

Improved key to the species of the *xylophilus* group of the genus *Bursaphelenchus* Fuchs, 1937

Helen Braasch¹ and Ute Schönfeld²

¹Kantstrasse 5, 14471 Potsdam (before: Federal Biological Research Centre for Agriculture and Forestry, Braunschweig, Germany) ² State Office for Rural Development, Agriculture and Landreallocation, Steinplatz 1, 15806 Zossen, Germany

Abstract

A correct determination of the pine wood nematode becomes more difficult, the more related or similar species are described. In view of the fact that a further spread of the nematode should be considered and continuously new *Bursaphelenchus* species are detected, identification keys must be adjusted accordingly. The recognition of this harmful quarantine pest using morphological characteristics in laboratories of the National Plant Protection Services is facilitated by a simplified key.

Based on the key published by Braasch (2008) and the revised intrageneric grouping of *Bursaphelenchus* by Braasch *et al.* (2009) an improved dichotome key including in the meantime newly described species (*B. paraluxuriosae*, *B. populi*, *B. macromucronatus*, *B. firmae*, *B. koreanus* and *B. gillanii*) is presented. The recently described similar species *B. tryphloei* and *B. masseyi* differ morphologically from the *xylophilus* group.

Table 1: Morphological key features of *Bursaphelenchus* species belonging to the *xylophilus* group































<i>Bursaphelenchus</i> species	Female tail	Male spicule shape
<i>B. xylophilus</i>		
<i>B. populi</i>		
<i>B. mucronatus kolymensis</i>		
<i>B. mucronatus mucronatus</i>		
<i>B. fraudulentus</i>		
<i>B. gillanii</i>		
<i>B. macromucronatus</i>		
<i>B. conicaudatus</i>		
<i>B. doui</i>		
<i>B. baujardi</i>		
<i>B. firmae</i>		
<i>B. koreanus</i>		
<i>B. paraluxuriosae</i>		
<i>B. luxuriosae</i>		
<i>B. singaporensis</i>		

Table 2: Morphological key for identification of species of the *xylophilus* group

1	Female tail conoid, $c' \geq 4$ (average), with or without mucro	2
	Female tail cylindrical or subcylindrical, $c' < 4$ (average), with or without mucro	8
2	Female tail with mucro	3
	Female tail without mucro	5
3	Female tail with small pointed mucro, spicule length $< 30 \mu\text{m}$ (av.)	<i>B. conicaudatus</i> *
	Female tail with long mucro (up to $7 \mu\text{m}$)	4
4	Mucro hairlike and pointed, $2.5\text{--}6.5 \mu\text{m}$ long, spicule length as bow $23\text{--}38 \mu\text{m}$	<i>B. macromucronatus</i>
	Mucro digitate with wide base, $5\text{--}7 \mu\text{m}$ long, spicule length as bow $31\text{--}37 \mu\text{m}$	<i>B. gillanii</i>
5	Spicule length as bow $41\text{--}48 \mu\text{m}$, spicules with prolonged medium part, stylet $14\text{--}17 \mu\text{m}$, female tail finally rounded	<i>B. singaporensis</i>
	Spicule length av. $< 40 \mu\text{m}$, female tail ventrally bent with irregular or roughened terminus	6
6	Spicule length $< 35 \mu\text{m}$	7
	Spicule length $35\text{--}44 \mu\text{m}$, stylet $15 \mu\text{m}$ long, condylus offset, $c' = 4.1$	<i>B. koreanus</i>
7	Spicule length $27\text{--}30 \mu\text{m}$, stylet $11\text{--}16 \mu\text{m}$ long, cucullus distinct	<i>B. luxuriosae</i>
	Spicule length $31\text{--}35 \mu\text{m}$, stylet $16\text{--}18 \mu\text{m}$ long, cucullus indistinct	<i>B. paraluxuriosae</i>
8	Female tail cylindrical, without or with mucro	9
	Female tail subcylindrical, terminus with mucro	10
9	Female tail terminus broadly rounded, without mucro, a part of the population may possess a short hairlike mucro less than $2 \mu\text{m}$ long, $a = 33\text{--}47$, excretory pore at level of nerve ring	<i>B. xylophilus</i>
	Cylindrical tail smoothly tapering to a tail tip with thick and blunt mucro, $3\text{--}5 \mu\text{m}$ long, $a = 27\text{--}36$, excretory pore at or posterior to bulb	<i>B. firmae</i>
10	Spicule length $< 34 \mu\text{m}$	11
	Spicule length $34\text{--}44 \mu\text{m}$ and with propped and almost straight middle part, excretory pore at level of median bulb	<i>B. doui</i>
11	Female tail, when isolated from wood, broadly rounded like in <i>B. xylophilus</i> , sometimes with a small irregular projection; in-vitro reared females with conical tail tapering to a finely rounded terminus, sometimes with $1\text{--}2 \mu\text{m}$ long projection, $a = 36\text{--}52$, vulval flap distinctly bent into sharp depression behind it	<i>B. populi</i>
	Female tail always with mucro, vulval flap differently shaped	12
12	Mucro $4\text{--}7 \mu\text{m}$ long, often pointed, usually as a continuation of the slightly conoid tail, spicule condylus dorsally usually not offset, capitulum distinctly concave, excretory pore usually posterior to median bulb	<i>B. mucronatus mucronatus</i>
	Mucro $\leq 5 \mu\text{m}$, spicule condylus dorsally offset, capitulum not distinctly concave	13
13	Excretory pore at or in front of median bulb	14
	Excretory pore at or posterior to median bulb, small mucro hair-like, mean $2.6\text{--}4$ (av. < 3) μm long, usually offset from tail	<i>B. xylophilus</i> (mucronate form)
14	Mucro short (ca. $2 \mu\text{m}$ long) and broad with a wide base, usually continuous with tail line, spicule condylus distinctly offset from dorsal line	<i>B. fraudulentus</i>
	Mucro usually not continuous with tail and variably shaped, often digitate, av. $> 4 \mu\text{m}$ long, spicule condylus usually offset from dorsal line, no capitulum depression	<i>B. mucronatus kolymensis</i>

* possibly synonymous with *B. baujardi*, $c' = 3\text{--}4$)

Table 3: Pictorial support of species identification









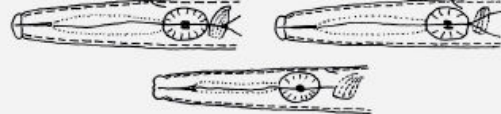




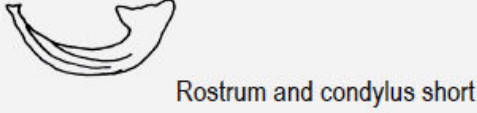
Character	Specific form	Picture
Female tail shape	conoid	
	cylindrical	
	subcylindrical	
Mucro	with or without	
	short or long	
	hairlike, digitate, wide base	
	offset or not	
Vulval flap	Bent or straight	
Excretory pore	Anterior, at, posterior bulb	
Spicules	prolonged middle part	
	With/ without cucullus	
	capitulum concave/not	
	condylus offset or not	
	Comparison to <i>B. tryphloeii</i> (and <i>B. masseyi</i>)	

Table 4: Simplified morphological key for identification of *Bursaphelenchus xylophilus*

<p>Beginning: aphelenchid nematodes found in wood or bark with offset cephalic region, big ovoid or rectangular median bulb, 10-20 μm long stylet, distinct anus, vulva posterior (70–80% of body length) with long vulval flap, long post-uterine sac, male tail strongly curved with terminal bursa (seen in dorso-ventral position), spicules large, rosethorn-shaped, arcuate, typically shaped like those of the <i>xylophilus</i> group (see Table 1)</p>		
1	Female tail cylindrical or subcylindrical, $c' < 4$ (average), with or without mucro	2
	Female tail conoid, $c' \geq 4$ (average), with or without mucro	Not <i>B. xylophilus</i>
2	Female tail cylindrical, with or without mucro	3
	Female tail subcylindrical, terminus always with mucro	4
3	Female tail terminus broadly rounded, without mucro, a part of the population may possess a short hairlike mucro less than 2 μm long, $a = 33-47$, excretory pore at level of nerve ring, vulval flap straight; found in coniferous wood	<i>B. xylophilus</i> (roundtailed form)
	Female tail, when isolated from wood, broadly rounded like in <i>B. xylophilus</i> , sometimes with a small irregular projection; in-vitro reared females with conical tail tapering to a finely rounded terminus, sometimes with 1–2 μm long projection, $a = 36-52$, excretory pore at or posterior median bulb, vulval flap bent its distal half sunken in a distinct depression; found in aspen trees	<i>B. populi</i>
4	Female tail terminus with short mucro (2–3 μm)	5
	Female tail terminus with longer mucro	Not <i>B. xylophilus</i>
5	Small mucro hair-like, mean 2.6–3.0 μm long, usually offset from tail, excretory pore at or posterior to median bulb, mean $c' \geq 3$	<i>B. xylophilus</i> (mucronate form)
	Mucro broad with wide base, ca. 2 μm long and usually continuous with tail line, mean $c' = 2.6$	<i>B. fraudulentus</i>

Conclusions: The keys presented here allow identification of species in the *xylophilus* group on the basis of key features. The consideration of several specimens is recommended because of variability. Molecular methods are additionally advisable to confirm a morphological diagnosis, particularly in case of identification of *B. xylophilus* or when only juveniles or very few specimens are available.

References: Braasch, H. (2008). The enlargement of the *xylophilus* group in the genus *Bursaphelenchus*. In: Pine wilt disease: a worldwide threat to forest ecosystems. (Ed. Mota, M. & Vieira, P.), Springer, p. 139–149. Braasch, H., Burgemeister, W. & Gu, J. (2009). Revised intra-generic grouping of *Bursaphelenchus* Fuchs, 1937 (Nematoda: Aphelenchoididae). *Journal of Nematode Morphology and Systematics* 12, 65–88. Gu, J., Zheng, W., Braasch, H. & Burgemeister, W. (2008). Description of *Bursaphelenchus macromucronatus* sp. n. (Nematoda: Parasitaphelenchidae) in packaging wood from Taiwan and India – a new species of the 'xylophilus' group. *Journal of Nematode Morphology and Systematics*, 11: 31–40. Gu, J., Wang, J., Braasch, H., Burgemeister, W. & Schröder, T. (2012). *Bursaphelenchus paraxyluniosae* sp. n. (Nematoda: Parasitaphelenchidae) in packaging wood from Indonesia. *Nematology* 14, 787–798. Gu, J., Wang, J. & Cheng, X. (2013). *Bursaphelenchus koreanus* sp. n. (Nematoda: Parasitaphelenchidae) in packaging wood from South Korea. *Nematology* (in press). Kanzaki, N., Maehara, N., Aikawa, T. & Matsumoto, K. (2012). *Bursaphelenchus firmiae* n. sp. (Nematoda: Aphelenchoididae) isolated from *Monochamus grandis* Waterhouse that emerged from *Abies firma* Sieb. et Zucc., in Japan. *Nematology* 11, 395–404. Schönfeld, U., Braasch, H., Riedel, M. & Gu, J. (2013). *Bursaphelenchus gilani* sp. n. (Nematoda: Aphelenchoididae) – a new species of the *xylophilus* group in packaging wood imported from China. *Nematology* (in press). Tomalak, M. & Filipiak, A. (2010). Description of *Bursaphelenchus populi* sp. n. (Nematoda: Parasitaphelenchidae), a new member of the *xylophilus* group from Aspen, *Populus tremula* L., from Europe. *Nematology* 12, 399–416. Tomalak, M. & Filipiak, A. (2011). *Bursaphelenchus trypophloeae* sp. n. (Nematoda: Parasitaphelenchidae) – an associate of the bark beetle, *Trypophloeus asperatus* (Gyll.) (Coleoptera: Curculionidae, Scolytinae), in aspen, *Populus tremula* L. *Nematology* 13, 619–636. Tomalak, M., Woral, J.J. & Filipiak, A. (2013). *Bursaphelenchus masseyi* sp. n. (Nematoda: Parasitaphelenchidae) – a nematode associate of the bark beetle *Trypophloeus populi* Hopkins (Coleoptera: Curculionidae, Scolytinae) in aspen, *Populus tremuloides* Michx. affected by sudden aspen decline in Colorado. *Nematology*. (in press).