BURSAPHELENCHUS VALLESIANUS SP. N. – A NEW SPECIES OF THE *BURSAPHELENCHUS SEXDENTATI* GROUP (NEMATODA: PARASITAPHELENCHIDAE)

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Summary. *Bursaphelenchus vallesianus* sp. n. has been found in wood samples from recently dead pine trees (*Pinus sylvestris*) in several locations in Canton Valais, Switzerland. The new species is described and illustrated. *Bursaphelenchus vallesianus* sp. n. is morphologically most similar to *Bursaphelenchus sexdentati* and *Bursaphelenchus borealis* and clearly belongs to the *Bursaphelenchus sexdentati* group, showing four lateral lines and the typical position of the caudal papillae of males. It can be differentiated from *Bursaphelenchus sexdentati* by the shorter stylet, a conical female tail with a more or less rounded terminus (bluntly rounded in *B. sexdentati*), shorter and slightly differently shaped spicules, the presence of a small cucullus and the lack of a distinct postvulval constriction of females. The post-uterine sac occupies 1/2-2/3 of the vulva-anus distance. *Bursaphelenchus vallesianus* sp. n. sometimes shows a slight hook-like condylus of spicules like *B. borealis*. It differs from *Bursaphelenchus borealis* by the shorter spicules and stylet, the presence of a cucullus, and the greater *c* value in males and females. The species differentiation is supported by the ITS-RFLP patterns of *Bursaphelenchus vallesianus* sp. n., *Bursaphelenchus sexdentati*, *Bursaphelenchus pinophilus*, *Bursaphelenchus borealis* and *Bursaphelenchus poligraphi*, which are all members of the *B. sexdentati* group.

The detection of the quarantine pest *Bursaphelenchus xylophilus* (Steiner *et* Buhrer, 1934) Nickle, 1970 (pine wood nematode) in Portugal (Mota *et al.*, 1999) indicates that the pine wilt disease is no longer just a South-East Asian problem, but now a pan-European pest problem. Although the nematode occurs in North America, indigenous conifer trees do not suffer from the pest there. Important European pines such as *Pinus sylvestris, P. pinaster* and *P. nigra* are, however, highly susceptible. There is a great need for systematic sampling in all European countries to find out more about the actual distribution of the *Bursaphelenchus* species and to be able, thus, to react quickly in case of an introduction of the pine wood nematode into other European regions. The surveys of the occurrence of *Bursaphelenchus* species carried out in southern and central Europe in recent years (Braasch *et al.*, 2000) and the monitoring of *B. xylophilus* in the member states of the European Union have contributed to our knowledge of the distribution of the genus *Bursaphelenchus* in Europe. Other European countries have also carried out monitoring surveys in order to ensure *B. xylophilus* is absent from their countries. The pest may not only cause damage to pine forests under suitable climatic conditions, but it may also lead to restrictions in trade with wood and wood products to fulfill international quarantine requirements.

Sampling for the presence of *B. xylophilus* is always advisable in European areas where pine trees (particularly *Pinus pinaster* Aiton, *P. sylvestris* L., *P. nigra* Arnold) die for unknown reasons. When attacked by this pest, trees may die quickly with average summer temperatures of 22/23 °C or more, but slowly with temperatures around 20 °C. In any suspicious case, the nematode has to be verified as the causative agent by reliable identification. Braasch (2001) registered 28 European *Bursaphelenchus* species in conifers in Europe, which have to be differentiated from *B. xylophilus*. When sampling for wood nematodes, a new *Bursaphelenchus* species of the *Bursaphelenchus sexdentati* group (Braasch, 2001) was found in pine wood from several locations in Canton Valais in Switzerland and is described in this paper.

MATERIAL AND METHODS

Wood samples were collected from 103 pine trees (*P. sylvestris*), which had recently died or suffered different levels of damage at six sites in CantonValais, Switzerland, in 2001-2003. In this area, pines grow between 500 and 1500 m a.s.l. Long term mean summer temperature at low altitude reaches up to 17 °C, but temperatures reached 19.2 °C in 1991-2000 and 22.0 °C in summer 2003. Mean annual precipitation is 500-600 mm. The new *Bursaphelenchus* species was described from three out of 23 isolates. The nematode isolates STN2 and ST42 were sampled from pine trees near Stalden, whereas the isolate SAN2 was taken from near Salgesch, about 50 km west of Stalden. Both sites are located at about 900 m a.s.l.

After felling a selected tree, discs about 5 cm thick were sawn from the lower, the middle and the upper part of the trunk. Small radial segments were cut from each disc, and the sapwood without bark was chopped into small pieces (ca. 1x1 cm). From each disc, 70-100 g of wood were separately analysed for the presence of nematodes using a modified Baermann funnel technique. The suspension was inspected with a Leica DM IL-(IMC) microscope. Nematodes were morphologically studied using a Zeiss Axioskop microscope and a Sony CCDmIRIS video camera. REM microphotographs were made with a scanning electron microscope Philipps SEM 515 with cryo preservation BAL-TEC. Measurements were made on TAF-fixed nematodes that had been cultured on non-sporulating *Botrytis cinerea* Pers. on malt agar.

The isolation of nematode DNA for ITS-RFLP analysis was carried out according to Roberts (1998) with modifications. Nematode samples (1 to 30 adult specimens) were placed in 5 µl of water using Eppendorf tubes and frozen at -20 °C until extraction. The sample was thawed, mixed with 10 µl of extraction buffer (0.2 M saccharose, 0.1 M Tris-HCl pH 9.2, 0.1 M NaCl, 50 mM EDTA, 0.5% sodium dodecylsulfate) and homogenized in the Eppendorf tube using a micropestle (Eppendorf). Thirty µl of extraction buffer was added on rinsing the pestle, and the sample was mixed and incubated for 15 min. at 70 °C. Then, 6 µl of 8 M potassium acetate was added, the sample was mixed and placed on ice for 15 min. A white precipitate of potassium dodecylsulfate formed, which was removed by centrifugation for 30 min. at 20,000 x g/4 °C. The supernatant was transferred to a fresh tube, 36 µl of ice-cold 2-propanol was added and the mixture was centrifuged for 30 min at 20,000 x g/4 °C to sediment the precipitated DNA. The sediment was washed with 500 µl of ice-cold 70% ethanol, centrifuged for 20 min. at 20,000 x g/4 °C and dried in a vacuum concentrator for 5 min. The DNA was then dissolved in 10 µl (for extraction of single animals) or 20 to 50 µl (for extraction of several to many animals) of TE buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA) and stored at -20 °C until use. DNA concentration was determined fluorimetrically using a DyNA Quant 200 fluorometer (Hoefer/Pharmacia) and the fluorescent dye, Hoe 33258.

ITS-RFLP analysis was carried out as described previously (Hoyer *et al.*, 1998; Mota *et al.*, 1999). A segment of nematode rDNA containing the internal transcribed spacer regions ITS1 and ITS2 was amplified by PCR using forward primer 5'-CGTAACAAGGTAGCTGTAG-3' (Ferris *et al.*, 1993) and reverse primer 5'-TTTCACTCGCCGTTACTAAGG-3' (Vrain, 1993). The PCR mixture (50 µl) contained 0.6 µM of each primer, 2 units Taq DNA polymerase (Stratagene), 10 mM Tris-HCl pH 8.8, 50 mM KCl, 2 mM $MgCl_2$, 0.1 mM dNTP's (Roche) and 2 ng DNA template. Amplification was carried out using a Perkin Elmer 9600 thermocycler employing an initial denaturation at 94 °C for 2.5 min., 40 reaction cycles of 94 °C for 1 min., 55 °C for 1 min., 72 °C for 2 min, and a final extension at 72 °C for 5 min. After completion of the PCR, 5 µl aliquots of the reaction mixture were resolved by electrophoresis in a 1.8% agarose gel and DNA fragments were visualized by staining in 1 µg/ml ethidium bromide. Suitable aliquots of the amplified DNA were digested with 3 units of the restriction endonucleases *Alu* I, *Hae* III, *Hinf* I, *Msp* I and *Rsa* I, following the manufacturer's instructions. Restriction fragments were resolved by electrophoresis in a 2.5% agarose gel and stained with ethidium bromide.

The *Bursaphelenchus* species used in ITS-RFLP analysis for comparison with *B. vallesianus* sp. n. were *B. sexdentati* Rühm, 1960, *B. borealis* Korenchenko,1980, *B. poligraphi* Fuchs, 1937 and *B. pinophilus* Brzeski and Baujard, 1997. They were cultured on *Botrytis cinere*a on malt agar.

DESCRIPTION

BURSAPHELENCHUS VALLESIANUS sp. n.
(Tables I and II; Figs 1-3)

The new species was found in 23 pine trees at six sites in varying numbers (maximum 1108 nematodes in 10 g of wood). More than 500 nematodes/10 g of wood were counted in four samples. *Bursaphelenchus vallesianus* sp. n. occurred only in trees that had recently died, mostly in the lower and middle part of the trunks, but occasionally also in the upper part and in thick branches.

Measurements and description

Female. These display all the features of the Aphelenchoidoidea according to Hunt (1993). Bodies are slim and slightly ventrally curved when killed by heat. Cuticle marked by fine annules. Lateral field about 3-4 µm wide with four lateral lines in midbody. Lip region convex, approx. 2.5-4 µm high, approx. 7-8 µm wide and offset by a distinct constriction, without labial annules. *En face* view of head showing six equal lips close to each other. Stylet without basal knobs, with slight swellings. Stylet cone about 43% of total stylet length. Median bulb angular, longer than wide. Excretory pore at anterior region of median bulb or at region of the bulb. Pharyngeal glands overlapping intestine for $1^1/_2$-3 body diam. Reproductive system prodelphic, gonad outstretched, occupying 1/3-1/2 of body length. Developing oocytes arranged in single file. Vulva-anus distance 199 ± 30 (138-260) µm. Postuterine branch occupying about 1/2-2/3 of vulva-anus distance. Vagina with a small vulval flap. Area posterior to vulval flap swollen, but no distinct postvulval constriction. Tail conical, gradually tapering, with variable terminus, more or less rounded or finely rounded, about two and a half times longer than anal body diam., sometimes with slightly digitate terminus.

Table I. Measurements of *Bursaphelenchus vallesianus* sp. n. males. Measurements in µm and in form: mean ± s.d. (range).

Parameter	Holotype	Paratypes	Isolate number		Mean ± SD (all isolates) (range of extremes)
		STN2	SAN2	ST42	
		(n = 10)	(n = 10)	(n = 10)	(n = 30)
L	755	753 ± 110 (613 – 918)	769 ± 100 (594 – 893)	725 ± 190 (513 – 1056)	749 ± 141 (513 – 1056)
Stylet	13	13 ± 0.9 (12 – 15)	13 ± 1.0 (12 – 15)	14 ± 1.2 (12 – 15)	13 ± 1.1 (12 – 15)
Oesophagus	62	72 ± 7.5 (63 – 83)	81 ± 9 (69 – 100)	72 ± 7.3 (60 – 81)	75 ± 9.8 (60 – 100)
Anterior end to bulbus	40	41 ± 3.5 (38 – 44)	55 ± 10 (44 – 75)	45 ± 8.7 (38 – 59)	48 ± 10.9 (38 – 75)
Tail length	20	26 ± 5.1 (19 – 37)	26 ± 2.0 (23 – 30)	26 ± 6.3 (20 – 37)	26 ± 4.9 (19 – 37)
a	32	29 ± 7.6 (22 – 46)	41 ± 4.2 (33 – 47)	38 ± 7.0 (27 – 48)	38 ± 7.5 (22 – 48)
b	12.2	10.2 ± 0.9 (9.2 – 12.2)	10.0 ± 1.2 (7.9 – 11.5)	10.1 ± 2.0 (7.8 – 14.1)	10.0 ± 1.6 (7.8 – 14.1)
c	38	30 ± 5.3 (20 – 37)	30 ± 4.6 (21 – 36)	28 ± 4.2 (23 – 36)	29 ± 4.9 (20 – 37)
c‘	1.7	1.9 ± 0.4 (1.5 – 3.0)	2.3 ± 0.3 (1.9 – 2.8)	2.1 ± 0.3 (1.6 – 2.8)	2.1 ± 0.4 (1.5 – 3.0)
Spicules	17	17 ± 1.5 (15 – 19)	16 ± 0.9 (14 – 17)	16 ± 1.1 (15 – 17.5)	16 ± 1.3 (14 – 19)

Table II. Measurements of *B. vallesianus* sp. n. females. Measurements in µm and in form: mean ± s.d. (range).

Parameter	Allotype	Paratypes	Isolate number		Mean±SD (all isolates) (range of extremes)
		STN2	SAN2	ST42	
		(n = 10)	(n = 10)	(n = 10)	(n = 30)
L	715	878 ± 110 (688 – 1063)	880 ± 70 (750 – 982)	743 ± 166 (573 – 1086)	834 ± 126 (573 – 1086)
Stylet	13	13 ± 1.1 (11 – 15)	13 ± 0.8 (12 – 14)	13 ± 1.1 (12 – 15)	13 ± 1.0 (11 – 15)
Oesophagus	66	72 ± 12 (59 – 100)	78 ± 5.6 (72 – 88)	67 ± 7.0 (56 – 75)	73 ± 9.5 (56 – 100)
Anterior end to bulbus	38	46 ± 8.0 (38 – 58)	53 ± 3.6 (46 – 56)	42 ± 6.5 (34 – 50)	46 ± 7.6 (34 – 56)
Tail length	20	26 ± 3.1 (19 – 31)	24 ± 1.2 (22 – 25)	24 ± 3.4 (20 – 31)	25 ± 2.6 (19 – 31)
V	73	73 ± 1.6 (71 – 75)	74 ± 1.3 (71 – 76)	72 ± 1.0 (71 – 74)	73 ± 1.4 (71 – 76)
Vulva-anus-distance	175	210 ± 25 (175 – 259)	204 ± 26 (158 – 237)	182 ± 39 (138 – 260)	199 ± 30 (138 – 260)
Post-uterine sac length	100	113 ± 31 (80 – 155)	119 ± 23 (81 – 156)	91 ± 23 (63 – 129)	107 ± 27 (63 – 156)
a	36	38 ± 3.1 (31 – 42)	39 ± 4.8 (31 – 47)	39 ± 5.5 (30 – 46)	39 ± 4.3 (30 – 47)
b	10.8	12.3 ± 1.8 (8.7 – 15.7)	11.3 ± 1.0 (9.8 – 12.9)	10.6 ± 0.9 (9.1 – 11.6)	11.0 ± 1.0 (9.1 – 12.9)
c	36	34 ± 4.0 (28 – 42)	37 ± 3.3 (33 – 43)	30 ± 3.7 (26 – 36)	34 ± 4.7 (26 – 43)
c‘	2.0	2.4 ± 0.3 (1.9 – 2.8)	2.5 ± 0.2 (2.1 – 2.8)	2.8 ± 0.5 (2.0 – 3.6)	2.6 ± 0.4 (2.0 – 3.6)

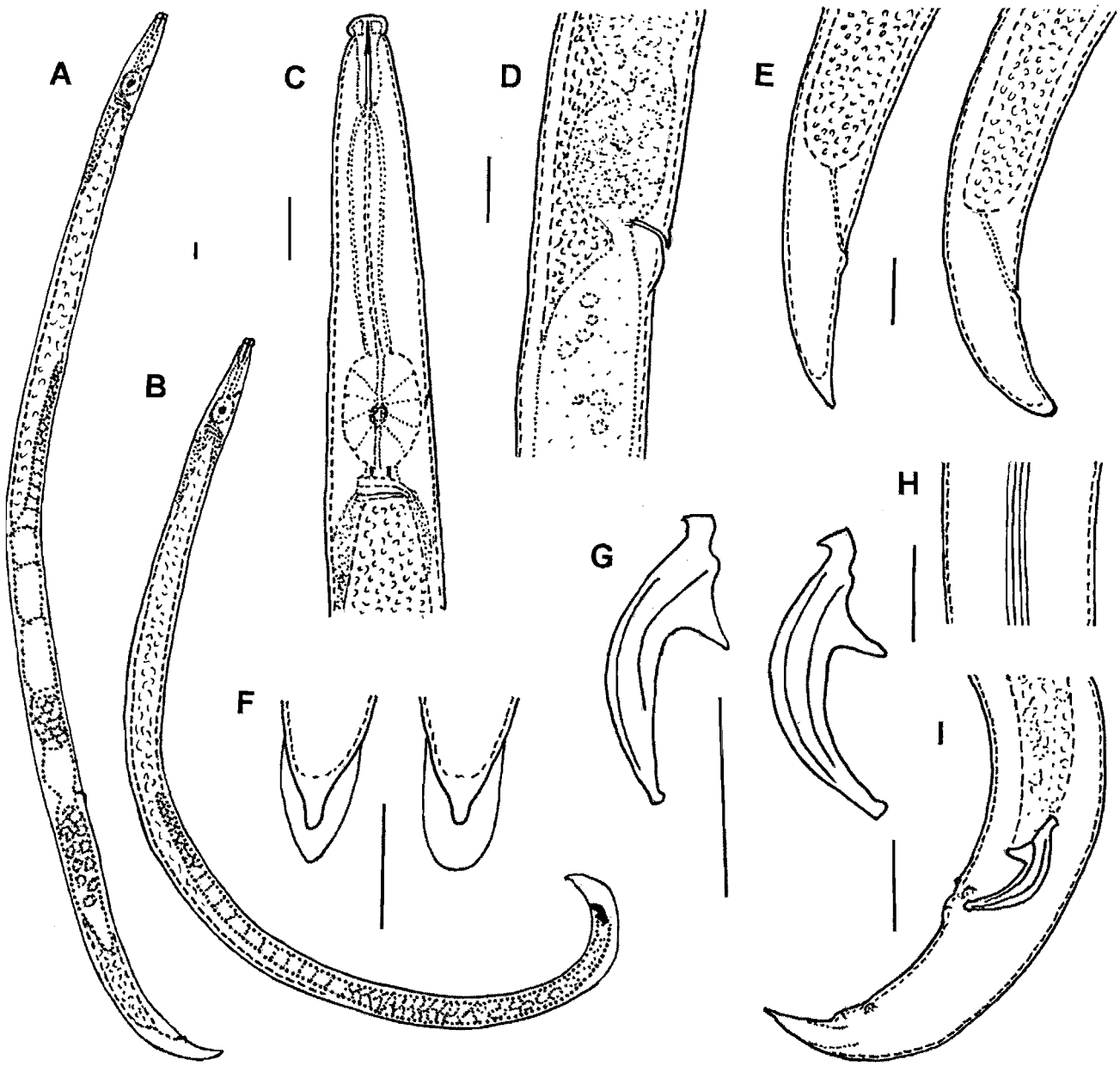

Fig. 1. *Bursaphelenchus vallesianus* sp. n. A: Female; B: Male; C: Female head region; D: Vulval region; E: Female tails; F: Bursa; G: Spicules; H: Lateral field; I: Mail tail. (Scale bars = 10 µm).

Male. Body C-shaped with curled tail when killed by heat. Anterior body region and cuticle similar to female. Testis usually outstretched, occupying 1/4-1/2 of body length. Spermatocytes arranged in multiple files. Spicules paired, strongly arcuate, not fused, with a more or less pointed, prominent rostrum of about 3 µm length, in proximal half of the spicules. Condylus approximately 5 µm high, with a ventral protuberance. Top of condylus slightly dorsally bent. Distance from tip of condylus to tip of rostrum 5-6 µm, usually no distinct angle in this line. Distal ends of spicules with small, often indistinct cucullus. Tail ventrally arcuate with a pointed, talon-like terminus bearing a small, oval or shovel-shaped, terminal 'bursa' of about 10 µm length. One single preanal and two subventral pairs of caudal papillae present; one pair preanal, one postanal, at about the middle of tail, and a ventral pair of papillae at anterior level of bursal flap.

Juveniles. Three juvenile forms of *B. vallesianus* sp. n. have been observed on *B. cinerea* culture. The smallest one (J_2) was 180-320 mm (n = 20), and the J_3 and J_4 were 350-480 mm (n = 20) and 480-580 mm (n = 20) in length, respectively. The smallest juveniles are round-tailed. The tail end of the J_3 juveniles is slightly conoid and rounded. The juveniles of the fourth stage show a more conoid tail than the third stage juveniles, with the terminus rounded. When the nematodes were cultured on *Botrytis* for several weeks without subculturing, the third juvenile stage became predominant. Some of these medium-sized juveniles were full of lipid granules.

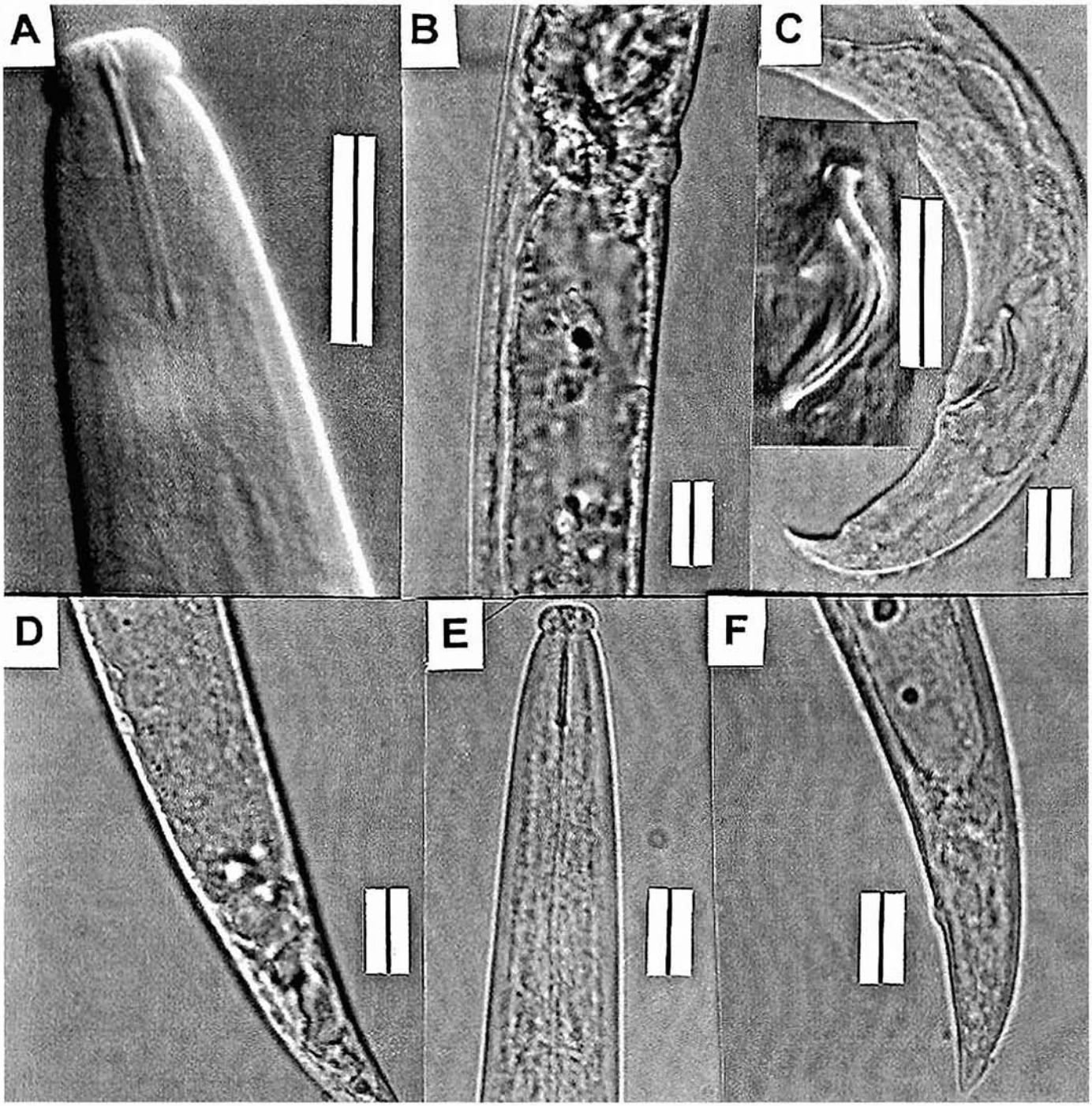

Fig. 2. Light microscope observations of *B. vallesianus* sp. n. A: Female head region with stylet; B: Vulval region; C: Male tail with spicules; D and F: Female tails; E: Anterior female body region (Scale bars = 10 µm).

Diagnosis and relationships. Bursaphelenchus vallesianus sp. n. is characterised by a relatively small stylet with basal swellings, a lateral field with four lines, an excretory pore before or at the median bulb. The female has a small vulval flap and a swelling behind the vulva, a conical female tail with a more or less rounded or finely rounded terminus. The male has distinct spicules with slightly dorsally bent condylus, more or less pointed rostrum, with small and sometimes indistinct cucullus and a dorso-ventral visible oval terminal 'bursa'.

Because of the presence of four lateral lines, the similarity in spicule shape, the presence of a small vulval flap and the position of caudal papillae, *B. vallesianus* sp. n. is affiliated to the *B. sexdentati* group within the genus *Bursaphelenchus*, which also includes *B. sexdentati, B. naujaci, B. pinophilus, B. poligraphi, B. borealis* and probably also *B. incurvus and B. piniperdae* (Braasch, 2001). These species can easily be differentiated from the *B. leoni* group, the *B. eggersi* group and the *B. hofmanni* group, which have three lateral lines (Braasch, 2001), and from species with two or six lateral lines. Species of the *B. xylophilus* group, which also

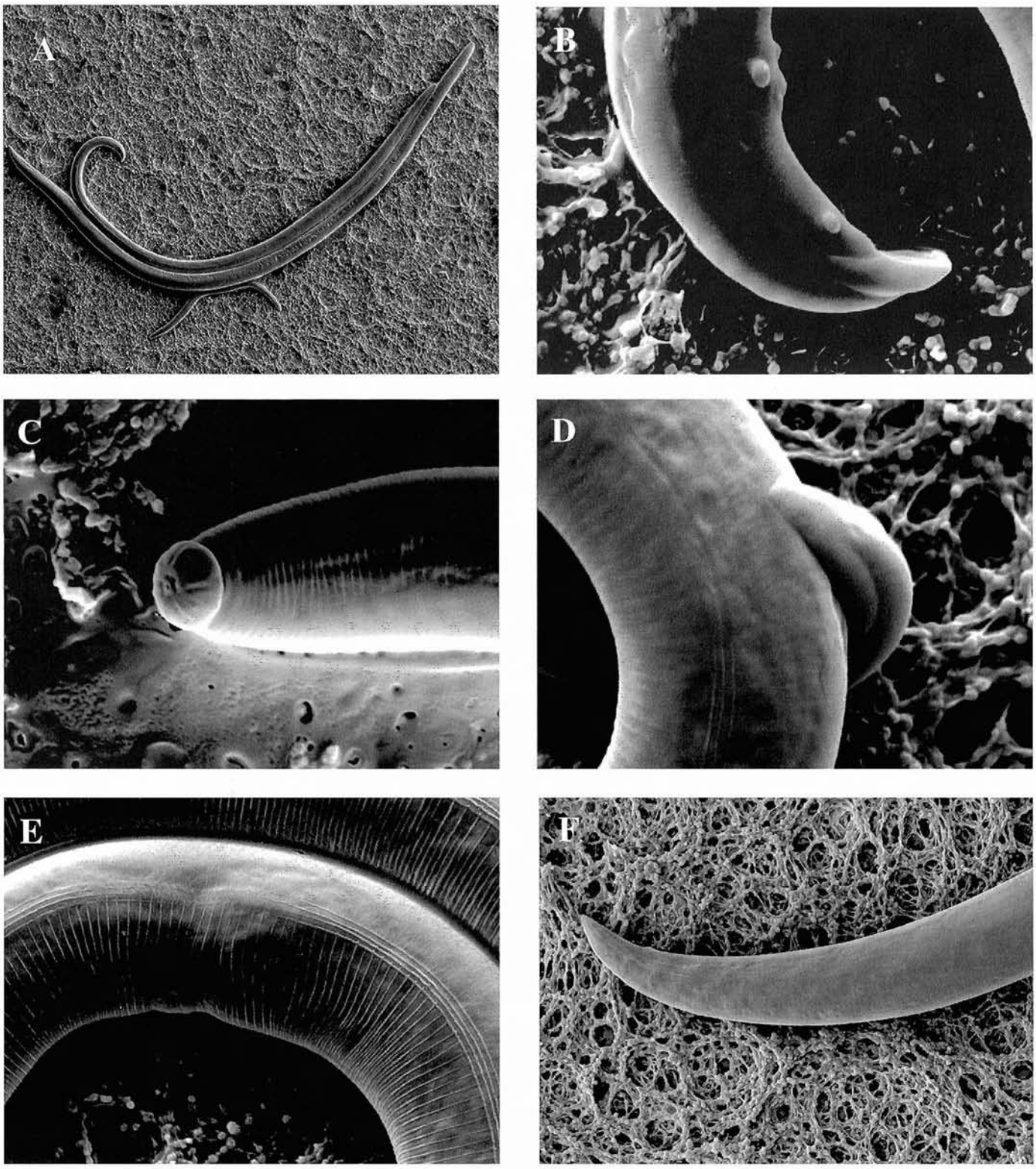

Fig. 3. Scanning electron microscope observations of *B. vallesianus* sp. n. A: Male, female and J2 juvenile complete; B: Male tail; C: Head; D: Bursa; E: Lateral field and vulval region; F: Female tail (Scale bars: A = 100 µm, B-F = 10 µm).

show four lateral lines, can easily be differentiated from the members of the *B. sexdentati* group by their distinctly different spicules with their typical shape, the different position of caudal papillae and the presence of a large vulval flap. *Bursaphelenchus fungivorus* also has four lateral lines, but another position for the caudal papillae and, moreover, the females have, unlike the species of the *B. sexdentati* group, a long, tapering, ventrally bent tail. Distinguishing the species of the *B. sexdentati* group morphologically from each other is, however, difficult.

Due to the shape of spicules, *B. vallesianus* sp. n. is most similar to *B. sexdentati* Rühm, 1960 and *B. borealis* Korenchenko, 1980. *Bursaphelenchus sexdentati* was

considered to be synonymous with *B. bakeri* Rühm, 1964 and *B. naujaci* Baujard, 1980 by Hunt (1993). The new species differs from *B. sexdentati* because of the shape of the female tail, which is less conoid and bluntly rounded in *B. sexdentati*, the lack of a distinct postvulval constriction, the shorter stylet (13 *vs* 18 µm), the shorter spicules (16 *vs* 19-22 µm), the less pointed rostrum of spicules, a ventral protuberance on the condylus, and the presence of a small cucullus at the spicules.

Bursaphelenchus vallesianus sp. n. differs from *B. borealis* Korenchenko, 1980 because of the shorter spicules (16 *vs* 19 µm), the shorter stylet (13 *vs* 15 µm), the different *c* values (males 29/females 34 *vs* 21/14 µm), the less distinctive bending of the condylus of spicules, the shovel-shaped bursa and the differently tapering tail of the females.

The new species differs from *B. poligraphi* Fuchs, 1937, which was found on spruce (Rühm, 1956), because of the shape of the female tail (wedge-shaped pointed in *B. poligraphi*), the higher *c* values (29/34 *vs* 17/15 µm) and the shape of the spicules (the rostrum of *B. poligraphi* is small and slender).

The finger-like tail of *Bursaphelenchus incurvus* Rühm, 1956, which was found on spruce as well, distinguishes it from *B. vallesianus* sp. n., as does the shorter stylet (13 *vs* 12-17/15-19 µm) of the new species, the shape of the *B. incurvus* spicules (rostrum almost in the middle of spicules) and its square-shaped bursa.

Bursaphelenchus vallesianus sp. n. can be distinguished from *B. piniperdae* Fuchs, 1937 (Rühm, 1956) mainly by the shape of the female tail (cylindrical and bluntly rounded in *B. piniperdae*), but it also lacks a distinct postvulval constriction, has a shorter stylet (13 vs 18-19/16-18 µm) and shorter spicules (16 *vs* 17-19 µm).

Bursaphelenchus vallesianus sp. n. differs from *B. pinophilus* Brzeski *et* Baujard, 1997 as it has a shorter post-uterine sac and shorter spicules (16 *vs* 18.5 µm), differently shaped spicules (*B. pinophilus* has a very variable condylus and long and strongly pointed rostrum), and the distance from the tip of condylus to the tip of rostrum is shorter (5-6 *vs* 8-10 µm) in the new species. Moreover, *B. pinophilus* has a pointed conoid and sometimes mucronate tail.

Molecular differentiation of B. vallesianus sp. n. from similar species

The pattern of DNA restriction fragments obtained in the ITS-RFLP analysis of *B. vallesianus* sp. n. is different from the patterns of the morphologically similar species *B. sexdentati*, *B. borealis*, *B. pinophilus* and *B. poligraphi* (Fig. 4, Table III). It is also distinct from the ITS-RFLP patterns of the following conifer-inhabiting European

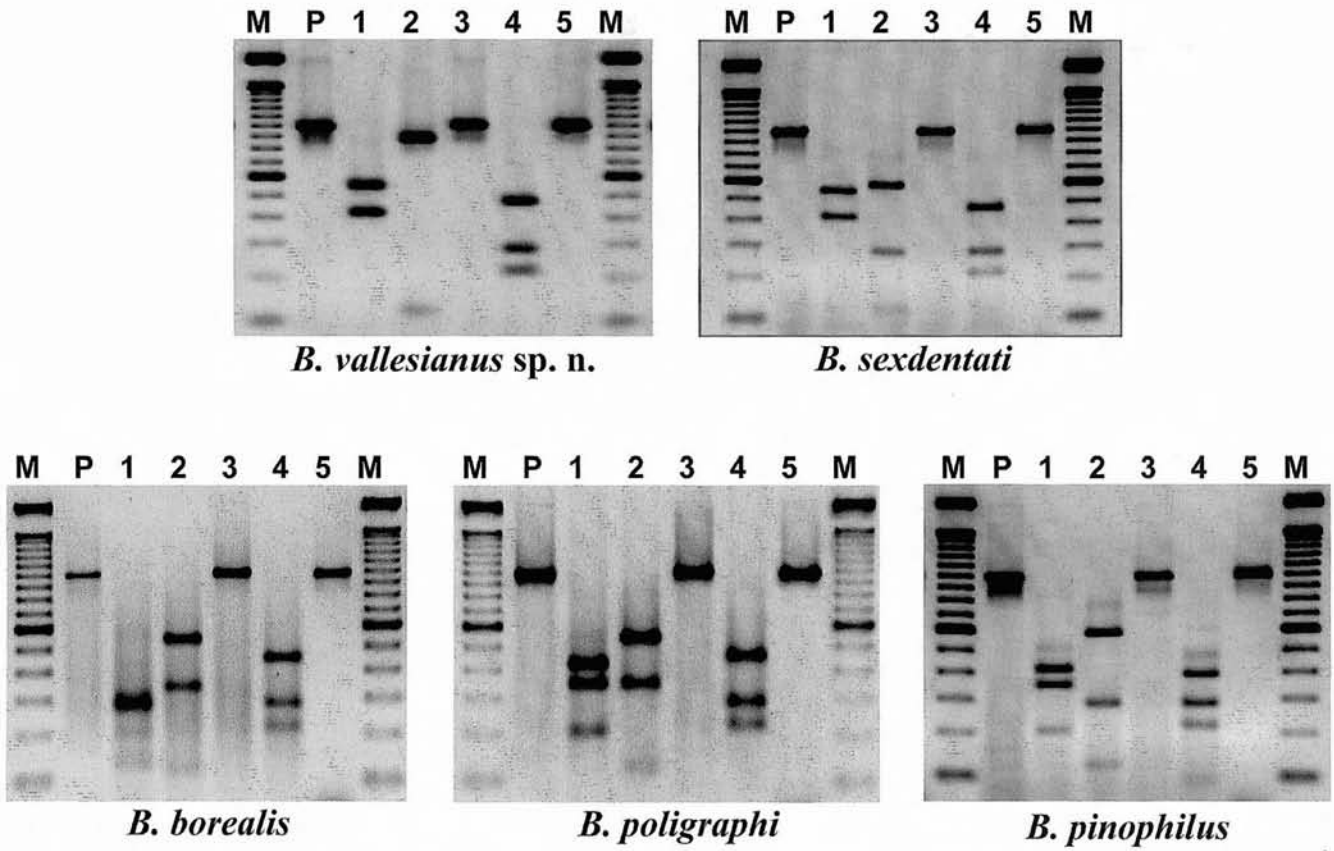

Fig. 4. ITS-RFLP patterns of *B. vallesianus* sp. n. and four morphologically similar *Bursaphelenchus* species. Restriction fragments were obtained by digestion of the amplified rDNA fragment (P) with *Rsa* I (1), *Hae* III (2), *Msp* I (3), *Hinf* I (4) and *Alu* I (5). M: DNA marker (100 bp ladder, Invitrogen Life Technologies).

Table III. Approximate size of DNA fragments observed in ITS-RFLP analysis of *B. vallesianus* sp. n. and four related species.

Bursaphelenchus species	PCR product (bp)	Restriction fragments (bp)				
		Rsa I	*Hae* III	*Msp* I	*Hinf* I	*Alu* I
B. vallesianus sp. n.	1000	570 430	880 120	1000	480 290 220	1000
B. sexdentati	1000	570 430	600 280 120	1000	480 290 220	1000
B. borealis	1000	290 220 130	560 350 120	1000	450 290 230	1000
B. poligraphi	980	430 340 210	530 340 110	980	470 290 220	980
B. pinophilus	1000	430 340 210	600 280 120	1000	400 290 220	1000

Bursaphelenchus species obtained in earlier investigations: *B. xylophilus*, *B. mucronatus*, *B. fraudulentus* Rühm, 1956, *B. leoni* Baujard, 1980, (see Hoyer *et al.*, 1998), *B. eggersi* Rühm, 1956, *B. fungivorus* Franklin and Hooper, 1962, *B. hofmanni* Braasch, 1998 (see Braasch *et al.*, 1999), *B. paracorneolus* Braasch, 2000 (= B. spec. DE-14(w) in Braasch *et al.*, 1999), *B. thailandae* Braasch and Braasch-Bidasak, 2002 (see Tomiczek *et al.*, 2003), *B. tusciae* Ambrogioni *et* Marinari Palmisano, 1998 and *B. silvestris* Lieutier *et* Laumond, 1978 (Burgermeister *et al.* unpublished). On the other hand, the DNA fragments shown in Fig. 4 and Table III exhibit some common features among the five species examined, which supports their close relationship. In all cases, a PCR product of the same size (about 1000 bp) is obtained, which contains no recognition sites for *Msp* I and *Alu* I. With other *Bursaphelenchus* species, a PCR product ranging from 800 bp to 1250 bp is obtained but it has recognition sites for *Msp* I and *Alu* I, as described in the publications cited above. *Bursaphelenchus vallesianus* sp. n. differs from *B. sexdentati* only in restriction fragments obtained with *Hae* III, from *B. borealis* and *B. poligraphi* in fragments obtained with *Rsa* I and *Hae* III, and from *B. pinophilus* in fragments obtained with *Rsa* I, *Hae* III and *Hinf* I. More detailed information on phylogenetic relationships of these species may be obtained by rDNA sequence analysis (work in progress).

Type locality and habitat. In the wood of a dead *Pinus sylvestris* tree aged about 30 years, in a pine forest near Stalden (900 m a.s.l.), Canton Valais, Switzerland.

Type material. Collected from a culture on *Botrytis cinerea*. Slides in the nematode collections of H. Braasch, Potsdam and in the Swiss Federal Research Institute WSL, Research Department Forests. One slide with paratypes in the USDA nematode collection, Beltsville, Maryland, USA.

DISCUSSION

Whereas the number of lateral lines and the position of caudal papillae allows a clear differentiation of the species of the *B. sexdentati* group from other *Bursaphelenchus* groups, the identification of the species within the *B. sexdentati* group poses some difficulties. As in the *B. xylophilus* group, an essential species characteristic is the shape of the female tail, but this may be variable in some species like *B. vallesianus* sp. n. Also, a certain variability of spicule shape is given, causing difficulties in species differentiation. Therefore, molecular biological methods are very helpful for reliable identification of the members of the *B. sexdentati* group. It has not been possible to establish the ITS-RFLP patterns of *B. naujaci, B. incurvus and B. piniperdae* so far, due to their lack of availability. A possible synonymy of these species with other species cannot be excluded. The pattern of *B. borealis* is based on a culture of German origin, which was identified as *B. borealis* without any opportunity to compare the nematodes with the Russian type material (Braasch *et al.*, 1999). It is also possible that more species of the *B. sexdentati* group may be found in Europe if further investigations are carried out and molecular biological methods used, for instance by employing more restriction enzymes in ITS-RFLP or by DNA sequencing.

Bursaphelenchus vallesianus sp. n. was found in recently dead trees at six different locations. Probably, it is widely distributed. The closely related species *B. sexdentati* is the most frequently found *Bursaphelenchus* species in southern Europe (Braasch *et al.*, 2000). It cannot be excluded that the new species was mistakenly identified as *B. sexdentati* in several cases. The vector of *B. vallesianus* sp. n. is unknown so far. Wood samples containing the nematode were obtained from trees colonised by various insects such as *Myelophilus minor, Orthotomicus longicollis* and *Ips sexdentatus*. Since the nematode was cultivated on *Botrytis cinerea*, it can be assumed that it is able to live fungivorously like most *Bursaphelenchus* species. However, its association with recently dead pine trees also raises the question of whether it may be involved in damaging pine and thus contributing to its decline. It could, however, also be possible that the nematode is vectored by a secondary invader. Further investigations are needed to find out more about the biology and possible harmfulness of this nematode.

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