 15 eggs g^{-1} of soil (Dale, 1988). Therefore, it is recommended to establish a strategy of combining cultural control methods including crop rotation with non-host crops and the use of resistant potato cultivars. The application of chemicals should be limited to avoid environmental pollution or deterioration of the physical properties of the soil.

Second-stage juveniles of *G. rostochiensis* had cylindrical bodies, tapering at both extremities, mostly posterior (Figure 1a). The tail was shortened, and the head was slightly offset with prominent cephalic sclerotization and rounded. The stylet was visible and anteriorly flattened to rounded knobs. The median bulb was ellipsoidal with a prominent valve. Esophageal glands extended ventrally for about 35

percent of body length (Figure 1b). Genital primordium located about 60 percent of body length. Tail tapered to a finely rounded terminus (Figure 1c).

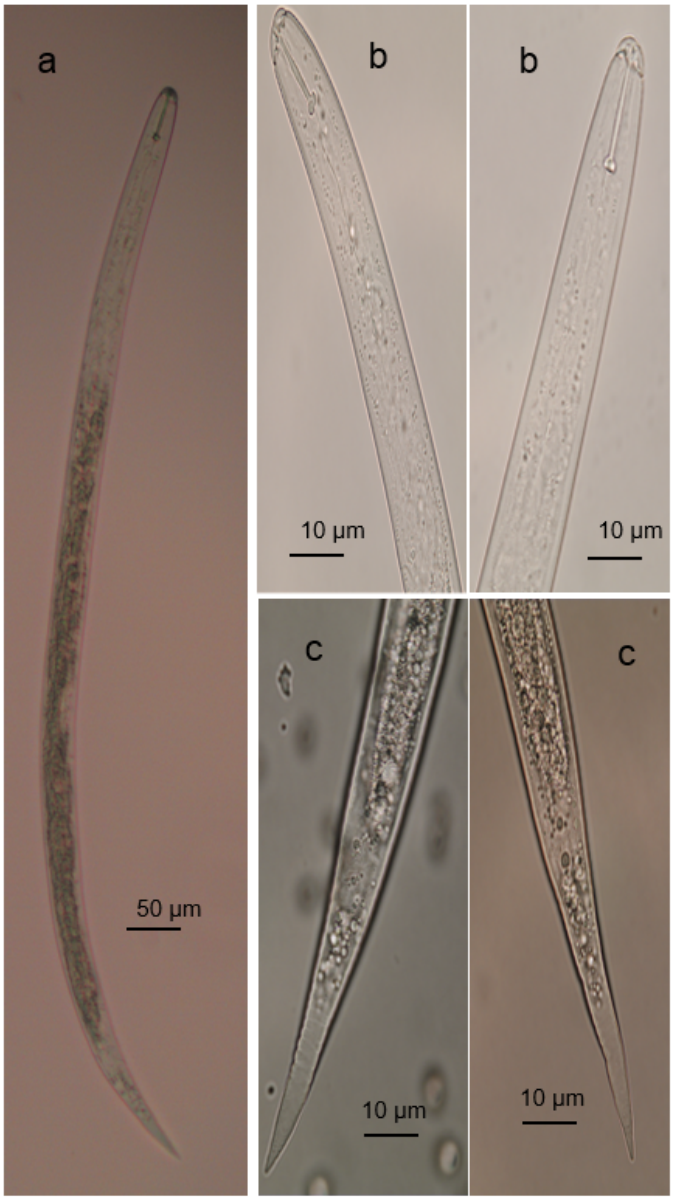

Figure 1. Second-stage juveniles of *Globodera rostochiensis* from İzmir. Specimens from İzmir: (a) body, (b) heads, and (c) tails.

In the Bozdağ populations, the body length of juveniles varied from 399 to 496 µm, and stylet length was 19 to 22 µm with rounded, slightly backward sloping stylet knobs (Table 2). Tail length and hyaline length were 38 to 46 µm and 17 to 28 µm, respectively. Among Ödemiş populations, the body length varied from 418 to 550 µm, and stylet length was 19-23 µm with anteriorly indented stylet knobs. Tail length and hyaline length were 32 to 56 µm and 21 to 30 µm, respectively (Table 2). Similarly, Wouts & Baldwin (1998) and Siddiqi (2000) stated that body length ranged from 445 to 510 µm, stylet length was 18 to 29 µm, tail length was 37 to 55 µm and the hyaline tail part was 21 to 31 µm for the second stages juveniles of *G. rostochiensis*, which was in accordance with our results.

Table 2. Morphometrics (in µm) of second-stage juveniles of *Globodera rostochiensis* from Izmir Province, Turkey

Characters	Bozdağ population			Ödemiş population		
	Range	Mean	SD	Range	Mean	SD
Body length	399-496	452.00	5.00	418-550	470.00	7.00
Stylet length	19-22	19.80	0.20	19-23	20.90	0.70
Tail length	38-46	41.40	1.12	32-56	47.26	2.30
Hyaline tail terminal length	17-28	20.64	1.14	21-30	27.80	1.55

The average values of morphological characters of second-stage juveniles were larger in populations of *G. rostochiensis* from Ödemiş than those from Bozdag (Table 2). However, the differences in body length and tail length between Ödemiş and Bozdağ populations were not significant ($P \leq 0.5$). The morphological average values of the J2 of *G. rostochiensis* were consistent with the range described in previous studies (Fleming & Powers, 1998; EPPO, 2013). For all 30 populations, the morphological characters of J2s, especially body length and tail length, were consistent with Wouts and Baldwin (1998). However, the measurements of the J2s slightly differed from those estimated by Golden (1986), who measured a longer tail length (42 to 67 µm) and a longer hyaline length (20 to 36 µm). According to their round shape and light-brown color cysts, populations were considered as *G. rostochiensis*.

The cysts were brown or yellow, spherical or subspherical in shape, with protruding necks and lacking a terminal cone. Cysts had a small projecting neck and with tanned brown skin. The cuticle surface had a zigzag pattern of ridges and a distinct D-layer was present. The perineal area consisted of a single circumfenestration around the vulval slit in vulval basin; underbridge and bullae were rarely present (Figure 2). Morphometric data for larvae also indicates similarity to *G. rostochiensis* (Table 3). Morphometrics of cyst perineal region *G. rostochiensis* (Table 3) provided clear differences between populations of Ödemiş and Bozdağ. The populations from Ödemiş had the largest values of vulva-anus distance, the number of cuticular ridges between vulva and anus, and Granek's ratio, fitting well within ranges of *G. rostochiensis* whereas populations from Bozdağı had small mean values of Granek's ratio and the number of cuticular ridges overlapping of *G. rostochiensis* (Subbotin et al. 2010).

Table 3. Morphometrics (in µm) of second-stage juveniles of *Globodera rostochiensis* from Izmir Province, Turkey

Characters	Bozdağ population			Ödemiş population		
	Range	Mean	SD	Range	Mean	SD
Body length excluding neck	423-592	521	7	469-712	587	8
Body with	389-545	472	12	446-610	534	10
Distance from anus to nearest edge of fenestra (anus-vulva)	52-68	58.4	1.4	56-71	66.6	2
Fenestra length	17-23	18.6	0.2	18-26	21.6	1.2
Number of cuticular ridges between vulva-anus	18-21	19	0.2	19-24	22	0.4
Granek's ratio	2.63-4.0	3	0.3	2.9-4.2	3.7	0.6

The cyst length of *G. rostochiensis* for Bozdağ populations varied from 423 to 592 µm, and body width was 389 to 545 µm with a spherical shape, with a short neck and no terminal cone. The anus to vulva and fenestra length were 52 to 68 µm and 17 to 23 µm, respectively. The number of cuticular ridges between vulva-anus and Granek's ratio were 18 to 21 and 2.63 to 4.00 (Table 3). The cyst length of *G. rostochiensis* for Ödemiş populations ranged from 469 to 712 µm and body width varied from 446 to 610 µm with a spherical shape, small projecting neck, lacking terminal cone. The anus to vulva and fenestra length were 56 to 71 µm and 18 to 26 µm, respectively. The number of cuticular ridges between vulva-anus and Granek's ratio were 19 to 24 µm and 2.9 to 4.2, respectively. Likewise, Wouts & Baldwin (1998), Siddiqi (2000) reported that cyst length without neck and width were 423 to 592 µm and 469 to 712 µm. The neck

length and mean fenestral diameter were 104±19 µm and 19.0±2.0 µm. The fenestral length and Granek's ratio were 20 µm and 3.0, respectively.

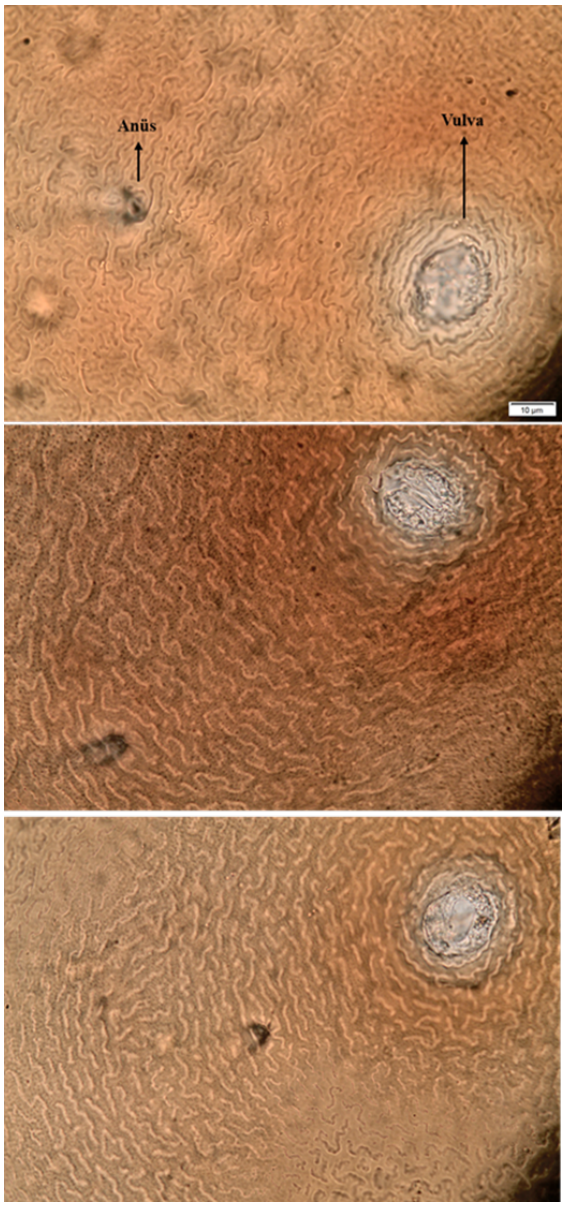

Figure 2. Photomicrographs of the anal-vulval regions of *Globodera rostochiensis* cysts from İzmir Province, Turkey.

The mean morphological and morphometric values of cysts were higher for Ödemiş populations than those of Bozdağ populations (Table 3). However, the differences between Ödemiş and Bozdağ populations were not significant ($P \leq 0.5$) for number of ridges and anus-vulva distance. Also, the average values associated with the morphological and morphometric features of the cysts were within the range described in previous studies (Fleming & Powers, 1998; EPPO, 2013). For all populations, the overlap was high in cysts morphological and morphometric characters especially fenestral length and Granek's ratio with Wouts & Baldwin (1998), but these values were slightly differed clearly from other Golden (1986) by slightly higher mean in the distance from anus to the nearest edge of fenestra 58.4 (52-68 µm).

All samples produced a single fragment of about 950 bp using F194 and F195 primers. The amplified sequences were used as BLAST queries against the NCBI database and had 99.89-100% nucleotide similarity with those of corresponding species recorded in GenBank. The result of the study, 30 cyst populations from Bozdağ and Ödemis locations compared with closely related cyst samples in GenBank and were all identified as *G. rostochiensis* based on their ITS sequences. The *G. rostochiensis* populations in this study were compared molecularly with international genotypes from different countries (Figure 3). The analysis involved 37 nucleotide sequences. The clustering of the populations on the phylogenetic tree that occurred according to their species levels based on genetic distance was constructed from the ITS sequence alignment (Figure 3).

Figure 3. Maximum likelihood tree was generated using the ITS sequences of populations of *Globodera rostochiensis* and reference populations from GenBank including their accession numbers and strain numbers. Numbers on the branches represent bootstrap values obtained from 1,000 bootstrap replications.

However, Madani et al. (2010) reported that *G. rostochiensis* populations from different locations in Canada were clustered in one group within the phylogenetic tree. Knoetze et al. (2013) reported that the ITS sequence alignment of *G. rostochiensis* populations from South Africa clustered together with a high bootstrap value similarity using the minimum evolution method. Different cyst nematode species were thought to be phylogenetically examined based on ITS sequences and this region was thought to be useful in identifying species (Subbotin et al., 2000). Therefore, the slight distances among *G. rostochiensis* population can be explained by high gene flow among potato cyst nematode populations.

Population diversity within the genus *Globodera* has often been used successfully for diagnostic purposes to discriminate *G. rostochiensis* populations from non-quarantine species (Baldwin & Mundo-Ocampo, 1991; Fleming & Powers, 1998; Manduric et al., 2004). This study is the first to provide a comprehensive molecular analysis of *G. rostochiensis* populations from Izmir and is complementary to other studies of Turkish potato cyst nematodes populations. This study emphasized the agroecological distribution of the potato cyst nematode species *G. rostochiensis* in Bozdağ and Ödemiş Districts in İzmir. The differences determined by morphological and morphometric techniques also demonstrated a variation among *G. rostochiensis* populations, indicating that climatic conditions of the mountainous area could influence variability. It is be concluded that the results of this study will help to investigate more of pathotypes of golden nematode and to gain a more complete understanding of the physiology, ecology and biology of the genus *Globodera* as agricultural pests for an effective management.

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