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First Record of *Bursaphelenchus vallesianus* Braasch, Schönfeld, Polomski, and Burgermeister in Turkey

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Abstract: *Bursaphelenchus vallesianus* isolated from a dead Scots pine, *Pinus sylvestris*, in the village of Bahçecik northeastern Turkey in an area managed by the Trabzon Regional Forestry Directorate is described and illustrated. The morphological characters match the original description. This is the first report of the species on pine wood in Turkey.

Key Words: *Bursaphelenchus vallesianus*, *Pinus sylvestris*, Pine wood nematode, Morphology, Morphometrics

***Bursaphelenchus vallesianus* Braasch, Schönfeld, Polomski ve Burgermeister'un Türkiye'de İlk Kaydı**

Özet: *Bursaphelenchus vallesianus*, Türkiye'nin kuzeydoğusunda, Trabzon Orman Bölge Müdürlüğü tarafından yönetilen alan içindeki Bahçecik köyünde ölü bir sarıçamdan, *Pinus sylvestris*, izole edilerek tanımlanmıştır. Morfolojik karakterler orijinal tanımlama ile uyşmaktadır. Bu türün Türkiye'de çam odunundan sağlanmasına ait ilk kayıttır.

Anahtar Sözcükler: *Bursaphelenchus vallesianus*, *Pinus sylvestris*, Çam odun nematodu, Morfoloji, Morfometrik ölçümler

Introduction

Pine wilt disease, caused by the pinewood nematode, *Bursaphelenchus xylophilus* (Steiner and Buhner, 1934) Nickle, 1970 (Nematoda: Parasitaphelenchidae), is a significant threat to susceptible conifer forests worldwide. This disease is native to North America, but has established itself widely in eastern Asia and more recently was introduced into Portugal (UK-CAB International, 1999). The pine wilt disease problem has increased scientific interest in this group of nematodes. Ryss et al. (2005) reported the existence of more than 75 *Bursaphelenchus* species worldwide. To date, with descriptions of several new species of the genus, the number of species has increased to about 90 (Akbulut et al., 2007).

The pinewood nematode was first detected in Europe (in Portugal) in 1999 (Mota et al., 1999). The detection

of this A1 quarantine organism (EPPO) in a member state of the European Union (EU) forced the implementation of specific measures to control and eradicate this nematode and its *Monochamus* beetle vectors (Coleoptera: Cerambycidae) from infested areas in Portugal. Surveys of the pinewood nematode within the pine forests of Portugal and other member states of the EU were implemented by directive (2001/219/EC). Turkey is not a member of the EU, but as a member of the EPPO it follows the phytosanitary regulations for quarantine organisms in Europe (Akbulut et al., 2006). Therefore, a survey should be carried out in order to ensure the absence of *B. xylophilus* in the pine forests of Turkey. It is also important to gather data on *Bursaphelenchus* species and their distribution in the forests of Turkey.

Due to these reasons, surveys of *B. xylophilus* in Turkey were started in 2003. Before this survey, the

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presence and distribution of *Bursaphelenchus* species in Turkey were not known. Some results of these surveys resulted in the genus being reported for the first time in association with conifer woods, and the results were presented and published in international meetings and journals (Vieira et al., 2003; Akbulut et al., 2006; Vieira et al., 2006; Akbulut et al., 2007). *Bursaphelenchus anatolius* Giblin-Davis, Hazır, Center, Ye, Keskin, Thorp, and Thomas was described from Ankara as an associate of bees in the genus *Halictus* (Giblin-Davis et al., 2005).

During one survey in 2004, wood samples were collected from forests managed by the Trabzon Regional Forestry Directorate of Turkey. A *Bursaphelenchus* species was found in a Scots pine tree (*Pinus sylvestris* L.) as a first record for Turkey. This species was identified as *Bursaphelenchus vallesianus* Braasch, Schönfeld, Polomski, and Burgermeister (Braasch et al., 2004). The original description of *B. vallesianus* was made by Braasch et al. (2004). They found this species in declining *P. sylvestris* trees in Canton Valais, Switzerland. *B. vallesianus* is a member of the *Bursaphelenchus sexdentati* group (Braasch, 2001). How *B. vallesianus* was similar to and different than other *Bursaphelenchus sexdentati* group members was previously described (Braasch et al., 2004). In the current study, the morphological and morphometric characteristics of the Turkish population of *B. vallesianus* found in a wilted *P. sylvestris* tree in the village of Bahçecik are given and compared to the original description (Braasch et al., 2004).

Materials and Methods

In 2004 a survey was conducted in Turkish forests managed by the Trabzon Regional Forestry Directorate. Wood samples weighing 40-80 g each were collected from the trunks of wilted *Pinus sylvestris* L. and *Picea orientalis* (L.) Link trees. Using a Pressler borer, samples were taken 1.5 m above the forest floor from opposite sides of each tree and were then stored in polyethylene bags. The nematodes were extracted from the pine wood fragments using the modified Baermann funnel technique (Southey, 1986) over the course of 48 h. *B. vallesianus* was isolated and multiplied on *Botrytis cinerea* Pars. (grown on malt agar) in petri dishes for 2 weeks at 25 °C. Nematode specimens reared on *B. cinerea* were collected, heat killed, fixed in TAF, and processed to

glycerol. The specimens that were mounted in permanent slides were used for morphological and morphometric observations under a Zeiss Axioskop 2 plus microscope with an Olympus DP-10 digital camera. For each character mean and standard deviation (range) were calculated.

Results

In all, 80 trees were sampled from forests managed by the Trabzon Regional Forestry Directorate (Table 1). A sample taken from a wilted *P. sylvestris* tree in a *P. sylvestris* stand (mature forest with 40% canopy closure) in the village of Bahçecik (lat 520130 N, long 4489633 E (ED 50, Zone 37) 1423 m elevation) contained *Bursaphelenchus vallesianus* Braasch, Schönfeld, Polomski, and Burgermeister, 2004.

This species is characterized by a relatively small stylet with basal swellings, a lateral field with 4 lines, an excretory pore located anterior to or at the median bulb (Braasch et al., 2004). *B. vallesianus* belongs to the *sexdentati* group (Braasch et al., 2004; Lange et al., 2007).

Females display all the features of the original description of *Bursaphelenchus vallesianus* given by Braasch et al. (2004). Body slightly curved when killed by heat. Body 696-914 µm long, lip region convex, offset, without labial annules, about 3.5-4.0 µm high × 6-7.5 µm wide. Lateral field with 4 lines and 3-3.5 µm wide. Stylet without feasible knobs and 12.7-15.1 µm long. Median bulb longer than wide. Excretory pore located at the median bulb. Esophageal glands overlapping intestine for 1.5-3 body diameters. Female has 1 prodelphic gonad with the vulva at 75% of the body length. Vulva-anus distance 178 ± 25.8 (144-200) µm. Vulva has small cuticular flap. The female body is slightly swollen behind the vulva and has a conical, rounded, or finely rounded tail terminus, 1.7-2.2 times longer than anal body diameter.

Males also display all the features of the original description of *Bursaphelenchus vallesianus* given by Braasch et al. (2004). Body C-shaped with curled tail when killed by heat. Anterior region of body and cuticle markers similar to female. Testis usually outstretched, tail ventrally arcuate and pointed, bearing a small terminal bursa (about 10 µm long); spicules paired with prominent rostrum, condylus dorsally bent.

Table 1. Location, tree species, aspect, and altitude (m) of each sampling point.

No	Forest District	Location	Tree Species	Aspect	Altitude (m)
1	Sürmene	Çamburnu	<i>P. sylvestris</i>	W	240
2	Sürmene	Çamburnu	<i>P. sylvestris</i>	S	173
3	Sürmene	Çamburnu	<i>P. sylvestris</i>	SW	197
4	Sürmene	Çamburnu	<i>P. sylvestris</i>	SW	197
5	Sürmene	Çamburnu	<i>P. sylvestris</i>	SW	197
6	Sürmene	Çamburnu	<i>P. sylvestris</i>	S	170
7	Sürmene	Çamburnu	<i>P. sylvestris</i>	S	170
8	Sürmene	Çamburnu	<i>P. sylvestris</i>	S	165
9	Sürmene	Çamburnu	<i>P. sylvestris</i>	S	165
10	Sürmene	Çamburnu	<i>P. sylvestris</i>	SW	170
11	Sürmene	Çamburnu	<i>P. sylvestris</i>	SW	175
12	Sürmene	Çamburnu	<i>P. sylvestris</i>	SW	175
13	Sürmene	Çamburnu	<i>P. sylvestris</i>	SW	152
14	Sürmene	Çamburnu	<i>P. sylvestris</i>	W	155
15	Sürmene	Çamburnu	<i>P. sylvestris</i>	W	150
16	Sürmene	Çamburnu	<i>P. sylvestris</i>	W	145
17	Sürmene	Çamburnu	<i>P. sylvestris</i>	W	129
18	Sürmene	Çamburnu	<i>P. sylvestris</i>	NW	30
19	Çaykara	Gökmar	<i>P. sylvestris</i>	NW	1430
20	Çaykara	Gökmar	<i>P. orientalis</i>	NW	1430
21	Çaykara	Gökmar	<i>P. sylvestris</i>	NW	1400
22	Çaykara	Gökmar	<i>P. sylvestris</i>	NW	1400
23	Çaykara	Gökmar	<i>P. sylvestris</i>	NW	1400
24	Çaykara	Gökmar	<i>P. orientalis</i>	S	1800
25	Çaykara	Gökmar	<i>P. orientalis</i>	SE	1620
26	Çaykara	Gökmar	<i>P. orientalis</i>	S	1580
27	Çaykara	Gökmar	<i>P. sylvestris</i>	S	1550
28	Çaykara	Gökmar	<i>P. sylvestris</i>	SE	1563
29	Çaykara	Gökmar	<i>P. sylvestris</i>	S	1450
30	Çaykara	Gökmar	<i>P. orientalis</i>	S	1450
31	Çaykara	Gökmar	<i>P. orientalis</i>	SW	1495
32	Çaykara	Gökmar	<i>P. orientalis</i>	SW	1500
33	Çaykara	Gökmar	<i>P. orientalis</i>	SW	1500
34	Çaykara	Gökmar	<i>P. orientalis</i>	E	1115
35	Çaykara	Gökmar	<i>P. sylvestris</i>	E	1115
36	Maçka	Zigana Tat.	<i>P. orientalis</i>	W	1600
37	Maçka	Zigana Tat.	<i>P. orientalis</i>	N	1616
38	Maçka	Zigana Tat.	<i>P. orientalis</i>	N	1668
39	Maçka	Zigana Tat.	<i>P. orientalis</i>	NE	1650
40	Maçka	Zigana Tat.	<i>P. sylvestris</i>	W	1640

Table 1 (Continued).

No	Forest District	Location	Tree Species	Aspect	Altitude (m)
41	Maçka	Mataracı	<i>P. orientalis</i>	NE	997
42	Maçka	Mataracı	<i>P. orientalis</i>	N	1005
43	Maçka	Mataracı	<i>P. orientalis</i>	NE	1010
44	Maçka	Mataracı	<i>P. orientalis</i>	NE	1010
45	Maçka	Mataracı	<i>P. orientalis</i>	NE	1010
46	Maçka	Mataracı	<i>P. orientalis</i>	NE	1010
47	Maçka	Mataracı	<i>P. orientalis</i>	NE	1010
48	Maçka	Mataracı	<i>P. orientalis</i>	NE	1010
49	Torul	Soğuksu	<i>P. sylvestris</i>	NW	1330
50	Torul	Soğuksu	<i>P. sylvestris</i>	SW	1340
51	Torul	Soğuksu	<i>P. sylvestris</i>	S	1450
52	Torul	Soğuksu	<i>P. sylvestris</i>	E	1524
53	Torul	Bahçecik	<i>P. sylvestris</i>	N	1265
54	Torul	Bahçecik	<i>P. sylvestris</i>	N	1265
55	Torul	Bahçecik	<i>P. sylvestris</i>	N	1380
56	Torul	Bahçecik	<i>P. sylvestris</i>	NW	1418
57 (T-47)	Torul	Bahçecik	<i>P. sylvestris</i>	E	1423*
58	Torul	Bahçecik	<i>P. sylvestris</i>	NE	1490
59	Torul	Bahçecik	<i>P. sylvestris</i>	N	1490
60	Trabzon	Merkez	<i>P. sylvestris</i>		30
61	Trabzon	Merkez	<i>P. sylvestris</i>		30
62	Trabzon	Merkez	<i>P. sylvestris</i>		30
63	Trabzon	Merkez	<i>P. sylvestris</i>		30
64	Trabzon	Merkez	<i>P. sylvestris</i>		30
65	Trabzon	Merkez	<i>P. sylvestris</i>		30
66	Trabzon	Merkez	<i>P. sylvestris</i>		30
67	Trabzon	Merkez	<i>P. sylvestris</i>		30
68	Trabzon	Merkez	<i>P. orientalis</i>		30
69	Tonya	Melikşah	<i>P. orientalis</i>	E	1027
70	Tonya	Melikşah	<i>P. orientalis</i>	N	945
71	Tonya	Melikşah	<i>P. orientalis</i>	N	945
72	Tonya	Kale	<i>P. orientalis</i>	W	1120
73	Tonya	Çayırıçı	<i>P. orientalis</i>	E	1007
74	Tonya	Derindere	<i>P. orientalis</i>	S	1618
75	Tonya	Sazalan	<i>P. sylvestris</i>	NE	1708
76	Tonya	Sazalan	<i>P. sylvestris</i>	NE	1709
77	Tonya	Kalıncam	<i>P. orientalis</i>	NW	1000
78	Tonya	Kalıncam	<i>P. orientalis</i>	SE	991
79	Tonya	Kalıncam	<i>P. orientalis</i>	SE	991
80	Tonya	Kalıncam	<i>P. orientalis</i>	S	959

*The sample in which *Bursaphelenchus vallesianus* was found.

The measurements (Table 2) and diagnostic features (Figure 1) of the samples found in this study are clearly similar to the original description of Braasch et al. (2004). Confirmation of the morphological description was made by H. Braasch (pers. Comm. with H. Braasch).

ITS-RFLP analysis of the nematodes was performed by W. Burgermeister in Germany and shown to be *B. vallesianus* (pers. comm. with W. Burgermeister). The ITS-RFLP patterns of *B. vallesianus* were previously published by Burgermeister et al. (2005); therefore, ITS-

RFLP patterns of *B. vallesianus* are not given in this paper in an effort to avoid duplication.

Discussion

Investigation of *Bursaphelenchus* species and clarification of their differences from *B. xylophilus* are important for the early detection of *B. xylophilus* (Braasch and Braasch-Bidasak, 2002). During this study *B. xylophilus* was not detected in any of the pine wood

Table 2. Measurements of *Bursaphelenchus vallesianus* females (mean \pm SD μ m (range)).

Parameter	Population from Turkey		Braasch et al. (2004)	
	Male	Female	Male	Female
n	10	10	10	10
L	796 \pm 48.1 (736-850)	810 \pm 80.5 (696-914)	753 \pm 110 (613-918)	880 \pm 110 (688-1063)
Stylet	13.6 \pm 0.7 (12.8-14.4)	13.6 \pm 0.8 (12.7-15.1)	13 \pm 0.9 (12-15)	13 \pm 1.1 (11-15)
Esophagus	74.6 \pm 4.4 (69-80)	116.2 \pm 12.9 (104-130)	72 \pm 7.5 (63-83)	72 \pm 12 (59-100)
Anterior end to bulb	39.2 \pm 1.4 (37-41)	60.5 \pm 5.5 (54.1-67.5)	41 \pm 3.5 (38-44)	46 \pm 8.0 (38-58)
Tail length	25.2 \pm 4.6 (20-31)	20.9 \pm 4.8 (17-27)	26 \pm 5.1 (19-37)	26 \pm 3.1 (19-31)
V		75.3 \pm 1.5 (73-77)		73 \pm 1.6 (71-75)
Vulva-anus distance		178 \pm 25.8 (144-200)		210 \pm 25 (175-259)
Post-uterine sac length		103 \pm 19.9 (91-133)		113 \pm 31 (80-155)
a	33.6 \pm 5.02 (28-41)	40.6 \pm 3.4 (36.5-46)	29 \pm 7.6 (22-46)	38 \pm 3.1 (31-42)
b	11.04 \pm 1.04 (9.5-12.0)	13.1 \pm 2.1 (10.3-15.6)	10.2 \pm 0.9 (9.2-12.2)	12.3 \pm 1.8 (8.7-15.7)
c	32.2 \pm 6.3 (25-42)	42.0 \pm 5.3 (37.6-49.8)	30 \pm 5.3 (20-37)	30.4 \pm 4.0 (28-42)
c'	2.2 \pm 0.4 (1.8-2.8)	1.9 \pm 0.2 (1.7-2.2)	1.9 \pm 0.4 (1.5-3.0)	2.4 \pm 0.3 (1.9-2.8)
Spicules	16.2 \pm 1.5 (14.4-18.0)		17 \pm 1.5 (15-19)	

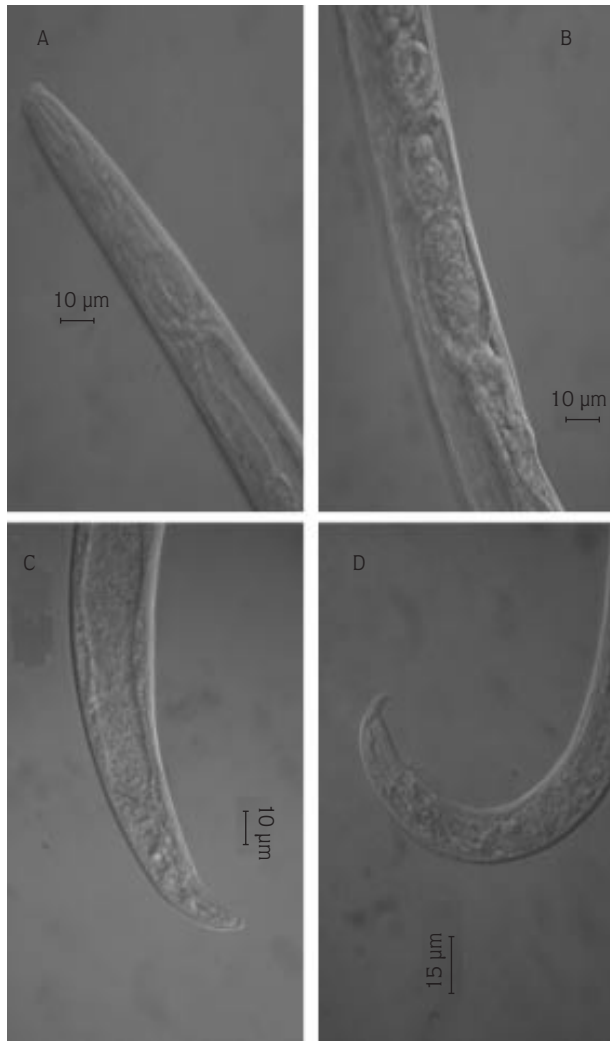


Figure 1. Light optical microscopy observations of *Bursaphelenchus vallesianus*. A: Anterior region; B: vulva region; C: female tail; D: male tail.

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samples. Previously, only a few species of *Bursaphelenchus* have been found in Turkey (Vieira et al., 2003; Giblin-Davis et al., 2005; Vieira et al., 2006; Akbulut et al., 2006, Akbulut et al., 2007). *B. vallesianus* was isolated from the wood of *P. sylvestris*. Morphological characteristics and measurements of Turkish populations of *B. vallesianus* are very similar to the original description of the species given by Braasch et al. (2004).

This is the first report of *B. vallesianus* from Turkey. This species was also found in *P. nigra* in Greece, and in *P. sylvestris* in Germany (Lange et al., 2007) and in Czech Republic (Gaar et al., 2006). Polomski et al. (2006) found 5 *Bursaphelenchus* in declining *P. sylvestris* trees in an alpine valley in Switzerland. *B. vallesianus* occurred in 75% of *Bursaphelenchus*-positive trees. They suggested that *B. vallesianus* could be a contributing factor in pine decline in Valais, Switzerland. It might also cause similar pine decline in Turkey. Further investigations are necessary to identify its vectors, geographic distribution, and pathogenic capacity in the pine forests of Turkey.

Detailed surveys may reveal the presence of more *Bursaphelenchus* species in Turkey, particularly in coniferous trees, as Turkey has expansive conifer forests, insect vector species, and suitable climatic conditions.

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