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First report of *Bursaphelenchus leoni* Baujard, 1980 (Nematoda: Parasitaphelenchidae) from Pine Forests of Turkey

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ABSTRACT

Several surveys have been performed in Turkey in order to determine a possible presence of *Bursaphelenchus xylophilus* (Steiner and Buhrer, 1934), Nickle, 1970. As a result, several *Bursaphelenchus* species have been reported. During a study aimed to identify potential insect vectors of these *Bursaphelenchus* species using the trap tree method in the western region of Turkey, one *Bursaphelenchus* species was isolated from a wood chip sample of a *Pinus brutia* trap log located in Bergama town of the city of İzmir. According to morphological and molecular studies, the species was determined as *Bursaphelenchus leoni* Baujard, 1980. Morphological characteristics of the species matched well with the original description made by Baujard in 1980. 18S and 28S rRNA genes of *B. leoni* were sequenced and a phylogenetic tree was constructed that showed *B. leoni* was clustered with *B. leoni* in the NCBI database. This is the first report of *B. leoni* from Turkey. To determine presence of other known *Bursaphelenchus* species in Turkey, new studies should be performed.

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ÖZET

Bursaphelenchus xylophilus (Steiner and Buhrer, 1934), Nickle, 1970'un tespiti için Türkiye'de bir kaç arazi calısmaları yapılmıştır. Sonuç olarak, bir kaç *Bursaphelenchus* türü rapor edilmiştir. Rapor edilen bu *Bursaphelenchus* türlerinin Türkiye'nin batı bölgesinde taşıyıcı böceklerinin tuzak ağaçları ile belirlenmesi icin yürütülen bir çalışmada, İzmir'in Bergama ilçesinde Kızılçam kütüklerinden bir *Bursaphelenchus* türü izole edilmistir. Morfolojik ve moleküler çalısmalara göre, bu tür *Bursaphelenchus leoni* Baujard, 1980 olarak belirlenmiştir. Bu türün morfolojik özellikleri 1980 yılında Baujard tarafından yapılan orjinal tanımlamayla eşleşmiştir. 18S and 28S rRNA genlerinin dizileyip filogenetik ağaç oluşturulmuş ve bu ağaç izole edilen *B. leoni*'nin NCBI veribankasındaki *B.leoni* türleri ile kümelendiğini göstermistir. Bu, *B.leoni*'nin Türkiye'den ilk kaydıdır. Bilinen diğer *Bursaphelenchus* türlerinin Türkiye'deki varlıklarının belirlenmesi için yeni çalısmalar yürütülmelidir.

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INTRODUCTION

The genus *Bursaphelenchus* Fuchs, 1937 contains over 125 described species worldwide and most of them are wood or soil inhabiting (Ryss et al., 2005;

Kanzaki and Giblin-Davis, 2018). Transmission of *Bursaphelenchus* species mainly occurs via insect vectors from a wide range of taxonomic insect groups (Ryss et al., 2005) and host trees of *Bursaphelenchus* species described so far range from conifer species to broad leaves (Ryss et al., 2005). Diversity in host tree and insect vectors of *Bursaphelenchus* species reflect adaptation to various environmental conditions and provide insights into evolution in nematodes.

Only two *Bursaphelenchus* species are known to cause sudden death of host plants under natural conditions: *B. cocophilus* (Cobb) Baujard, the pathogen of red ring disease of palm trees (Griffith et al., 2005) and *B. xylophilus* (Steiner and Buhrer, 1934) Nickle, 1970 the causal agent of pine wilt disease (Mamiya, 1983; Futai, 2013). *B. xylophilus*, the pinewood nematode (PWN), has been causing extensive damage in susceptible pine forests of Japan since the beginning of the 1900's (Futai 2013). In addition to Japan, it has been distributed in China, Korea, Taiwan, and Portugal (Futai 2013; Mota et al., 1999). In recent years, limited occasions of the PWN have been reported from Spain (Abelleira et al., 2011).

Turkey possesses around 22 million ha forest area, of 27 percentage of the total land area. The majority of forest area is composed of conifer trees, mainly pine species with 30% coverage (Atalay et al. 2014). In additon to a wide range of pine forests, diverse climatic conditions have contributed biological richness of Turkey (Atalay, 1995).

In Turkey, the survey studies of *B. xylophilus* has been ongoing since 2002 (Akbulut et al., 2010). At the end of these surveys, with the exception of *B. xylophilus*, several *Bursaphelenchus* species were reported (Akbulut et al. 2006, 2007, 2008a, 2008b; Dayi et al. 2014). During the last survey to detect insect vectors of *Bursaphelenchus* species in conifer forests of the western region of Turkey in 2012, *Bursaphelenchus* species were isolated from logs of *Pinus brutia* Ten. in the İzmir Regional Forestry Directorate. To identify the isolated *Bursaphelenchus* species morphological and molecular studies were performed.

MATERIAL and METHOD

Collection and isolation of Nematodes

A survey was conducted to investigate the insect vectors of *Bursaphelenchus* species in pine forests of the western regions of Turkey in 2012 (Dayi et al, 2014). During the survey, pine trees cut (wood stocks) by İzmir Regional Forestry Directorate for timber production were investigated for the presence of *Bursaphelenchus* species and possible insect vectors. Approximately 40–80 g wood chip samples were taken from each log with insect activity to check the presence of the nematode. To take wood chip samples, a wood auger was used and collected samples put into polyethylene bags and brought into the laboratory. We extracted nematodes using a modified Baermann Funnel Technique (Hooper 1986) and handpicked using Olympus SZX-12 microscope and transferred into petri dishes of Botrytis cinerea Pers. (1794) grown on malt agar at 25 °C to obtain a laboratory culture. After the culture developed, the nematodes were washed with sterile water, and collected nematodes were used for identification studies.

To prepare permanent slides, nematodes extracted from the log samples were killed at 65 °C and fixed in TAF solution [7 ml formalin (40% formaldehyde), 2 ml triethanolamine and 91 ml pure water] (Hooper, 1986). Then, nematodes were transferred to solution I (1 part glycerol and 79 parts pure water) at 35-40 °C for 12 h and later in solution II (5 parts glycerin and 95 parts 96% ethanol) at 40 °C for 3 h. Nematodes were put in a desiccator for the period of time required for all remaining water to evaporate (Seinhorst, 1959). The nematodes were kept in pure glycerin and mounted on glass slides using the wax-ring method (Hooper, 1986).

Morphological studies for identification of nematodes

Nematodes were identified by morphological, morphometric characters and molecular methods. Each sample has 10 female and 10 male nematodes on slides. A Leica DM 4000B microscope was used for microscopic observations and pictures (Ryss et al, 2005).

Molecular Identification of *B. leoni*

DNA extraction from nematodes

DNA was extracted from nematodes (1 to 5 individuals of *B. leoni*) and later were rinsed for 5 min in autoclaved Milli-Q water before transferring into a 1.5 ml micro tube containing 50 µL of DNA Extraction Buffer (DEB) included 0.25 mg Proteinase K (Fisher Scientific: BP-1700-500) per 1 mL of 1x PCR buffer (Thermo Fisher Scientific: BP6112). A sterile micro pestle was used to crash nematodes before inclubating for 2.5 h in a water bath at 60 °C followed by 15 min incubation at 95 °C for inactivation of the Proteinase K. We cooled the tubes in ice for 5 min and stored at -20 °C to use at PCR (Polymerase Chain Reaction) (Thermo Fisher Scientific: BP6112).

Amplification of 18S and 28S rRNA and Sequencing

We used 2.0 µL DNA as template for PCR reactions, 0.4 µL of each primer (forward and reverse), 1.25 µL dNTPs, 2.5 µL of 10x Buffer, 2.0 µL of Titanium Taq, 18.25 µL of H2O and 25 µL was the final volume. We used the primers M13-18S-1-2A- (Forward

primer:TGTAAAACGACGGCCAGTCGATCAGATAC CGCCCTAG) and M13-18S-r2b- (Reverse primer:CAGGAAACAGCTATGACTACAAAGGGCAG GGACGTAAT) to amplify 18S rRNA with the following cycle conditions; 94 °C for 3 min for initial denaturation, 94 °C for 30 sec for deneration, 57 °C for 30 sec 40 cycles for anneling, 68 °C for 1 min 40 cycles for extension, and 68 °C for 3 min, and 40 cycles for the final extension. To amplify 28S rRNA we used the primers M13 D2A-28S- Forward primer: TGTAAAACGACGGCCAGTACAAGTACCGTGAGGG AAAGT, and M13 D3B-28S- Reverse primer: CAGGAAACAGCTATGACTGCGAAGGAACCAGCTA CTA and appplied same PCR conditions used to amplify 28S rRNA. After PCR reactions, we purified PCR and sequenced using the Sanger Sequencing (Sanger et al.1977).

Phylogenetic Analyses

Phylogenetic analyses were performed using 18S sequence data of *B. leoni*. We included other *Bursaphelenchus* and *Aphelenchoides* species 18S sequences downloaded from the NCBI (National Center for Biotechnology Information) to construct phylogenetic trees. Alignments of 18S sequences of species used for phylogenetic analyses were performed using MUSCLE (v3.8.31) (Edgar CR, 2004), and then trimmed using trimAl (1.2rev59) (Capella-Gutierrez S et al. 2009). For choosing the best substitution model, jModelTest v2 (Darriba et al. 2012) was used. Maximum Likehood Phylogenetic analysis was performed using RAxML v8 (Stamatakis, 2014). with 500 bootstrap and GTRGAMMA model based on jModelTest v2 result. The phylogenetic tree was visualized using TreeView (Page RD, 1996).

Locality

Bursaphelenchus leoni was found in the Bergama location of İzmir city, Forest Enterprise of İzmir Regional Forestry Directorate (N: 39o 14' 08" E:27 o 07' 42" and 650 m asl).

Deposition of Material

A total of 10 slides *Bursaphelenchus leoni* samples used for morphological and morphometric measurements in this manuscript were deposited at the Department of Plant Protection, Çukurova University, Adana, Turkey.

RESULTS and DISCUSSION

Systematics and Differential Diagnosis

B. leoni belongs to the leoni group which are known to have large vulval flap, mostly seven caudal papillae in male nematodes with the exception of *B. silvestris*. Females of this group has long and thin tails. Described species so far have three incisures in the lateral field (Braasch et al. 2009).

Bursaphelenchus leoni was found in *Pinus brutia* samples in the Bergama location of İzmir. Specimens collected from the Bergama location corresponds matched well with the original description by Baujard (1980) and reports of Philis and Braasch (1996) and Li et al. (2020) (Table 1 and Table 2).

The morphological characteristics and allometric criteria of male and female individuals were given in Figure 1. The body of *B. leoni* had typically slender with a distinct off-set lip region on head and Aphelenchoid median bulb (Figure 1a, b).

Male: Body cylindirical, showing vertrally curved and J-shaped structure when killed by heat and fixed. Lip region hemispheric, offset distictly from body. Cuticle with invisible, vey fine annulation. The stylet is 14,4 µm (12,8-16,0) long with very small basal thickenings (knobs). The excretory pore was located ½ to 1 body diameter behind the median bulb. Males have paired spicules showing same shape as described by Baujard (1980) and indicaed by Philis and Braasch (1996) and Li et al. (2020). Spicules curved dorsally with a prominent rostrum without cucullus at the distal end, condylus of the cuticulum distictly curved dorsally (Figure 1 c,d,e). One single and one pair caudal papillae located at preanal and one pair caudal papillae at postanal located ca. middle of the tail are present (Figure 1 c,d,e). It is known that the bursa shape is variable in some *Bursaphelenchus* species (Braasch et al. 1998). The bursa tightly covered only the anus aperture, bursal flap spade like with posterior margin truncate or irregular as indicated by Li et al. (2020).

Female: Body cylindirical, slightly curved at vulva when killed by heat and fixed. Stylet is 14,8 µm (10,5-20,8) long with very small basal thickenings (knobs). The anterior vulval lip of females developed as a small vulval flap (Figure 1 f,g). Anterior body region and annulation same as male. Single gonad outstreched to anteriorly. The post uterus branch is long and sometimes reached ca. 60% of the vulva-anus distance. Size of female tails is a relatively long ended conoid to finely rounded end with slight ventral curvature. Females of *B. leoni* have conical tail. (Figure 1 h,i).

Bursaphelenchus leoni occurs mainly in warm regions and reported from many Mediterranean countries as well as from South Africa (Philis and Braasch, 1996; Braasch et al., 1998).

Molecular Characterization and Phylogeny

The 18S rRNA sequence length was 868 bp, matched with the *B. leoni* (accession number MN907406) (99.63 % identify, e-value 0.0 and 0% gaps). Besides, and the 28S rRNA length was 649 bp and matched

with the *B. leoni* (accession number MN907407) (99.20 % identity, e-value 0.0 and 0% gaps). Sequences of 18S and 28S of *B. leoni* were deposited into Genbank with the access numbers MW073442 and MW075383, respectively. The phylogenetic tree of the 18S sequence showed that *B. leoni* is clustered with *B.leoni* (accession number MN907406) in NCBI database (Figure 2) and the phylogenetic tree of 28S clustered *B. leoni* as a sister species to other *B. leoni* isolates from China and Germany (Figure 3).

Table 1. Morphometric data for female *Bursaphelenchus leoni* from Bergama location of İzmir city-Turkey* and comparison with other isolates from Cyprus, France, South Africa and China.

Çizelge 1. Türkiye İzmir Bergama bölgesinden Bursaphelenchus leoni dişi bireylerin Güney Afrika, Fransa, Kıbrıs ve Çin'deki diğer örneklerin morfometrik ölçümleriyle kıyaslanması.

Characteristics	Female (Izmir-Turkey)	Cyprus (Philis and Braasch. 1996)	France (Baujard, 1980)	South Africa (Braasch et al., 1998)	China (Li et al., 2020)
n	10	10	38	15	15
L (µm)	770.6±116.3(672-955)	750 (700-850)	700 (580-860)	799 (740-900)	920±42.6(848-1001)
a	39.3±4.9 (30.5-50.2)	39 (29-45)	39 (33-44)	36 (29-45)	44.0±2.3(40.5-48.1)
b	8.1±0.9 (6.7-9.4)	9 (8-10)	10 (8-13)	6.2 (5.3-7.4)	10.8±0.4 (9.8-11.4)
b'	5.4±0.8 (4.5-7.7)	-	-	-	6.0±0.4 (5.4-6.8)
c	12.7±2.3 (9.6-16.4)	11 (9-13)	12 (10-15)	12 (11-14)	11.9±0.7 (11.0-13.4)
c'	5.8± 1.2 (2.8-7.5)	-	-	-	6.1±0.4 (5.3-6.9)
Tail (µm)	63.3±17.1 (32-83)				77±6.6 (65-90)
V (%)	67.8± 6.6 (51-77)	69 (63-71)	71 (69-74)	70 (62-72)	-
Stylet (µm)	14.8±2.8 (10.5-20.8)	15 (13-17)	13 (12-17)	15 (14-18)	13.8±0.9 (12.2-15.5)
Vulva/anus distance (µm)	165.3±18.4 (140.8-201.6)				180±9.5 (163-196)
Body width at anus (µm)	13.4±4.3(6.4-20.8)	-	-	-	12.8±0.6 (11.5-13.7)
Distance from anterior end to junction of oseophagus and intestine (µm)	95.1±14.3 (70.4-120)	-	-	-	85±3.8 (80-90)
Distance from anterior end to posterior end of oseophageal glands (µm)	140.5±18.9 (115-176)	-	-	-	155±11.9 (135-172)
Distance from anterior end to base of median bulb (µm)	75.7±10.0 (65.6-96.6)	-	-	-	-
Oesophageal glands overlapping intestine length (µm)	44.7±19.5 (19.2-88)	-	-	-	-
Oesophageal glands overlapping intestine length/ Body with to junction of oesophagus and intestine stylet length	2.5±0.9 (1.3-4.5)	-	-	-	-
Distance from anterior end to excretory pore (µm)	96.6±9.1 (83.2-113.6)	-	-	-	112±5.1 (104-122)
Distance from anterior end to hemizonid (µm)	104.2± 8.5 (94.4-120)	-	-	-	114±4.9 (107-123)
Anterior genital branch length (µm)	342.8±51.7(268.8-456)	-	-	-	417±89.0 (297-650)
G1 (%)	46±15.4 (38-86)	-	-	-	-
Post-uterine branch length (µm)	118.3±21.5 (88-158)	-	-	-	79±12.8 (59-97)
G2 (%)	15.4±3 (10.9-21)	-	-	-	-
Post-uterine branch lenght/ Vulva-anus distance (%)	64.9±5.9 (60-71)	-	-	-	43.7±6.9 (31.6-53.5)
Body diameter at vulva	19.4±2.9(17.6-27.2)	-	-	-	20.9±1.2 (18.5-22.4)
Post-uterine branch length / Body diameter at vulva	6.5±1.7(4-9.6)	-	-	-	-

* All measurements were calculated as µm and mean± Standart Deviation.

The genus *Bursaphelenchus* has gained importance since *Bursaphelenchus xylophilus* was reported as the causative agent of pine wilt disease in susceptible conifer forests of the world. *Bursaphelenchus* species are mostly known as minor or nonpathogenic organisms associated with living trees and feed on fungus in wood.

Table 2. Morphometric data for male *Bursaphelenchus leoni* from Bergama location of İzmir city-Turkey* and comparison with other isolates from Cyprus, French, South Africa and China.

Çizelge 2. Türkiye İzmir Bergama bölgesinden Bursaphelenchus leoni erkek bireylerin Güney Afrika, Fransa, Kıbrıs ve Çin'deki diğer örneklerin morfometrik ölçümleriyle kıyaslanması.

Characteristics	Female (Izmir-Turkey)	Cyprus (Philis and Braasch. 1996)	France (Baujard, 1980)	South Africa (Braasch et al., 1998)	China (Li et al., 2020)
n	10	10	29	15	15
L (µm)	709.4±37.6 (654.4-771.2)	670 (610- 720)	640(510-1060)	699 (620-699)	799±62.7(695-893)
a	39.3±4.5 (34.6-51.1)	34 (26-43)	42 (36-56)	36 (30-45)	42.7±2.7(38.1-47.5)
b	7.0±0.4(6.3-7.5)	7 (7-8)	9 (7-15)	6.5 (4.6-8.3)	9.8 ± 0.8(8.5-11.2)
b'	4.7±0.2(4.3-5.1)				5.1 ± 0.7(3.3-6.1)
c	20.1±1.7 (16.3-21.9)	28 (26-30)	20 (16-26)	27 (24-30)	21.9 ± 1.7(19.4-25.2)
c'	2.5±0.2 (2.2-3)				2.6 ± 0.2(2.2-2.9)
Tail (µm)	35.5±3.5 (30.4-43.2)				37 ± 3.0 (32-43)
Stylet (µm)	14.4±0.8(12.8-16)	14 (12-14)	13.5 (13-17)	14 (13-15)	14.0± 0.8 (12.4-15.4)
Spicule (µm) (curved median line)	18.0±1.0(16-19.2)	18 (15-20)	15(10-20)	19 (18-21)	14.5 ± 0.9(13.4-16.9)
Spicule (chord)	-	-	-	-	16.8 ± 1.0(15.2-18.9)
Anterior end to pharyngo-intestinal junction (µm)	101.6±5 (96-113.6)	-	-	-	81 ± 2.6 (77-86)
Anterior end to pharyngeal gland end (µm)	151.2±5 (144-160)	-	-	-	158 ± 27.6(136-248)
Distance from anterior end to base of median bulb (µm)	71.8±0.5 (68.8-73.6)	-	-	-	-
Oesophageal glands overlapping intestine length (µm)	49.0±5.3 (38.4-57.6)	-	-	-	-
Oesophageal glands overlapping intestine length/ Body with to junction of oesophagus and intestine stylet length	3.0±0.4 (2.4-3.8)	-	-	-	-
Anterior end to excretory pore (µm)	96.0±5.0 (84.8-104)	-	-	-	106 ± 6.8 (97-122)
Anterior end to hemizonid (µm)	105.8±3.3 (100.8-112)	-	-	-	108 ± 6.7 (94-123)
Testis length (T)	407.2±77.2 (310.1-467)	-	-	-	479 ± 62.8 (375-604)
Anal or cloacal body diam.(µm)	14.6±1.3 (12.8-16.8)	-	-	-	14.3± 1.3 (12.3-16.5)
Lip diam.	-	-	-	-	6.5± 0.3(6.2-7.1)
Lip height	-	-	-	-	3.2± 0.3(2.7-3.6)
Median bulb diam.	-	-	-	-	11.1 ± 0.7(10.1-12.3)
Median bulb length	-	-	-	-	16.6 ± 0.7(15.2-18.1)
Median bulb length: diam.	-	-	-	-	1.5 ± 0.1

* All measurements were calculated as µm and mean± Standart Deviation.

The original description of *B. leoni* was given by Baujard (1980). It was isolated from the wood of Pinus pinaster subsp. *atlantica* in France and characterized by the presence of a vulval flap, the form of the female tail, morphology of spicules and bursa in the male (Baujard, 1980). Recently it was suggested that *B. leoni* and *B. borealis* are conspecific based on essential morphological features such as long slender bodies, lip region and stylet structure and position of excretory pore and these two species belong to the leoni-group (Li et al. 2020).

Philis and Braasch (1999) suggested that *B. leoni* is a typical species of warm climate regions. *B. leoni* occurs mainly in warm regions and reported from many Mediterranean countries as well as from South Africa (Philis and Braasch, 1996; Braasch et al., 1998). So far, it was reported from France (Baujard, 1980), Italy (Palmisano and Ambrogioni, 1994), Cyprus (Philis and Braasch, 1996), Greece (Skarmoutsos and Skarmoutsos, 1999), and less frequently in Southern Germany and Austria (Braasch et al., 1999, Tomiczek, 2000, Braasch and Philis, 2002). In addition to Europe and Mediterranean regions, this species was also reported from South Africa (Braasch et al., 1998) and China (Li et al., 2020). Braasch et al. (1998) isolated *B. leoni* from a *P. radiata* D. Don tree in South Africa for the first in the southern hemisphere. They suggested that *B. leoni* is distributed widely in Southern Europe and an indigenous species for the Mediterranean region. They suggested that the presence of *B. leoni* in South Africa was due to similarities in climatic conditions between the Western Cape Province, South Africa and the Mediterranean Region (Braasch et al.,

1998). In the current study, *B. leoni* was found in the İzmir Regional Forestry Directorate located in the western part of Turkey. The city of İzmir has similar climatic conditions with both Southern Europe and the Mediterranean region. This supports the idea of *B. leoni* being a species of warm climate regions (Philis and Braasch, 1996).

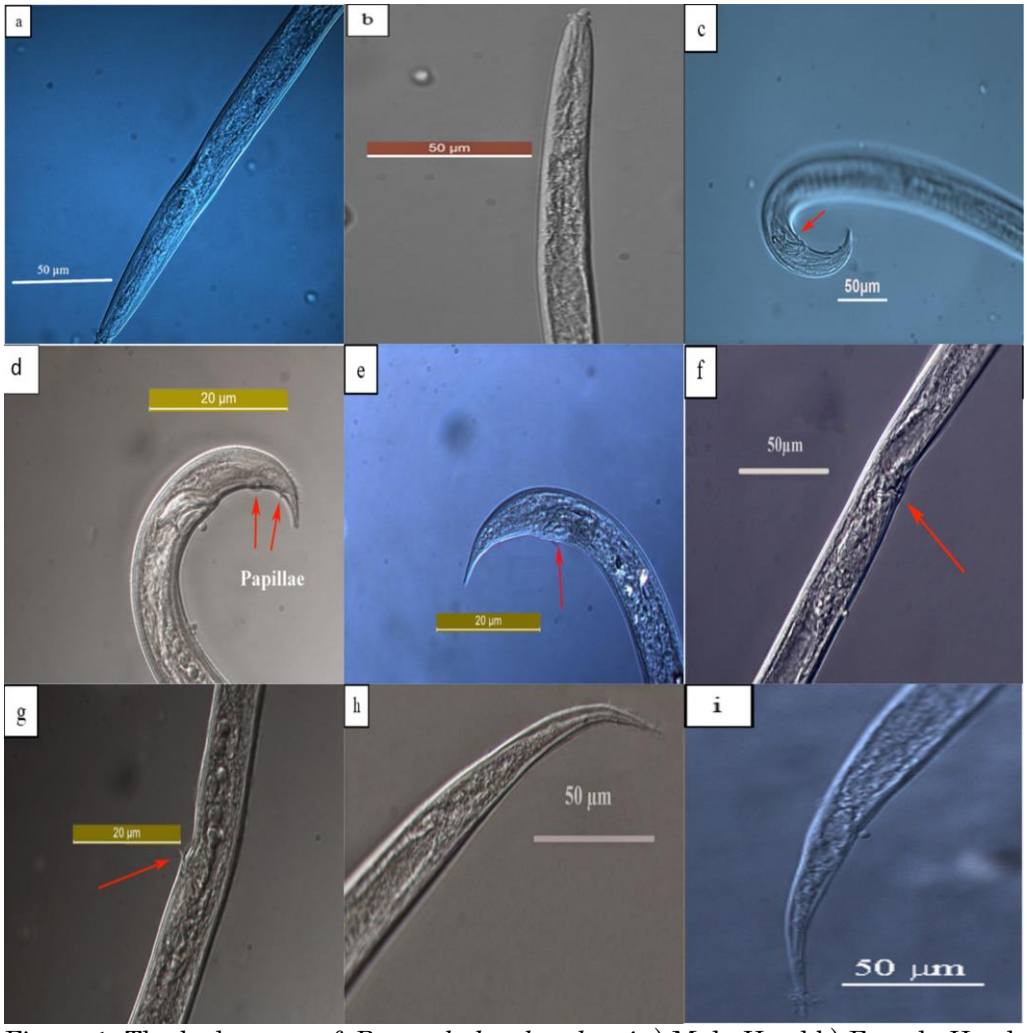

Figure 1. The body parts of *Bursaphelenchus leoni* a) Male-Head b) Female-Head c-d-e) Spicule and Male Tail in lateral view, d) Papillae f-g) Vulva h-i) Female Tail in lateral view

Şekil 1. Bursaphelenchus leoni vücut kısımları a) Erkek-Baş b) Dişi-Baş c-d-e) Spikul ve Erkek Kuyruğunun lateral görünümü, d) Papillalar f-g) Vulva h-i) Dişi kuyruğunun lateral görünümü

CONCLUSIONS

In this study, *B. leoni* was extracted from wood chips of *P. brutia*. According to other reports from different countries, the host species of *B. leoni* are *Pinus* species i.e. *P. pinaster* in France (Baujard, 1980), *P. pinaster*, *P. pinea*, *P. halepensis*, and *P. sylvestris* in Italy (Ambrogioni et al., 1994; Caroppo et al., 1998; Ambrogioni and Caroppo 1998), *P. brutia*, *P. pinea*, and *P. nigra* in Cyprus (Philis, 1996; Philis and Braasch, 1996; Braasch and Philis, 2002), *P. brutia, P. nigra, P. pinaster, P. radiata* and *P. halepensis* in Greece (Skarmoutsos and Skarmoutsou, 1999; Michalopoulos Skarmoutsos et al., 2004), *P. halepensis* and *P. pinea* in Spain (Escuer et al., 2002; Escuer et al., 2004) and *P. radiata* in South Africa (Braasch et al., 1998). In general, *B. leoni* has been isolated from dead or dying trees, but there is no certain proof that *B. leoni* may cause tree death as *B. xylophilus* does (Braasch et al., 1998), and no connection was found in wilting cases in Cyprus (Philis, 1996). Skarmoutsos and Michalopoulos-Skarmoutsos (2000) studied the pathogenicity of several *Bursaphelenchus* species on 3-year-old pine seedlings. It was reported that 55% of *B. leoni* inoculated *P. halepensis* seedlings wilted with low numbers of re-isolated nematodes. In the current study, the nematode was isolated from a wilted *P. brutia* tree, but no connection was found between the presence of *B. leoni* and wilting incidence of pine trees. So far, there is no record about the insect vectors of *B. leoni* (d'Errico et al., 2015). In Cyprus,

most of the *B. leoni* infested trees were attacked by bark beetles (Philis and Braasch, 1996). In Turkey, new studies are required to find out distribution areas, host tree species (addition to *P. brutia*), insect vectors and pathogenic potential of *B. leoni*.

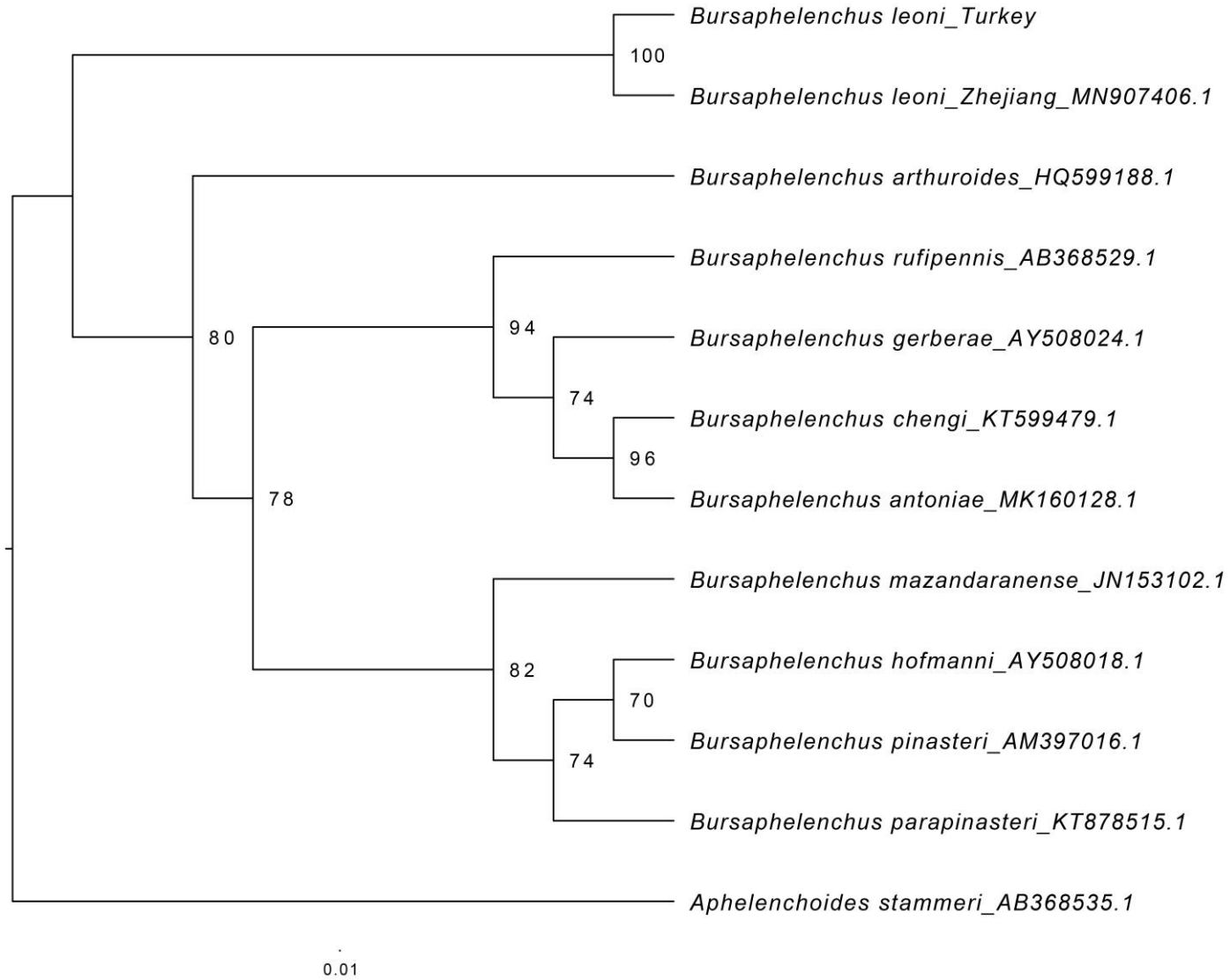

Figure 2. Maximum Likehood tree inferred from 18S rRNA gene under GTRGAMMA model by RAxML. Bootstrap values exceeding 50% are shown on appropriate clades. *Aphelenchoides stammeri* was used as outgroup species.

Şekil 2. RAxML tarafından GTRGAMMA model altında 18S rRNA geninden oluşturulan Maximum Likehood ağacı. %50'yi gecen destek degerleri ilgili grup icin gosterilmektedir.

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The authors declare that they have contributed equally to the article.

Conflicts of Interest Statement

None of the authors had any financial or personal relationships with other individuals or organizations that might inappropriately influence their work during the submission process.

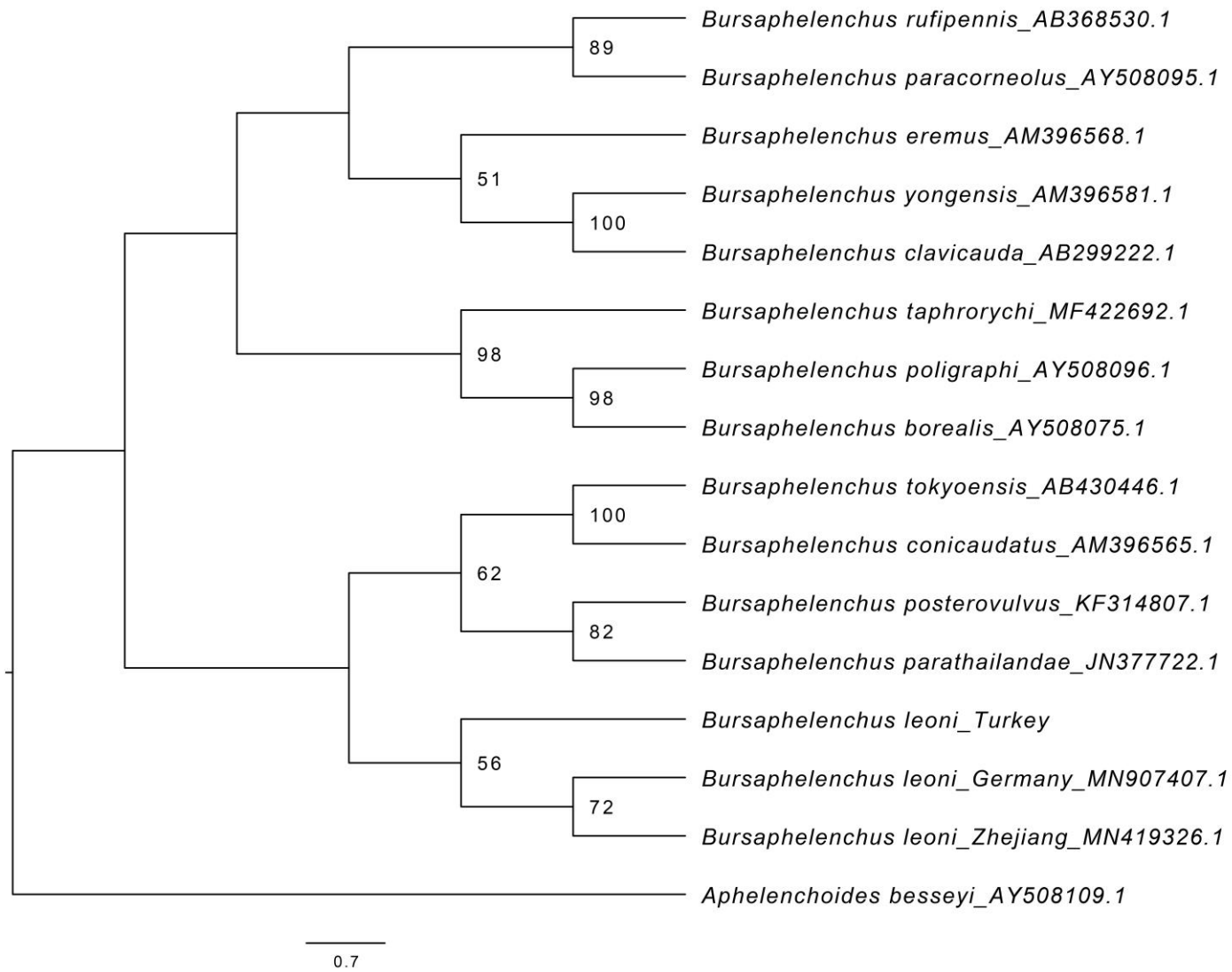

Figure 3. Maximum Likehood tree inferred from 28S rRNA gene under GTRGAMMA model by RAxML. Bootstrap values exceeding 50% are shown on appropriate clades. *Aphelenchoides besseyi* was used as outgroup species.

Şekil 3. RAxML tarafından GTRGAMMA model altında 28S rRNA geninden oluşturulan Maximum Likehood ağacı. %50'yi gecen destek degerleri ilgili grup icin gosterilmektedir.

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