9
Taxonomy and Systematics of the Nematode Genus *Bursaphelenchus* (Nematoda: Parasitaphelenchidae)

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9.1 Introduction

Nematodes in the genus *Bursaphelenchus*, which are mycophagus or plant parasitic, or both, have been considered a potential risk to cultivated plants, especially conifers, since the end of the 1970s. The reason for this is that the genus contains two virulent plant pathogens, the pine wood nematode (PWN), *B. xylophilus*, and the red ring nematode, *B. cocophilus*. To date almost 90 *Bursaphelenchu*s species have been described (Hunt 1993; Ryss et al. 2005; Kanzaki 2006; see Table II.1), especially from Europe (Rühm 1956; Braasch 2001) and the USA (Massey 1974), as associates of coleopteran beetles; however, because of the finding of the PWN in Portugal (Mota et al. 1999), the practical importance of the taxonomy of this genus has been re-evaluated worldwide. Recently, probably because of the global interest in this nematode genus, the number of newly described species from Asian countries such as China, Thailand and Japan, where in the past only a few *Bursaphelenchus* nematodes have been reported, has increased (Braasch and Braasch-Bidasak 2002; Braasch et al. 2005; Gu et al. 2005, 2006a, b; Kanzaki 2006; Kanzaki and Futai 2007).

The main purposes of this chapter is: (1) to bring together information on the taxonomy and systematics of the genus *Bursaphelenchus*, that is, the taxonomic status of the genus within the family Parasitaphelenchidae and the superfamily Aphelenchoidoidea, (2) review the morphological characteristics for identification and molecular systematics, and (3) discuss the morphological and ecological evolution of the genus.

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Table II.1 Species list of the genus *Bursaphelenchus*

Species name	Original description	Ryss et al. (2006) grouping	Molecular profiles[h]
B. aberrans	Fang et al. (2002a)	aberrans	—[i]
B. abietinus	Braasch and Schmutzenhofer (2000)	piniperdae	RS
B. abruptus	Giblin-Davis et al. (1993)	xylophilus	RS
B. africanus	Braasch et al. (2006d)	africanus[e,f]	RS
B. anamurius	Akbulut et al. (2007)	piniperdae[f]	R
B. anatolius	Giblin-Davis et al. (2005)	hunti[f]	S
B. antoniae	Penas et al. (2006a)	piniperdae	RS
B. arthuri	Burgermeister et al. (2005a)	hunti[f]	RS
B. baujardi	Walia et al. (2003)	xylophilus	—
B. bestiolus	Massey (1974)	piniperdae	—
B. burgermeisteri	Braasch et al. (2007)	africanus[f]	RS
B. borealis	Korenchenko (1980)	borealis	RS
B. chitwoodi	Rühm (1956)	piniperdae	—
B. cocophilus	Cobb (1919)	hunti	S
B. clavicauda	Kanzaki et al. (2007)	piniperdae[f]	RS
B. conicaudatus	Kanzaki et al. (2000)	xylophilus	RS
B. conjunctus[a]	Fuchs (1930)	—[g]	—
B. conurus[a]	Steiner (1932)	—[g]	—
B. corneolus	Massey (1966)	piniperdae	S
B. crenati	Rühm (1956)	xylophilus	—
B. cryphali[a]	Fuchs (1930)	borealis	—
B. curvicaudatus	Wang J et al. (2005)	piniperdae[f]	R
B. debrae	Hazir et al. (2007)	hunti[f]	S
B. digitulus	Loof (1964)	eidmanni	—
B. dongguanensis	Fang et al. (2002b)	hunti	—
B. doui	Braasch et al. (2005)	xylophilus[f]	RS
B. eggersi	Rühm (1956)	piniperdae	RS
B. eidmanni	Rühm (1956)	eidmanni	—
B. elytrus	Massey (1971a)	aberrans	—
B. eproctatus	Sriwati et al. (2008)	piniperdae	—
B. eremus	Rühm (1956)	piniperdae	RS
B. eroshenkii	Kolossova (1997)	xylophilus	—
B. erosus	Kurashvili et al. (1980)	eidmanni	—
B. eucarpus	Rühm (1956)	piniperdae	—
B. fraudulentus	Rühm (1956)	xylophilus	RS
B. fuchsi	Kruglik and Eroshenko (2004)	piniperdae	—
B. fungivorus	Franklin and Hooper (1962)	hunti	RS
B. georgicus	Devdariani et al. (1980)	piniperdae	—
B. gerberae	Giblin-Davis et al. (2006a)	piniperdae[f]	S
B. glochis	Brzeski and Baujard (1997)	piniperdae	—
B. gonzalezi	Loof (1964)	hunti	—
B. hellenicus	Skarmoutsos et al. (1998)	piniperdae	RS
B. hildegardae	Braasch et al. (2006b)	piniperdae[f]	RS
B. hofmanni	Braasch (1998)	piniperdae	RS
B. hunanensis	Yin et al. (1988)	piniperdae	—
B. hunti	Steiner (1935)	hunti	—
B. hylobianum	Korenchenko (1980)	piniperdae	RS
B. idius	Rühm (1956)	aberrans	—
B. incurvus	Rühm (1956)	piniperdae	—

(continued)

Table II.1 (Continued)

Species name	Original description	Ryss et al. (2006) grouping	Molecular profiles[h]
B. kevini	Giblin et al. (1984)	hunti	S
B. kolymensis	Korentchenko (1980)	xylophilus	—[j]
B. leoni	Baujard (1980)	borealis	—
B. lignophilus[b]	Körner (1954)	—[g]	—
B. lini	Braasch (2004)	piniperdae	RS
B. luxuriosae	Kanzaki and Futai (2003a)	xylophilus	RS
B. maxbassiensis	Massey (1971b)	piniperdae	—
B. minutes	Walia et al. (2003)	piniperdae	—
B. mucronatus	Mamiya and Enda (1979)	xylophilus	RS[j]
B. naujaci	Baujard (1980)	piniperdae	—
B. newmexicanus	Massey (1974)	piniperdae	—
B. nuesslini	Rühm (1956)	piniperdae	—
B. paracorneolus	Braasch (2000)	piniperdae	RS
B. parvispicularis	Kanzaki and Futai (2005)	piniperdae	R[k]S
B. pinasteri	Baujard (1980)	piniperdae	RS
B. piniperdae	Fuchs (1937)	piniperdae	—
B. pinophilus	Brzeski and Baujard (1997)	piniperdae	R
B. pityogeni	Massey (1974)	piniperdae	—
B. platzeri	Giblin-Davis et al. (2006b)	hunti[f]	S
B. poligraphi	Fuchs (1937)	piniperdae	RS
B. rainulfi	Braasch and Burgermeister (2002)	piniperdae	RS
B. ratzeburgii	Rühm (1956)	piniperdae	—
B. ruehmi[c]	Baker (1962)	—[g]	—
B. sachsi	Rühm (1956)	piniperdae	—
B. scolyti	Massey (1974)	piniperdae	—
B. seani	Giblin and Kaya (1983)	hunti	RS
B. sexdentati	Rühm (1960)	piniperdae	RS
syn. *B. bakeri*	Rühm (1964)		
B. silvestris	Lieutier and Lamond (1978)	borealis	—
B. sinensis	Palmisano et al. (2004)	aberrans	RS[i]
B. singaporensis	Gu et al. (2005)	xylophilus[f]	R[l]S
B. steineri	Rühm (1956)	eidmanni	—
B. sutoricus	Devdariani (1974)	piniperdae	—
B. sychnus	Rühm (1956)	piniperdae	—
B. talonus	Thorne (1935) and Kaisa (2003)[d]	piniperdae	—
B. teratospicularis	Kakuliya and Devdariani (1965)	eidmanni	—
B. thailandae	Braasch and Braasch-Bidasak (2002)	piniperdae	RS
B. tritrunculus	Massey (1974)	piniperdae	—
B. tusciae	Ambrogioni and Palmisano (1998)	borealis	RS
B. typographi	Kakuliya (1967)	piniperdae	—
B. uncispicularis	Zhou et al. (2007)	borealis	—
B. vallesianus	Braasch et al. (2004)	piniperdae	RS
B. varicauda	Thong and Webster (1983)	piniperdae	—
B. wakuae	Kurashvili et al. (1980)	piniperdae	—
B. wilfordi	Massey (1964)	piniperdae	—

(continued)

Table II.1 (Continued)

Species name	Original description	Ryss et al. (2006) grouping	Molecular profiles[h]
B. willi	Massey (1974)	piniperdae	—
B. willibaldi	Schönfeld et al. (2006)	hunti[f]	RS
B. xerokarterus	Rühm (1956)	piniperdae	—
B. xylophilus	Steiner and Buhrer (1934)	xylophilus	RS
syn. *B. lignicolus*	Mamiya and Kiyohara (1972)		
B. yongensis	Gu et al. (2006a)	piniperdae[f]	RS

[a] "Species inquirendae" in Hunt (1993)
[b] "Species incertae sedis" in Hunt (1993)
[c] "Species indeterminatae" in Hunt (1993)
[d] Redescription
[e] New group proposed by Braasch et al. (2006d)
[f] Described after Ryss et al. (2005)
[g] Excruded from the genus by Ryss et al. (2005)
[h] R: ITS-RFLP profiles are reported in Burgermeister et al. (2005); S: DNA sequence(s) is (are) available from GenBank Database
[i] "*B. aberrans*" in Burgermeister et al. (2005) was corrected to *B. sinensis* by Kanzaki and Futai (2007)
[j] *B. kolymensis* might be a junior synonym of *B. mucronatus* European type
[k] Shown in Kanzaki and Futai (2005)
[l] Shown in Gu et al. (2005)

9.2 Taxonomic Status of the Genus *Bursaphelenchus*

The genus *Bursaphelenchus* was established by Fuchs (1937) with the type species, *B. piniperdae*. According to Hunt (1993), the genus is a member of the Family Parasitaphelenchidae, Superfamily Aphelenchoidoidea, Suborder Aphelenchina, Order Aphelenchida. Although the Superfamily Aphelenchoidoidea contains some predatory nematodes, obligate plant parasites and insect parasites, most *Bursaphelenchus* species are free-living mycophagus species inhabiting soil or dead plant material, including dead wood. Many species are also known as entomophilic (phoretic) nematodes. The families and genera belonging to the superfamily are listed below.

Currently, the taxonomy of the phylum Nematoda is changing drastically, and the order Aphelenchida, to which the genus *Bursaphelenchus* belongs, is now considered as belonging to the Superfamily Aphelenchoidea, Infraorder Tylenchomorpha, Order Rhabditida based on molecular phylogenetic analyses conducted by Blaxter et al. (1998) and De Ley and Blaxter (2002). However, the construction of the new taxonomic system has not been completed; consequently, the widely accepted system proposed by Hunt (1993) is used here. The main feeding habits are indicated after each family name.

Order Aphelenchida
Superfamily Aphelenchoidoidea

Family Aphelenchoididae: mycophagus, entomoparasitic, plant parasitic
Subfamily Aphelenchoidinae
Genera: *Aphelenchoides*, *Laimaphelenchus*, *Megadorus*, *Ruehmaphelenchus*, *Schistonchus*, *Sheraphelenchus*, *Tylaphelenchus*
Subfamily Anomyctinae
Genus *Anomyctus*
Family Seinuridae: predator
Subfamily Seinurinae
Genera *Seinura*, *Aprutides*, *Papuaphelenchus*, *Paraseinura*
Family Ektaphelenchidae: entomoparasitic, mycophagus (?)
Subfamily Ektaphelenchinae
Genera *Ektaphelenchus*, *Cryptaphelenchus*, *Cryptaphelenchoides**, *Ektaphelenchoides*
Family Acugutturidae: entomoparasitic
Subfamily Acugutturinae
Genus *Acugutturus*
Subfamily Noctidonematodae
Genera *Noctidonema*, *Vampyronema*
Family Parasitaphelenchidae: entomoparasitic, mycophagus, plant parasitic
Subfamily Parasitaphelenchinae
Genus *Parasitaphelenchus*
Subfamily Bursaphelenchinae
Genera *Bursaphelenchus*, *Rhadinaphelenchus***
Family Entaphelenchidae: entomoparasitic, mycophagus (?)
Subfamily Entaphelenchidae
Genera *Entaphelenchus*, *Peraphelenchus*, *Praecocilenchus*, *Roveaphelenchus*

Note: *Cryptaphelenchoides** and *Rhadinaphelenchus*** are now considered synonyms of *Ektaphelenchus* and *Bursaphelenchus,* respectively (Baujard 1989; Giblin-Davis et al. 1989; Ryss et al. 2005; Ye et al. 2007; Hunt 2008), and the possibility of mycophagy of the families Ektaphelenchidae and Entaphelenchidae, followed by "?", has been suggested, but has not been confirmed.

Hunt (1993) noted the genus *Bursaphelenchus* as a "home to a considerable assemblage of species, some of which have been placed in separate genera", and stated the necessity of further taxonomic work. Actually, the morphological definition of this genus has been extended many times by several authors (e.g., Braasch 2004; Kaisa 2005; Ryss et al. 2005), and overlaps with the other genera belonging to the same superfamily. Several important features are excerpted from the current generic morphological definition (Hunt 1993), and are listed below. The features are also illustrated in Fig. II.1. Some exceptions are shown in parentheses.

Genus *Bursaphelenchus*
Body: slender, ventrally arcuate when killed with heat, 0.3–1.4 mm in length, lateral field with 2–6 incisures present but not described in several species.
Lip: well-developed, separated in six equal-sized lips (exceptions: *B. lini* and *B. eproctatus*, two lateral lips are narrower than the other four), constricted clearly at posterior end (several species, e.g., *B. platzeri*, have weak constriction).

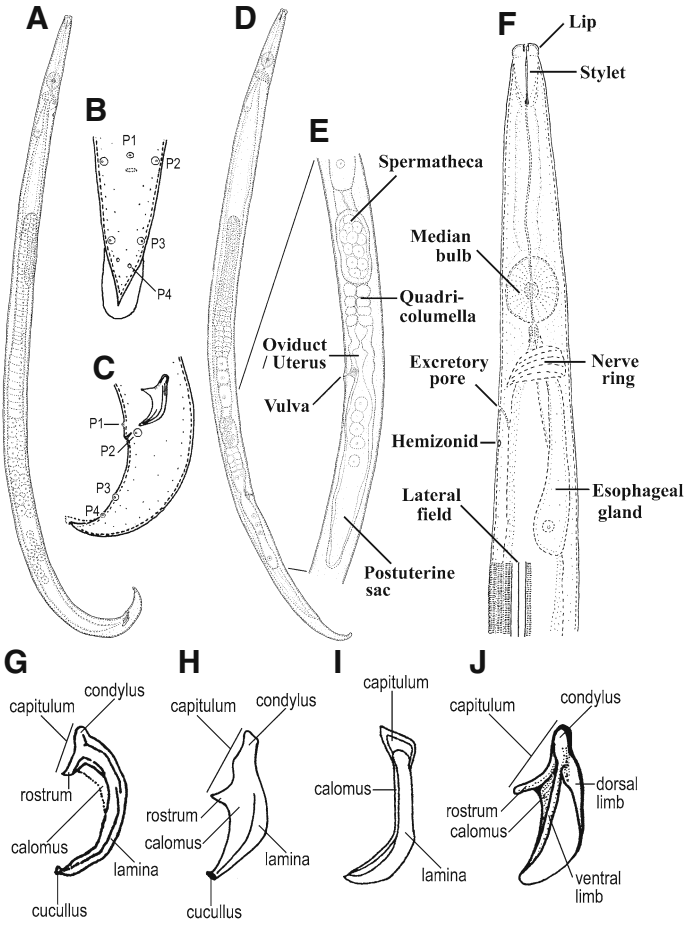


Fig. II.1 General morphology of *Bursaphelenchus* nematodes. Adult male (**A**), ventral view of male tail (**B**), lateral view of male tail (**C**), adult female (**D**), female reproductive organ (**E**), anterior part of female (**F**) and spicules of *B. conicaudatus* (**G**), *B. corneolus* (**H**), *B. aberrans* (**I**) and *B. seani* (**J**), modified after Kanzaki (2006), with permission

Stylet: well-developed, 12–20 µm in length but sometimes reaches 26 µm, basal swelling called "basal knob" usually present (several species, e.g., *B. lini*, lacking the knob).

Metacorpus: well-developed, muscular, spherical- or oval-shaped.

Excretory pore: usually conspicuous, located at various positions, that is, anterior to metacorpus to posterior to nerve ring, but usually located at the same level as or just posterior to the metacorpus.

Female reproductive organ: monodelphic, *V* value usually 70–80%, vulva various, that is, without any flap, with short (=side) flap or long (=real) vulval flap, most species probably possess a pair of three-celled structures at the

junction of the uterus and postuterine sac; postuterine sac is usually well-developed, 3–6 body diameter. Length, occupying more than 50% of the vulva–anus distance, but very short in some species.

Female tail: varies from short to elongated, rounded to pointed tip, mucronated in some species.

Male spicule: usually separated (but fused in some species), variable in size and shape, usually arcuate.

Male bursa: present, variable in shape.

Male caudal papillae: 4 (2 pairs) to 11 (single ventral one +5 pairs) have been reported.

As suggested in the above parentheses, there are many exceptions in generic definitive characteristics. In particular, the distinction between *Bursaphelenchus* and its sister taxa, *Parasitaphelenchus*, is very vague. These two genera are separated mainly by the presence or absence of the parasitic juvenile stage, body length, *V* value and fused or separated male spicule; however, some *Bursaphelenchus* nematodes have a *Parasitaphelenchus*-like parasitic juvenile stage, for example, *B. hylobianum* (Korentchenko 1980), a large *V* value, for example, *B. dongguanensis* (*V* value = 86–92; Kaisa 2005), and fused spicule, for example, *B. platzeri* (Giblin-Davis et al. 2006b), and some *Parasitaphelenchus* species have a small body, for example, *P. oldhami* has a body length of ca. 1 mm (Hunt and Hague 1974). Thus, an integrated generic revision of these two taxa is needed in the future.

9.3 Methods of Taxonomy Identification and Systematics of the Genus *Bursaphelenchus*

9.3.1 Morphological Taxonomy and Identification

Many authors have attempted to systematize the genus *Bursaphelenchus*, which contains many species. Tarjan and Baeze-Aragon (1982) and Yin et al. (1988) proposed a pictorial key for species based on morphological characteristics; however, their main purpose was construction of the pictorial key, and they did not propose any taxonomic system to divide the genus into subsets. After their pictorial keys, as the results of efforts to organize the taxonomic system by several authors, currently, the genus is divided into subsets called “groups”, which is not a formally accepted taxonomic unit, but is roughly equivalent to “subgenus”. The “group” is defined by spicule morphology, and species belonging to the same “group” are distinguished by the other morphological traits, for example, arrangement of caudal papillae, vulval structure, female tail shape and morphometrics.

The original concept of the “group”, the assemblage of species, which share characteristic spicule morphology, was proposed and tested by Giblin and Kaya (1983), but they did not apply this concept to all species. Braasch (2000) expanded this concept, taking the number of lateral incisures, the number and arrangement of

caudal papillae, morphology of male bursa and female vulval structure into consideration, and divided 28 European conifer-inhabiting species into 10 groups, while Ryss et al. (2005) used spicule morphology as the most important feature, because only spicules are described in all species evenly. They also proposed detailed morphometrics about spicule morphology, for example, ratio of spicule length and maximum width, capitulum length and ratio of capitulum and spicule length, and divided the genus into six groups according to systematic analysis based on their morphometrics and other morphological traits. This system proposed by Ryss et al. (2005) could be considered as a well-constructed integrated system based on the "group" concept (Giblin and Kaya 1983) and systematic analysis (Tarjan and Baeze-Aragon 1982); however, their major purpose was the construction of an "identification system", thus the system is still too typological to systematize the genus. Hence, as the authors remarked, their system still contains some arbitral clades, because they constructed the system based on original descriptions, which contain many misinterpretations in morphological observation, and did not evaluate the weight (= importance in evolutionally systematics) of each characteristic. Nevertheless, their efforts could serve as a starting point for integrated discussion, because they listed all nominal species at that point, and proposed the digitalization and generalization of morphological traits, especially spicule morphology.

9.3.2 Molecular Systematics and Identification

Molecular techniques have many advantages in identification compared to morphological methods, for example, they do not require special training in morphological observation and the methods are applicable to juveniles, which do not have specific diagnostic morphological characteristics. Also the cost and labor involved in molecular techniques is now becoming reasonable.

Many of the molecular techniques that have been developed and used with other *Bursaphelenchus* nematodes have also been used to identify the PWN, for example, RFLP (Webster et al. 1990; Abad et al. 1991; Beckenbach et al. 1992; Tarés et al. 1992), satellite DNA probe (Tarés et al. 1993, 1994; Harmey and Harmey 1994), species specific PCR (Matsunaga and Togashi 2004; Takeuchi et al. 2005), RAPD–PCR (Braasch et al. 1995), PCR–RFLP of ITS rDNA (Hoyar et al. 1998; Iwahori et al. 1998, 2000; Burgermeister et al. 2005b) and DNA sequencing (Iwahori et al. 1998, 2000; Kanzaki and Futai 2002a; Ye et al. 2007). Among these molecular techniques, PCR–RFLP profiles of ITS rDNA (=ITS–RFLP) and DNA base sequencing of several genetic loci have recently been widely employed.

The PCR-RFLP technique has been applied to many nematode species to identify them at species or strain level (Harris et al. 1990; Ferris et al. 1993; Ibrahim et al. 1994; Orui 1996). This technique was introduced for *Bursaphelenchus* nematodes by Hoyar et al. (1998) and Iwahori et al. (1998) to identify the isolates of *B. xylophilus* and *B. mucronatus* using ITS rDNA. Reference profiles of more than 30 *Bursaphelenchus* species have been provided by several authors (e.g.,

Burgermeister et al. 2005; Kanzaki and Futai 2005; details given in Table II.1). The advantages of ITS–RFLP is its simplicity and speed and relatively low cost; however, it is difficult to apply the technique to systematic analyses, and reference patterns to compare with the query profile are required every time. Thus, ITS–RFLP is less general than DNA sequencing and its utilization may be limited to identify certain species or strains (isolates).

DNA sequencing may become the standard tool for molecular taxonomy and identification, as suggested by Ye et al. (2007), because of the expansion of DNA sequence databases, for example, GenBank. Databases also enable us to compare query sequences with all other sequences stored in the database using a computer system, and to estimate the phylogenetic relationships if proper genetic loci and analytical algorithms are chosen. The cost of sequencing, the largest disadvantage of this technique, is now becoming lower, similar to that for ITS–RFLP.

Several ribosomal DNAs, that is, 18S rDNA (SSU) (Kanzaki and Futai 2002a; Ye et al. 2007), ITS region (Iwahori et al. 1998, 2000; Kanzaki and Futai 2002a) and 28S rDNA (D2/D3 LSU) (Kanzaki and Futai 2007; Ye et al. 2007), and mitochondrial COI (Kanzaki and Futai 2002a, 2002b; Ye et al. 2007) have been applied to molecular systematic analyses of *Bursaphelenchus* nematodes at various levels. Each molecular region has its own substitution rate and inherent characteristics, for example, mitochondrial DNA is inherited maternally and has a relatively high substitution ratio, thus DNA sequences are applicable to various levels of comparison if a proper genetic loci, for example, stable loci for higher taxa and variable loci for lower taxa, are chosen for analysis. Kanzaki and Futai (2002a) and Ye et al. (2007) compared the features of those genetic loci, and defined the applicable range of each locus. The characteristics of each locus are summarized as follows. The sequences of universal primers are summarized in Fig. II.2.

ITS Region

Internal transcribed spacer (ITS) region consists of ITS 1, 5.8S ribosomal DNA and ITS 2. ITS 1 and 2 are kinds of "intron" sequences located between small and large subunits of ribosomal RNA coding regions. Sequence mutations accumulate easily in this region, therefore, the ITS region is suitable for analyses of intraspecific, that is, isolate group phylogeny (Iwahori et al. 1998, 2000; Kanzaki and Futai 2002a, b); however, it is not applicable for interspecific phylogeny because sequence divergence is too high in this locus (Kanzaki and Futai 2002a). The sequence length, that is, length of PCR products amplified with universal primer sets, of this region varies so much among species, ranging from 0.7 to 1.2 kbps.

D2D3 LSU

D2D3 LSU, which consists of highly variable D2 and relatively stable D3, is a part of 28S ribosomal DNA (large subunit ribosomal RNA). This locus is applicable to

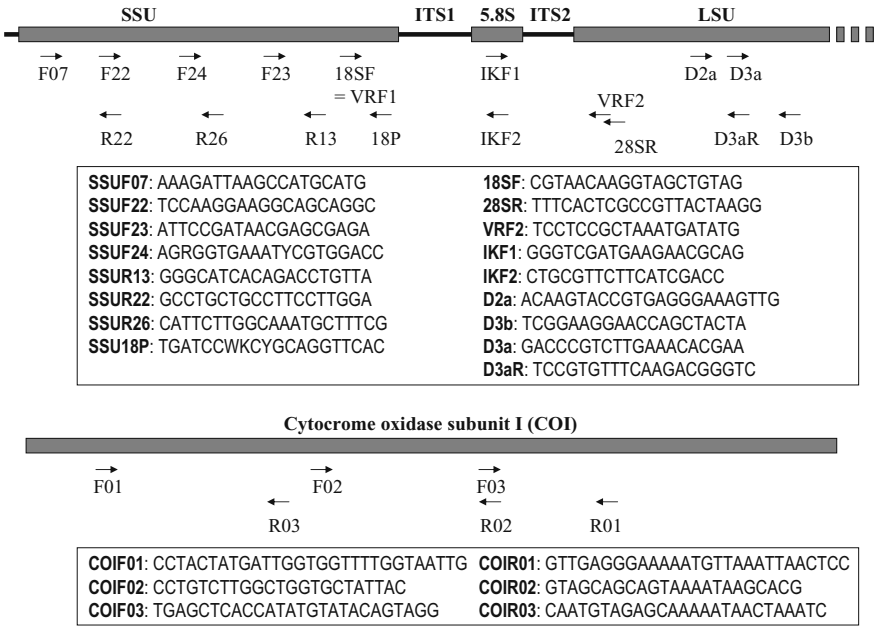


Fig. II.2 Sequences and locations of universal PCR primers applicable to *Bursaphelenchus* nematodes. *Arrows* indicate the direction and position of primers

relatively wide-ranging phylogenetic analyses, that is, among closely related species to among genera (Ye et al. 2007). To date only Ye et al. (2007) have applied this region to the genus *Bursaphelenchus*, however, this region seems very effective to analyze interspecific variation within the genus, and is expected to be a standard region for molecular profiles of *Bursaphelenchus* spp. The sequence length of this region is about 0.75 kbps.

18S rDNA

The 18S ribosomal DNA is a coding region of 18S ribosomal RNA (SSU: small subunit). This locus is highly stable, and is used for molecular systematics of higher taxa, for example, class and order level (Blaxter et al. 1998; Floyd et al. 2003). In the genus *Bursaphelenchus* and related nematodes, this region is very useful for molecular systematics among groups, genera and families, because of its stability. Many molecular sequences of this region are also available in databases (Blaxter et al. 1998; Giblin-Davis et al. 2005; Kanzaki and Futai 2005; Ye et al. 2007); however, the SSU is not applicable in the analyses of intraspecific variations because this region contains few intraspecific variations (N. Kanzaki, unpublished data). Many authors (e.g., De Ley et al. 2002) have developed

universal primers for this region, and many of those primer sequences are available at Prof. Dr. Blaxter's website (http://www.nematodes.org/barcoding/sourhope/nemoprimers.html). The sequence length of this region is relatively long, ca. 1.7 kbps.

Mitochondrial COI Gene

Cytochrome oxidase subunit I (mtCOI) gene, a part of the mitochondrial genome, is suitable to analyze intraspecific variations and variations among closely related species, because of its high sequence diversity (Kanzaki and Futai 2002a, b). This gene is transcribed to protein, thus, the amino acid sequences of this gene could be available for higher level analysis, for example, among groups (Kanzaki and Futai 2002a, 2003a). About 1.0 kbps of DNA sequence is available with the primer set developed by Kanzaki and Futai (2002a).

9.4 Comparison of Molecular Phylogeny, Morphology and Life History of *Bursaphelenchus* Nematodes

DNA sequences of SSU and D2D3 LSU of several *Bursaphelenchus* species downloaded from the GenBank database were compared using maximum likelihood analysis. The morphological and life history traits of the nematodes were then plotted on phylograms (Figs. II.3, II.4, II.5).

About 30 species available for this comparison fell into three large clades (Figs. II.3, II.4):

Clade I contains only one species, *B. abruptus*, which is outside of the phylogram, and is distinct from the other clades. Morphologically, *B. abruptus* is similar to "xylophilus" group species, belonging to clade III (subclade III-d), because of its very characteristic spicule shape, relatively large spicule possessing narrow lamina and calomus, and well-developed condylus and rostrum, and a long female vulval flap (Giblin-Davis et al. 1993). Also, the biological traits of *B. abruptus*, associated with a soil-dwelling bee, *Anthophora abrupta*, are similar to those of *B. anatolius*, *B. kevini* and *B. seani*, which belong to clade III (subclades III-b and III-c) (Giblin and Kaya 1983; Giblin et al. 1984; Giblin-Davis et al. 1990, 1993, 2005). However, Giblin-Davis et al. (1993), who identified this species, pointed out the possibility of morphological and biological convergence, because *B. abruptus* has a unique lip structure, lacking head annulations and possessing a circular oral depression, which is clearly different from other *Bursaphelenchus* nematodes. Molecular analysis based on SSU and D2D3 LSU suggests that these morphological and biological traits of clade I (=*B. abruptus*) are convergent characteristics, which occurred independently from those of clade III. *B. abruptus* may be separated from the other *Bursaphelenchus* nematodes. Generic or subgeneric reconstruction might be considered following detailed morphological observations.

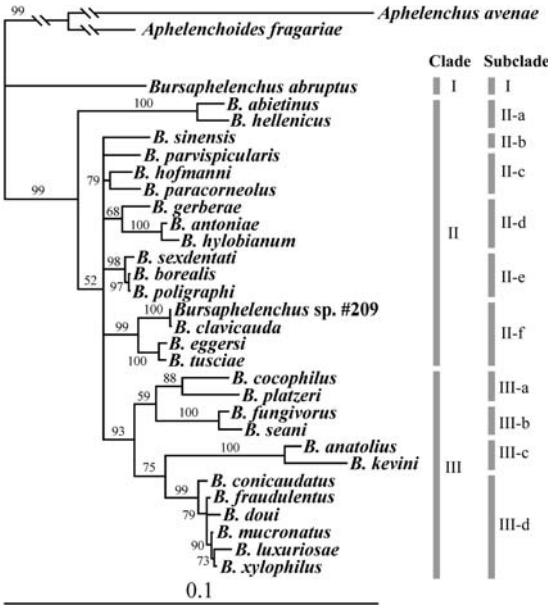


Fig. II.3 Phylogenetic relationship among 29 *Bursaphelenchus* nematodes based on small subunit. *Aphelenchus avenae* and *Aphelenchoides fragariae* served as outgroup species. *Bursaphelenchus* spp. #209 assumed to be similar to *B. eremus* and *B. yongensis* is now being identified by the author

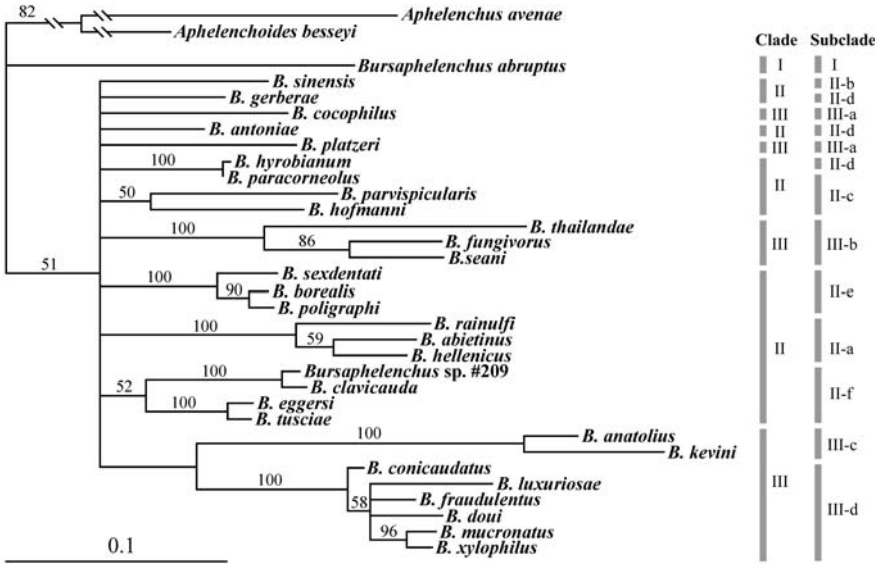
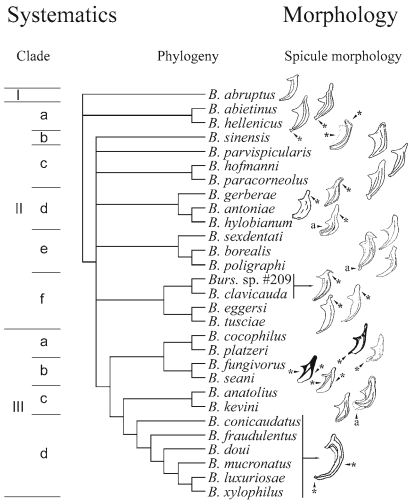


Fig. II.4 Phylogenetic relationship among 31 *Bursaphelenchus* nematodes based on D2/D3 expansion segment of large subunit. *Aphelenchus avenae* and *Aphelenchoides fragariae* served as outgroup species. *Bursaphelenchus* spp. #209 assumed to be similar to *B. eremus* and *B. yongensis* is now being identified by the author



LL	CP	VF	3C	Habitat/Host	Vector/Host
4	4L	L	−	Soil	Bee*
2[a]	4S, no P1?	S	N/R	Conifer	Bark beetle
3	4S	S	N/R	Conifer	Bark beetle
2[a]	4S + ds[a]	S	+	Conifer	*unknown*
3	4S	S	N/R	Broad-leaved tree	Bark beetle
3	4S	S	N/R	Conifer	Bark beetle
3	4S	S	N/R	Conifer	*unknown*
3	4S	S	+	Grass (palm)?	Weevil*
3	4S	S	N/R	Conifer	Weevil*
3	4S	S	N/R	Conifer	Weevil*
4	4S	S	N/R	Conifer	Bark beetle
4	4S	S	N/R	Conifer	Bark beetle
4	4S	S	N/R	Conifer	Bark beetle
3	4S	S	N/R	Conifer	Bark beetle
3	4S	S	N/R	Broad-leaved tree	Bark beetle
3	4S	S	N/R	Conifer	Bark beetle
3	4S	S	+	Conifer	*unknown*
4	4L	S?	N/R	Grass (palm)?	Weevil[a]
3[a]	4L (+P0)	No	+	Rotten fruit	Nitidurid[a]
4	4L	No	+	Fungi? / Conifer[a(?)]	*nuknown* / Bark beetle[a(?)]
4	4L	No	+	Soil	Bee
4	4L + P5*	No	+	Soil	Bee
4	4L + P5*	No	+	Soil	Bee
4	4L	L	−	Broad-leaved tree	Cerambycid*
4	4L	L	−	Broad-leaved tree	*unknown*
4	4L	L	−	Conifer	Cerambycid*
4	4L	L	−	Conifer	Cerambycid*
4	4L	L	−	Broad-leaved tree	Cerambycid*
4	4L	L	−	Conifer	Cerambycid*

Fig. II.5 Comparison among phylogeny based on small subunit, morphology and biological traits of 29 *Bursaphelenchus* nematodes. *LL* number of lateral lines, *CP* arrangement of caudal papillae, *4L* large (conspicuous) P4 papillae, *4S* small P4 papillae, *ds* dot-like sensilla, *P0* an extra pair at pre-anal, *P5* an extra pair around tail tip, *VF* vulval flap structure, *3C* a three-celled structure at uterus/post-uterine sac junction; *N/R* not reported, + present, − absent. Common traits shared within each subclade are indicated by an *asterisk*. Derived character is indicated as *a* (= alternation)

The species belonging to Clade II have relatively small and stout spicules, tiny P4 papillae and a short vulval flap (side flap: see Giblin-Davis et al. 2006a). This clade is divided into six subclades, II-a to II-f (Fig. II.5). Morphologically, clade II consists of the "aberrans" group, "borealis" group and "piniperdae" group sensu Ryss et al. (2005) or "abietinus" group, "eggersi" group, "hofmanni" group and "sexdentati" group sensu Braasch (2001); however, neither of these classifications corresponds to the phylogenetic clades clearly, mainly resulting from the polyphyogeny of "piniperdae" group sensu Ryss et al. (2005) and "hofmanni" group sensu Braasch (2001). As Braasch (2001) pointed out, the species belonging to clade II are difficult to classify clearly, because they have relatively small spicules similar to each other and few conspicuous morphological characteristics. The following morphological characteristics of each clade can be mentioned: lack of condylus, rostrum and cucullus in spicule (II-b: *B. sinensis*); relatively stout spicule and three lateral lines (II-c, II-d); relatively stout spicule with more or less recurved condylus and four lateral lines (II-e); relatively stout spicule with recurved and more or less pointed condylus (II-f); however, clear diagnostic morphological characteristics to distinguish these molecular phylogenetic clades have not been identified so far.

Interestingly, three alternations on the number of lateral lines and a convergence of spicule morphology are found in clade II. The numbers of lateral lines are two in *B. abietinus* and three in *B. hellenicus* (subclade II-a), two in *B. sinensis*, four in *B. aberrans* (subclade II-b: *B. aberrans* was not available for this molecular

comparison, but based on their very unique spicule shape, there is no doubt that *B. aberrans* and *B. sinensis* are sister species.), three in *B. platzeri* and four in *B. cocophilus* (Fig. II.5). These alternations occurred within each phylogenetic clade, while with the spicule shape, a large and dorsally recurved condylus with a pointed end, which was considered as a key characteristic of the "borealis" group sensu Ryss et al. (2005), was shared with two phylogenetic subclades, that is, *B. borealis* (II-e) and *Bursaphelenchus* sp. #209 which is close to *B. eremus* and *B. yongensis* and is now being identified (N. Kanzaki et al., unpublished data). This characteristic morphology may be a convergent characteristic or an ancestoral characteristic of a common ancestor of subclades II-e and II-f. Detailed re-observation to figure out clade-specific characteristics is necessary in the future.

The biological traits of clade II species are similar to each other. All were isolated from various kinds of dead wood, that is, conifers or broad-leaved trees, or coleopteran insects, or both, inhabiting shallow (=beneath the bark) dead wood. There was no clear preference for tree species. Vector preferences seems to correspond to phylogenetic clades, that, tree species may be explained by host preferences of the vector beetles. Species in subclade II-d, *B. gerberae*, *B. antoniae* and *B. hylobianum*, have been isolated from weevils, and the others arre associated with bark beetles (family Scolytidae), although vector insects have not been identified for *B. sinensis*, *B. paracorneolus*, *B. tusciae* and *B. fraudulentus*. The weevil associate is probably derived from a vector-switching event, which occurred in an ancestor of subclade II-b. Further, *B. hylobianum*, which was originally described as a member of the genus *Parasitaphelenchus*, has a parasitic juvenile stage, which is very similar to that of *Parasitaphelenchus* spp. Parasitism of vector weevil may have evolved independently in this species or as the re-emergence of an ancestoral characteristic.

Clade III is very variable in morphology and life history, thus, it is difficult to identify the common traits of this clade morphologically and/or biologically. The only common morphological trait is conspicuous P4 caudal papillae. This clade, containing "hunti" group sensu Ryss et al. (2005) and "xylophilus" group sensu Braasch (2001), is divided into four subclades. Subclades III-a (*B. cocophilus* and *B. platzeri*), III-b (*B. fungivorus* and *B. seani*), III-c (*B. anatolius* and *B. kevini*) and III-d ("xylophilus" group sensu Braasch 2001). The morphological characteristics of each subclade are as follows: III-a has a fused and semi-circle-shaped male spicule and lacks a real female vulval flap; III-b has a broad spicule with conspicuous ventral and dorsal limbs, and is totally lacking a female vulval flap; III-c has a relatively broad spicule and extra (small P5 pair) caudal papillae in males and very short post uterine sac in females, and totally lacks a female vulval flap; III-d has a long, slender and strongly arcuate spicule with well-developed condyles and rostrum in males and long vulval flap in females. Morphologically, subclades III-a, III-b and III-c are similar in their spicule shape, thus, spicule morphology of the "xylophilus" group is assumed to be a derived characteristic occurring in the ancestor of this subclade, and the ancestoral spicule morphology of clade III may be semi-circular.

The biological traits of clade III are also very variable. Common biological traits within subclade III-a and III-b are not identified clearly. In subclade III-a, *B. cocophilus*, the red ring nematode, is vectored by a species of palm weevil, *Rhynchophorus palmarum*, and inhabits and feeds on palm tissue. This species has a unique feeding habitat, obligate plant parasite, and entomoparasitism is also suspected (Griffith 1987; Gerber and Giblin-Davis 1990), while *B. platzeri*, another member of III-a, is vectored by a nitidulid beetle, *Carpophilus humeralis*, and inhabits rotten fruit, feeding on many species of fungi (Giblin 1985; Giblin-Davis et al. 2006b). Both of these two species have different hosts, vectors and feeding habitat preferences from each other and from other *Bursaphelenchus* species. Thus, the ancestoral characteristics and origins of these unique biological traits are still unknown. *B. fungivorus* and *B. seani*, members of III-b, are morphologically similar, but *B. seani* is associated with a soil-dwelling bee, *Anthophora bomboides*, and inhabits the vector's nest, feeding on fungi (Giblin and Kaya 1983), while *B. fungivorus* was described from a species of broad-leaved tree, *Gardenia* sp. affected by *Botrytis cinerea* in a greenhouse (Franklin and Hooper 1962), and was recently isolated from a species of bark beetle, *Orthotomicus erosus* emerging from a dead pine tree (Arias et al. 2005). Although *B. fungivorus* may consist of several cryptic species, which are morphologically identical but genetically different, if *B. fungivorus* is one species, it may have flexible habitat and vector preferences. The biological traits of *B. anatolius* and *B. kevini* (III-c) are also similar to each other: both are associates of soil-dwelling bees, *Halictus* spp., and inhabit their vector's nest, feeding on various fungi (Giblin et al. 1984; Giblin-Davis et al. 2005). The life histories of "xylophilus" group species (III-d) are similar, and are unique to the genus, thus, the characteristics are assumed to have occurred in the common ancestor of this subclade. They inhabit relatively deep wood of dead or dying trees, feeding on various fungi. The species in the "xylophilus" group have an unique dauer stage, fourth-stage dispersal juvenile, that is, most of *Bursaphelenchus* nematodes have third-stage dauers, and dauer juveniles are vectored by longicorn beetles of the tribe Lamiini, entering the vector's tracheal system (Kanzaki and Futai 2003b).

The ancestors of the genus *Bursaphelenchus* may be soil-inhabiting mycophagus nematodes such as species of *Aphelenchus* and *Aphelenchoides*, the outgroup species of the phylogram. A comparison of the morphological and biological traits and the phylogenetic relationship suggest many radiations and convergences within the genus (Fig. II.5).

Regarding vector preference, bee associations occurs at least twice, that is, in clade I and III (the origins of the associations in subclade III-b and III-c are not specified as the same or different). Weevil associations also occur at least twice, that is, II-d and III-a. Bark beetle association, which is widely distributed through clade II, longicorn beetle association ("xylophilus" group = subclade III-d) and nitidulid association (*B. platzeri*: subclade III-a) may have occurred at least once. Regarding morphology, there are several species-specific alternations on spicule morphology, which has been the primary taxonomic characteristic of the genus *Bursaphlenchus* (e.g., Ryss et al. 2005). A strongly bent lamina/calomus complex

of *B. hylobianum*, a distal projection (cucullus?) of *B. borealis* and a sac-like structure of *B. anatolius* may be species-specific morphologies. In the present analysis, the other morphological and biological characteristics, that is, structure of caudal papillae and vulval flap and associated vectors, also seem to correspond to phylogenetic clades.

Currently, about a third of nominal *Bursaphelenchus* nematodes are available for molecular analysis. To construct an integrated taxonomic system, many more species should be added to the molecular analysis, and the morphological and biological characteristics must be re-evaluated.

9.5 Future Taxonomic Issues

9.5.1 Old Descriptions without Type Material

Type material is very important for taxonomic studies; however, the type material for many *Bursaphelenchus* species are not available, because in the original descriptions type specimens were not designated for some old species and the type material of other species have been lost or have limited availability due to problems at collection institutions.

Species lacking type materials should be re-isolated and re-described, and neotypes should be designated. Re-isolation, identification and new type designation may be possible for clearly described species; however, it may be almost impossible to identify old and poorly described species. For example, Hunt (1993) considered *B. pinasteri* (Baujard 1980; with type materials) as a junior synonym for *B. sachsi* (Rühm 1956; without type materials = without type designation), while Ryss et al. (2005) treated both of them as valid species. In this case, the description of *B. sachsi* is not sufficient, although it was sound enough at the time of description; therefore, a conclusion, the same or different, cannot be reached. In a similar case, *B. kolymensis* is suspected to be a junior synonym for *B. mucronatus* (Magnusson and Kulinich 1996; Braasch et al. 2005). In this case, type materials of both species are available. Further, many isolates of *B. mucronatus* are available as cultures, and biological information about both species is described very well (Mamiya and Enda 1979; Korentchenko 1980). Although the morphological re-observation by Magnusson and Kulinich (1996) did not provide a taxonomic conclusion, re-isolation of *B. kolymensis* followed by molecular analysis and hybridization tests may be possible, and the relationship between these two species could be clarified in the future.

Species descriptions often contain misinterpretations. If a description without type designation contains misinterpretations, the situation becomes complicated. These misinterpretations allow fictional species to remain in the species list and pictorial and text keys, and may cause misidentification of synonyms. Continuous efforts to re-isolate and re-describe old species, culture preservations at a reliable

institute, and rules for specimen voucher are needed. It is also necessary to obtain and accumulate molecular profiles.

The morphological traits easily misinterpreted are as follows:

Spicule morphology. Spicule morphology is one of the most important taxonomic characteristics, because the spicule has been described for all nominal species within the genus; however, spicule morphology is three-dimensional, and its shape seems to differ depending on the microscopic focal plane and direction (angle) of the spicule. Furthermore, there are some, usually slight, morphological variations among individuals; so, it is becoming difficult to understand spicule shape based on just one drawing or photograph. Figure II.6 shows morphological variations of spicules within *B. parvispicularis*, *B. gerberae* and *B. clavicauda*. Also, almost identical spicule shapes are sometimes interpreted as different. Figure II.7 shows the

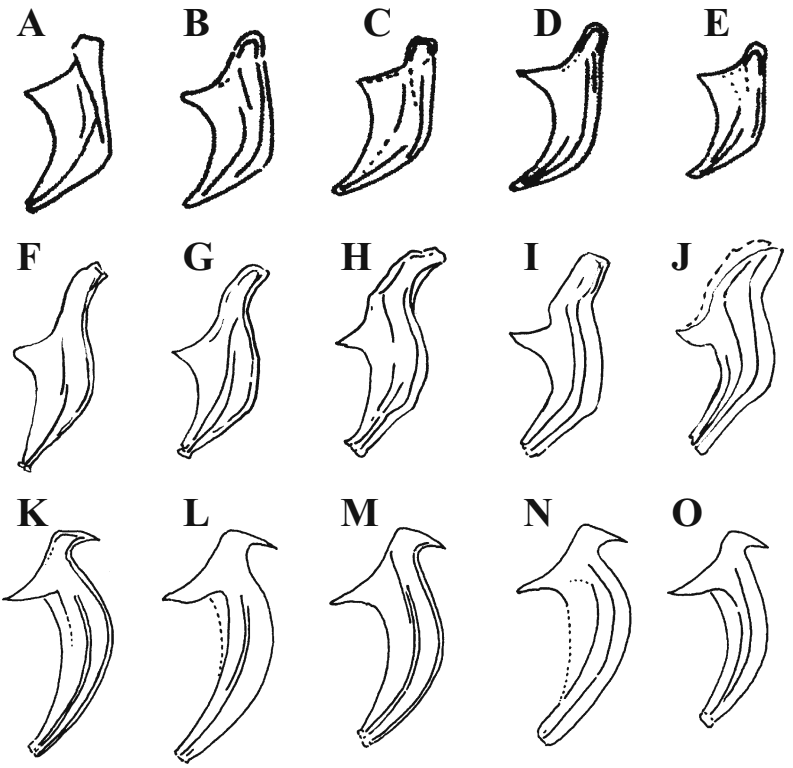


Fig. II.6 Intraspecific variation of spicule morphologies. **A–E** *Bursaphelenchus parvispicularis*; **F–J** *B. gerberae*; **K–O** *B. clavicauda*. Modified after Kanzaki and Futai (2005) (**A–E**), Giblin-Davis et al. (2006b) (**F–J**); and **K–O** Kanzaki et al. (2007)

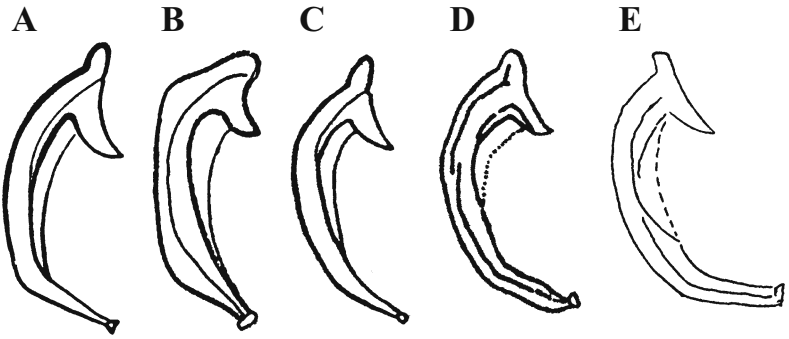


Fig. II.7 Spicules of five (four?) "xylophilus" group species. **A** *B. xylophilus*; **B** *B. kolymensis* (=*B. mucronatus*?); **C** *B. mucronatus*; **D** *B. conicaudatus*; **E** *B. luxuriosae*. The spicule morphologies of these five species are basically the same; however, they are somewhat different from each other in rostrum and condylus morphologies. Modified after Mamiya and Kiyohara (1972) (**A**), Korentchenko (1980) (**B**), Mamiya and Enda (1979) (**C**), Kanzaki et al. (2000) (**D**) and Kanzaki and Futai (2003a) (**E**)

descriptions of *B. xylophilus*, *B. kolymensis* (synonym for *B. mucronatus*?), *B mucronatus*, *B. conicaudatus* and *B. luxuriosae*. These spicule shapes are almost identical; however, the drawings appear to be different. Similarly, the spicule of *B. eremus*, drawn by Rühm (1956) in the original species description, seems totally different from that of the re-isolated culture reported by Braasch et al. (2006c).

Female vulval flap. Generally, the vulval flap is described as "present" or "absent", and when present, "long" or "short"; however, the structure of the vulval flap (or vulva) is roughly classified into three types (Fig. II.8). The species belonging to subclades III-a, III-b and III-c lack a vulval flap, and the anterior and posterior vulval lips seem a little protuberant (Fig. II.8). The ventral view of the vulva on SEM micrograph looks like a simple horizontal slit (Fig. II.8), while the vulval flap of the "xylophilus" group and *B. abruptus* is obviously long and conspicuous; however, other species have a short vulval flap, which is referred to as a "side flap" (see Giblin-Davis et al. 2006a). These species have a dome-shaped expansion just posterior to the vulva, and the flap covers both sides of the vulva, but not the central part (Fig. II.8). This flap sometimes seems like a short flap in the lateral view (Fig. II.8). Re-observation and confirmation are necessary for the species where a short flap was described. For example, the short vulval flaps of *B. hylobianum*, *B. paracorneolus*, and *B. hofmanni* were confirmed as side flaps by Giblin-Davis et al. (2006a).

The number and arrangement of male caudal papillae. Generally, males of *Bursaphelenchus* nematodes have seven caudal papillae, P1 to P4 (Fig. II.1). Within these papillae, P2 and P3 are observed easily with a light microscope; however, P1 is sometimes located at the same level as P2, thus it is often masked by the P2 pair when observed in lateral view, or confused with the cloacal structure or spicule, or

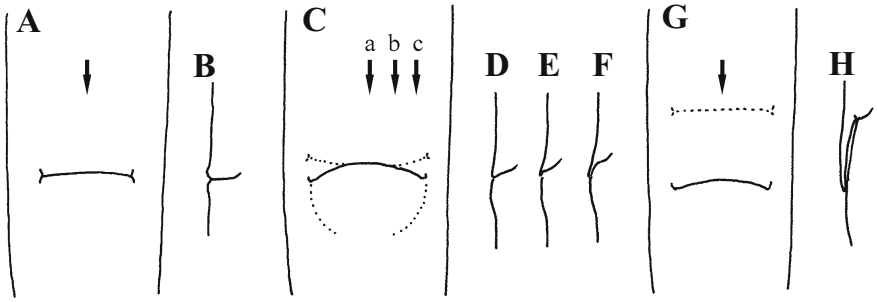


Fig. II.8 Variation of female vulval flap structure. Without the vulval flap, ventral view (**A**) and lateral view (**B**). Short vulval flap (=side flap) in ventral view (**C**) and lateral views (**D**–**F**). Long vulval flap in ventral view (**G**) and lateral view (**H**). *Arrows* in ventral view indicate the focal plane of corresponding lateral views. The *a*, *b* and *c* in **C** correspond to **D**, **E** and **F**, respectively

both, when observed in the ventral view. Therefore, P1 is sometimes missed in the species description, and is confirmed by re-observation with a high-resolution microscope (e.g., Kanzaki and Futai 2002c; Giblin-Davis et al. 2006a). P4 is also missed or easily confused. The structure of the P4 pair is different among phylogenetic clades, that is, the species in clades I and III have relatively large and distinctive P4 located at almost the same level as P3 (e.g., Kanzaki and Futai 2002c), and those in clade II have a small P4 pair, referred to as glandpapilla in Ryss et al. (2005), located on a rounded square cuticle raise plate (see Giblin-Davis et al. 2006a, b). Thus, the P4 pair of clades I and III is sometimes confused with P3 and missed in the lateral view in the original description (e.g., Kanzaki et al. 2000, *B. conicaudatus*; Mamiya and Kiyohara 1972, *B. lignicolus* = *B. xylophilus*; Rühm 1956, *B. fraudulentus*). In clade II, P4 paired papillae are located close to each other and asymmetrically on the right and left. Thus, a P4 pair is sometimes confused as two pairs in the lateral view. Also, the edges of the cuticle plate are sometimes misinterpreted as papillae (Fig. II.9). Actually, this characteristic was corrected in several species by re-observation of type materials and/or living cultures using SEM or a high-resolution light microscope. The number, arrangement and structure of caudal papillae are very important taxonomic characteristics and should be observed carefully, and the possibility of misinterpreting old descriptions should be taken into account.

Besides the basic pattern (seven: P1–P4), various numbers and arrangements of caudal papillae have been reported: four (P2 and P3: many species); six (P2–P4: many species); eight [P2–P5: *B. georgicus* (Devdariani et al. 1980); *B. gonzalezi* (Loof 1964)]; nine [P1–P5: *B. hylobianum* (Korentchenko 1980); *B. kevini* (Giblin et al. 1984); *B. anatolius* (Giblin-Davis et al. 2005)] and 11 [P1–P6: *B. piniperdae* (Fuchs 1937) and *B. poligraphi* (Rühm 1956)]. Within this variation, most of "four" and "six" are assumed to have missed P1 and P4. Only *B. lini* and *B. eproctatus* were confirmed to have just four papillae using SEM (Braasch et al. 2006a; Sriwati et al. 2008). While most of "8" to "11" may have been confused with a cuticle plate

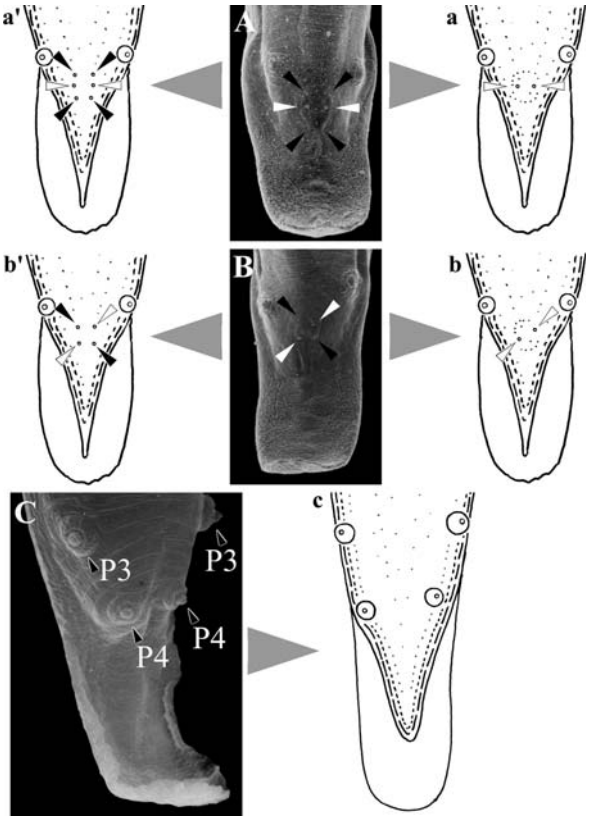


Fig. II.9 Caudal papillae structure. A pair of small P4 papillae (A, B, a, b) easily misinterpreted as three pairs (a′) or two pairs (b′) of papillae with misinterpretation of the edge of cuticular raised plates (A, B) and conspicuous P4 paired papillae. *Solid arrow* edge of cuticular plate, *white arrow*: P4 papillae. Modified after Giblin-Davis et al. (2006a) (A, B, a, b, a′, b′) and Giblin-Davis et al. (2006b) (C and c′)

as mentioned above. Only two species, *B. kevini* and *B. anatolius* have been shown to have an extra pair on photographs (Giblin et al. 1984; Giblin-Davis et al. 2005). Also, some individuals of *B. platzeri* have an extra pair of caudal papillae just anterior to P1 (Giblin-Davis et al. 2006b).

Number of lateral lines. Generally, *Bursaphelenchus* nematodes have a longitudinal tape-like structure on both sides of the lateral body, which is called the "lateral field". The lateral field usually has several incisures called "lateral lines". The number of lateral lines are usually two to four, and a few species have more than five (Rühm 1956; Braasch 2001). The number is mostly difficult to count with a light microscope, and is sometimes missing from the species description. In some old descriptions, only the presence of the lateral field is mentioned without giving the number of lines, or the lateral field is totally missing from the description. The

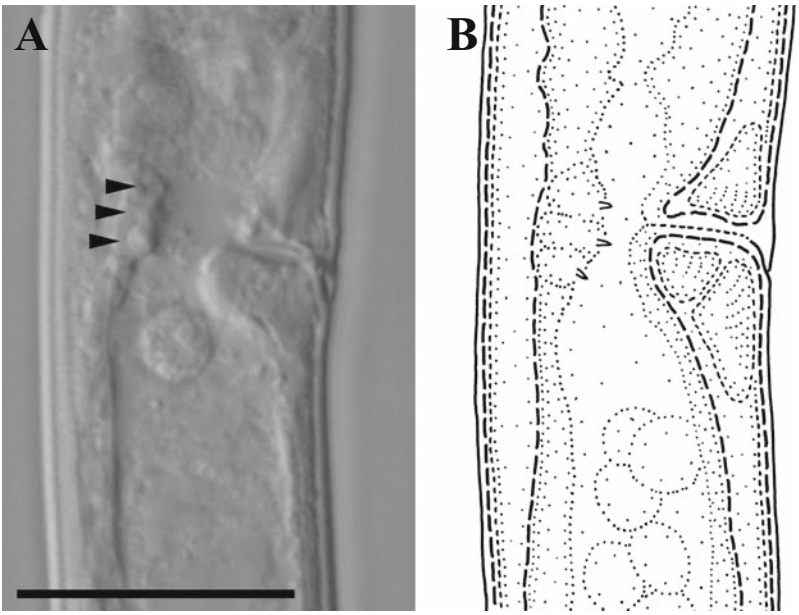


Fig. II.10 A pair of three-celled pronged structures of *Bursaphelenchus sinensis*. **A** light microscopy; **B** drawing. The structure is indicated by *arrows*. Modified after Kanzaki and Futai (2007)

number may correspond relatively well to the phylogenetic relationship, although several alternations are suspected (Figs. II.3, II.4, II.5), but at present is difficult to use as a taxonomic characteristic.

A three-celled structure at uterus-postuterine sac junction. In several species, a pair of three-celled structures are reported at the uterus-post uterine sac junction (Fig. II.10). The presence of the structure is known in just a few species; however, this structure may be present in most *Bursaphelenchus* nematodes, because it has been found in many phylogenetic groups (Kanzaki and Futai 2007). The function of the structure has not been clarified, but it probably has some role concerning oviposition. Kanzaki and Futai (2007) reported that this structure was absent in subclade III-d. Probably, the structure was lost, or altered morphologically in a common ancestor of the "xylophilus" group.

9.5.2 Species Morphologically Overlapping with Other Genera and Families

Several members of the genus *Bursaphelenchus* diverge from general generic definitions. Some have very unique characteristics and others overlap with other genera or families in their essential morphological features. The systematic positions of these species within the genus, or a suitable home of these species has not

determined so far. For example, Baujard (1989) and Giblin-Davis et al. (1989) considered *B. cocophilus* as a member of the genus *Bursaphelenchus*, based on its seven caudal papillae and rounded bursa, while Hunt (1993) placed this species in another genus, *Rhadinaphelenchus*, based on its obligate plant parasitism, extraordinarily slender body and fused male spicule. Similarly, *B. hylobianum* was originally described as a member of the genus *Parasitaphelenchus*, although the adult morphological traits fit the generic definition of *Bursaphelenchus*, because this species has a special parasitic stage, which has a head hook and a tail hook, characteristic of the parasitic third juvenile of *Parasitaphelenchus* species (Hunt 1993; Korentchenko 1980).

In the case of *B. aberrans*, Fang et al. (2002a) reported an elongated median bulb, similar to those of *Ektaphelenchus* spp., and Braasch and Braasch-Bidasak (2002) reported a "parasitic adult" of *B. aberrans*, which is very similar to *Ektaphelenchus* species, that is, the "parasitic adult" of *B. aberrans* has a flattened lip and stylet with a wide lumen. As *B. sinensis*, a sister species of *B. aberrans*, clearly belongs to the genus *Bursaphelenchus* (Figs. II.3, II.4, II.5; Kanzaki and Futai 2007), *B. aberrans* is assumed to belong to the genus; however, the origin of this *Ektaphelenchus*-like parasitic stage is still unknown, that is, as to wheather it developed independently from *Ektaphelenchus* or is derived from a common ancestor.

Fortunately, living material is available for the above three species, and they were confirmed to be included clearly in the inner clades of the genus, based on their molecular phylogenetic positions; however, no living material is available for the other species with unique characteristics. *B. dongguanensis* and *B. digitulus* share several adult morphological traits with the genus *Parasitaphelenchus* although a parasitic juvenile has not been reported for these species, for example, large (more than 85%) *V* value (Loof 1964; Fang et al. 2002b; Kaisa 2005) and weak curvature of the male tail when killed by heat (Loof 1964; Kaisa 2005). Besides *B. aberrans*, *B. lini* and *B. eproctatus* also share several essential characteristics with the family Ektaphelenchidae, that is, elongated (long-oval) median bulb (common in *Ektaphelenchus*), lacking anus and rectum (common in Ektaphelenchidae) and four (two pairs) caudal papillae in males (common in *Cryptaphelenchus*) (Braasch 2004; Braasch et al. 2006a; Sriwati et al. 2008). Braasch (2004), who described *B. lini*, mentioned these features, unique in *Bursaphelenchus* and common in Ektaphelenchidae, and placed this species in the genus *Bursaphelenchus* because this species has a clear male bursa. In the generic description of the *Cryptaphelenchus* (Ekitaphelechidae), Rühm (1956) stated that the bursa is absent in this genus; however, he drew a bursa-like flap at the male tail tip in his illustration. Up to the present, 19 species have been described as members of the genus *Cryptaphelenchus*, but unfortunately, there is no type material available for them; therefore, the presence or absence and structure of the bursa-like flap drawn by Rühm (1956) is still unknown.

The phylogenetic affiliation of those species, as well as the integrated definition, diagnoses and phylogenetic relationship among *Bursaphelenchus*, *Parasitaphelenchus*, *Ektaphelenchus* and *Cryptaphelenchus* remain important taxonomical subjects. Re-isolation, followed by molecular analyses, are needed for these confusing species without type materials.

9.6 Concluding Remarks on the Taxonomy and Systematics

The present situation of the taxonomy, systematics and evolutional hypotheses of the genus *Bursaphelenchus* are summarized in this chapter. Similar to other nematode groups, the taxonomic system of the genus *Bursaphelenchus* is still incomplete, and the border of the genera is still unclear, because of the many problems remaining in morphological and molecular taxonomic systems. These problems are mainly the result of old and unclear descriptions, and the lack of type materials; however, in the future these issues will be addressed individually.

Since the finding of the PWN in Portugal (Mota et al. 1999), which warned the world about the PWN threat, the importance of the taxonomy and identification of *Bursaphelenchus* nematodes has increased rapidly. Further, the pathogenicity of several *Bursaphelenchus* nematodes on *Pinus* spp. has been reported recently (e.g. Skarmoutsos and Michalopoulos-Skarmoutsos 2000), and that has increased the importance of the genus for plant quarantine. This trend may help to solve the taxonomical problems of the genus.

There are two important issues to reconstruct the generic taxonomic system. Primary, old and unclear species are to be organized and verified again, that is, correct the mistakes occurring in the original descriptions and designations of lectotype or neotypes, and secondly, a standard method of description and deposition is necessary.

To reconstruct the taxonomic system, the following procedure is proposed, as described in the last half of this chapter. First, construction of a temporal taxonomic system based on detailed observation of type materials or living specimens, or both, and molecular analysis. Here, morphological and partial biological information may be plotted for phylogenetic groups. Then, re-isolation of unclearly described species based on their biological information, for example, host, habitat or locality, ascription or correction of their morphological traits and plotting on the phylogenetic tree. At present, a temporal system seems to be under construction by several research groups (Braasch 2001; Ryss et al. 2005; Kanzaki 2006; Ye et al. 2007), and several corrections of old species have been proposed (Braasch et al. 2006c; Giblin-Davis et al. 2006a, b; Kanzaki and Futai 2007). The reconstructed generic taxonomy is expected to be proposed in the near future.

Conversely, to standardize the description, although there are general rules suggested in the International Code of Zoological Nomenclatures, besides a proper deposition system for morphological specimens, a system for molecular specimens (=DNA vouchers) is also essential. At present, neither a molecular voucher system nor molecular barcode region has been standardized. The establishment of molecular or culture vouchers may be an important area in the future.

The manuscript of this chapter was written in August 2007, and updated in December 2007. After the last update, taxonomic framework of the superfamily Aphelenchoidea, formerly called "order Aphelenchida", was updated by Hunt (2008). The latest taxonomic system and species list are provided in the paper; Hunt DJ (2008) A check list of the Aphelenchoidea (Nematoda: Tylenchina). J Nematode Morph Syst 10:99–135