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Diversity and Plant Pathogenicity of *Bursaphelenchus* and Related Nematodes in Relation to Their Vector Bionomics

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Abstract

Purpose of Review The nematode genus *Bursaphelenchus* is a highly divergent group containing fungal feeders and obligate and facultative plant parasites. The genus is also known as the phoretic (and parasitic) associates of many groups of insects. Further, two major plant pathogens, *B. xylophilus* and *B. cocophilus*, are members of this genus, and several other species are suspected to be weak to moderate plant pathogens. Here, the diversity of vector insects and host/habitat/plant species interactions, as well as phylogenetic relationships, are summarized for *Bursaphelenchus* species. The hypothetical origins of plant pathogenicity for different species of *Bursaphelenchus* are then discussed in relation to the bionomics of their vector insects.

Recent Findings Phylogenetic analyses suggest that the genus is separated into four clades, and the basal clade will be further separated into two new genera. Based on a literature survey, the phoretic host association patterns appear different among the other three true *Bursaphelenchus* clades, i.e., one clade is mostly associated with bark beetles that occupy relatively dry niches (under bark), whereas the other two clades are associated with various groups of insects inhabiting diverse niches in other relatively humid conditions.

Summary Plant pathogenicity for a few members is hypothetically derived from the nematodes' tolerance to the static resistance of host plants, i.e., the nematode species vectored by the insects attacking or interfacing with live plant tissue were provided an opportunity and, in a few cases, possessed the means to manifest plant pathogenicity with serious consequences, i.e., *B. xylophilus* and *B. cocophilus*.

Keywords *Bursaphelenchus* · Evolution · Life history · Pathogenicity · Phoretic host · Phylogeny

Introduction

Pine wilt disease is one of the most important forest diseases globally. It was first reported in the early twentieth century [1] and has devastated pine forests in East Asia (Japan, China, Korea, and Taiwan) [2–5]. Recently, the disease spread to Europe (Portugal and Spain) where the impacted area is expanding [6, 7, 8•, 9].

The disease has two important characteristics. First, it is caused by a species of nematode, *Bursaphelenchus xylophilus*, commonly known as pine wood nematode [10, 11, 12••], and manifests systemic wilting symptoms derived from tissue damage occurring in large areas of water-conductance tissues [13]. Plant nematodes are usually obligate parasites, e.g., root-knot nematodes (*Meloidogyne* spp.) and cyst nematodes (*Heterodera* spp. and *Globodera* spp.), and sometimes kill their host plants through malnutrition [14],

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i.e., the nematodes modify or destroy the plant tissue by their feeding. However, the pine wood nematode causes a kind of "sudden death" symptom probably derived from the hypersensitive reactions of the host tree [11, 12••, 13]. So far, only two nematode species, *B. xylophilus* and *Bursaphelenchus cocophilus* (red ring nematode), the pathogen of the red ring disease of palms, are known to cause this type of sudden death on plant hosts [15, 16]. Second, *B. xylophilus* is an invasive pathogen introduced from North America to Asia and Europe [17, 18]. In its native range, the nematode is vectored by native North American longhorn beetle species, *Monochamus carolinensis* (Olivier) (Coleoptera: Cerambycidae), *Monochamus mutator* LeConte in Agassiz (Coleoptera: Cerambycidae), *Monochamus scutellatus* (Say) (Coleoptera: Cerambycidae), and *Monochamus titillator* (Fabricius) (Coleoptera: Cerambycidae) [19, 20], and in the Asian and European countries, the nematode is vectored by native longhorn beetles in each area, *Monochamus alternatus* Hope (Coleoptera: Cerambycidae) and *Monochamus galloprovincialis* (Olivier) (Coleoptera: Cerambycidae), respectively [21–23]. This vector switching within the *Monochamus* genus suggests that the nematodes can establish their population in newly introduced areas regardless of their vector-borne (dependent) transmission mode, which is always similar between the various pine sawyer species.

Because of these two major (*B. xylophilus* and *B. cocophilus*) and several minor plant pathogens, e.g., *Bursaphelenchus mucronatus* and *Bursaphelenchus sexdentati* which sometimes kill their native host pine tree hosts [24–29], all *Bursaphelenchus* species are regarded as potential plant pathogens and an important group for plant quarantine [30, 31].

In addition to its economic importance, the genus *Bursaphelenchus* is ecologically and evolutionarily an interesting group of nematodes. For example, the alternation of feeding mode has occurred several times in the genus. Most *Bursaphelenchus* species are fungal feeders, and they are phoretically associated with various groups of insects, mostly wood-inhabiting bark beetles [32–35]. However, obligate plant parasitism, e.g., *Bursaphelenchus sycophilus* and *B. cocophilus* [16, 36], and facultative insect parasitism, e.g., *Bursaphelenchus masseyi* and *Bursaphelenchus piceae* [37, 38], have evolved several times independently from fungal feeders.

In this article, the diversity, phylogeny, and some characteristic biological traits of the genus are introduced. The general biological characters of some plant parasites/pathogens are summarized for each species. Thereafter, the origin of plant parasitism and plant pathogenicity is discussed in relation to their plant and insect associations.

Diversity and Phylogeny of *Bursaphelenchus* and Its Close Relatives

General Biology of *Bursaphelenchus*

The genus *Bursaphelenchus* consists of 125 mostly mycophagous species inhabiting newly dead wood or soil and utilizing various groups of insects as their phoretic hosts (= transportation carrier without direct nutritional relationship) [34, 39–41]. The most common life history of *Bursaphelenchus* spp. is as follows (Fig. 1) (e.g. ,[28, 29, 42]). From spring to summer, carrier insects, typically the wood-boring beetles such as bark beetles, weevils, and longhorn beetles, emerge from dead wood, where nematodes enter the tracheal system, reproductive system, or bottom surface of the elytra of the vectors. Nematodes are associated with their carrier insects as dispersal (dauer) juveniles, the dormant stage specialized for long-term survival and transportation. Thereafter, nematodes enter the live, wilting, or dead tree concurrent with the vectors' feeding (bark beetles, weevils, and longhorn beetles), colonization (bark beetles), or oviposition sites (weevils and longhorn beetles) in the host. The nematodes exit from the vector insect, molt to the propagative form to build up their population while feeding on fungi around the vector larva's tunnel, and enter the next generation of teneral adults of carrier insect just before their emergence.

Thus, most species of *Bursaphelenchus* are basically dead wood-inhabiting saprophytes which do not cause any economic impacts.

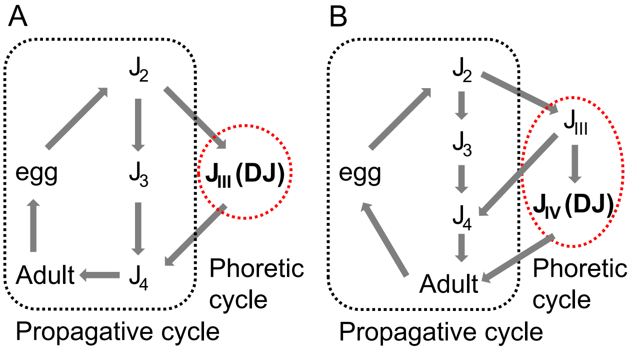


Fig. 1 Common life cycle of *Bursaphelenchus* nematodes. **a** General life cycle of bark and other beetle phoretic species. The nematodes hatch as second-stage juveniles (J_2) and develop to third (J_3)- and fourth (J_4)-stage juveniles and adults feeding on fungi in the propagative cycle, but to phoretically associate the vector insects, J_2 develop to third-stage dispersal (J_{III}) or dauer juveniles (DJ) which are specialized to be transported by the vector beetles. **b** Life history of *xylophilus* group species. The nematodes develop to third-stage dispersal juveniles (J_{III}) before developing to the fourth-stage dispersal (J_{IV}) or dauer juveniles (DJ), the insect-phoretic stage

Taxonomy and Phylogeny

The genus *Bursaphelenchus* belongs to the subfamily Parasitaphelenchinae (Nematoda: Tylenchomorpha: Aphelenchoididae) which consists of four genera, *Bursaphelenchus*, *Parasitaphelenchus*, *Ruehmaphelenchus*, and *Sheraphelenchus* [35, 40, 43] (Suppl. 1). However, regardless of their traditional taxonomic status, these four genera are not phylogenetically distinguished from each other, i.e., the other three genera are located as inner clades within *Bursaphelenchus* as currently defined [35, 43] (Fig. 2).

As depicted in Fig. 2, the subfamily is separated into four phylogenetic groups: a basal clade consisting of *Bursaphelenchus abruptus* and *Aphelenchoides stammeri*, several *Bursaphelenchus* spp. and *Ruehmaphelenchus* (clade I), several *Bursaphelenchus* spp. and *Parasitaphelenchus* (clade II), and several *Bursaphelenchus* spp. and *Sheraphelenchus* (clade III). Further, clades I–III consist of several intrageneric species groups, practically treated as subgenera (Fig. 2; Suppl. 2, 3). Within those clades, the *B. abruptus* + *A. stammeri* clade is clearly separated from the other clades, and these two species are also clearly separated from each other. Thus, these two species represent two undescribed genera.

The plant and insect associations seem different among these three clades (Fig. 2, Suppl. 2). Although there are several exceptions, the basal clade is associated with a soil-dwelling bee (*B. abruptus*) [44, 45] and a rotten, wood-inhabiting longhorn beetle, *Spondylis buprestoides* (L.) (Coleoptera: Cerambycidae) (*A. stammeri*) [46]; clade I is mostly associated with bark beetles and weevils [34, 47]; clade II is associated with various groups of beetles, e.g., weevils, ambrosia, nitidulid and stag beetles, soil-dwelling bees, and fig wasps [34, 48–54]; and clade III is primarily associated with longhorn beetles, with some isolated from bark beetles and soil-dwelling bees [55–60] (Fig. 2). As a general tendency, the clade I species are associated with the vectors occupying niches under bark (relatively dry conditions), whereas the other three clades occur in deep wood, rotten plants, or soil (in relatively humid conditions), i.e., the phoretic relationship occurs in these environmental conditions. The detailed isolation source and phylogenetic status of sequenced members of these clades are summarized in Suppl. 2.

In addition to their diversity in habitat and insect associations, several species show characteristic biological traits, e.g., hermaphroditism and dimorphism of feeding structures.

Currently, two species are known to reproduce without males, i.e., hermaphroditic or parthenogenetic reproduction mode. *Bursaphelenchus okinawaensis* isolated from a longhorn beetle, *Monochamus maruokai* Hayashi (Coleoptera: Cerambycidae), from the southeastern islands of Japan has been confirmed to be a hermaphroditic species [57, 61]. Although many parthenogenetic species have been reported in the family, *Bursaphelenchus okinawaensis* is tentatively the only hermaphroditic species experimentally confirmed. The other species, *Bursaphelenchus arthroides*, was isolated from packing material at the plant quarantine inspection in China, and can also reproduce without males, although the detailed reproductive mode, hermaphroditic or parthenogenetic, has not been confirmed [62].

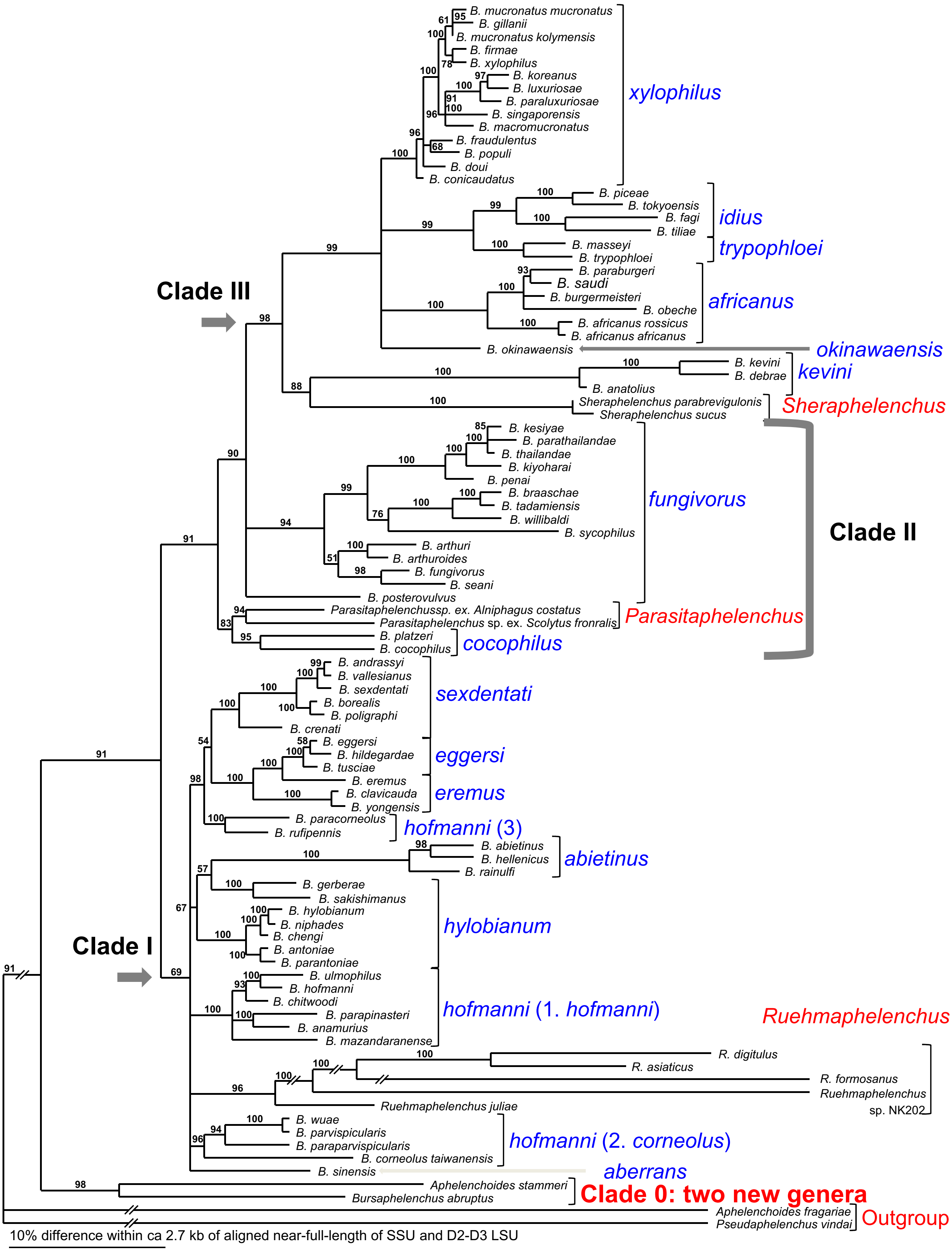
Dimorphism (polymorphism) of feeding structures has been reported in the nematode family, Diplogastridae, and it is a good model to analyze the genetic basis of phenotypic plasticity [63, 64], but the phenomenon has not been investigated in other nematode groups. However, a *Bursaphelenchus* species, *Bursaphelenchus sinensis*, is suspected to have two different types of stylet, a needle-like feeding structure in the Aphelenchoididae. The regular form of the stylet is the typical mycophagous form, which is the same as that of the other *Bursaphelenchus* species, i.e., relatively thin and possessing small basal swellings [65–67]. However, the species seems to have another type of stylet termed a “parasitic form” which is suspected to be a predatory form, which manifests as relatively thick and without basal swellings [67]. Although the detailed mechanism and ecological functions have not been analyzed, this could also represent some of the range of physiological plasticity within the genus.

Insect parasitism has also been suspected. For example, *B. cocophilus* is suspected to parasitize its main vector weevil, *Rhynchophorus palmarum* (L.) (Coleoptera: Curculionidae) [16]; *B. piceae* has been isolated from the Malpighian tubules of older larvae, pupae, and adult vector beetles [38], and the dauer juveniles of *Bursaphelenchus ulmophilus* have been recovered from the hemocoel, fat body, and trachea of dissected female adults and larvae of its vector beetles, *Scolytus multistriatus* (Marsham) (Coleoptera: Curculionidae) and *S. scolytus* (Fabricius) (Coleoptera: Curculionidae) [68].

Plant-Parasitic and Pathogenic Species in the Genus

Economically, the most important character of the genus is plant parasitism and pathogenicity. The *Bursaphelenchus* spp. are basically facultative plant parasites, i.e., they are fungal feeders, and can feed on plant cells as well, and some species are putative obligate plant parasites. Within those facultative or obligate parasites, some species cause systemic damages to the tissues on tree species, i.e., pathogenic.

Currently, in addition to *B. cocophilus* (red ring nematode) and *B. xylophilus* (pine wood nematode), 10 species have been experimentally confirmed to have weak to moderate plant pathogenicity, namely *B. mucronatus*, *Bursaphelenchus firmae*, *Bursaphelenchus luxuriosae*, *B. sexdentati*, *Bursaphelenchus leoni*, *Bursaphelenchus hellenicus*, *Bursaphelenchus anamurius*, *Bursaphelenchus vallesianus*, *Bursaphelenchus yongensis*, and *Bursaphelenchus kiyoharai.* However, none of these species have been associated with


B. mucronatus mucronatus
B. gillanii
B. mucronatus kolymensis
B. firmae
B. xylophilus
B. koreanus
B. luxuriosae
B. paraluxuriosae
B. singaporensis
B. macromucronatus
B. fraudulentus
B. populi
B. doui
B. conicaudatus
xylophilus
B. piceae
B. tokyoensis
B. fagi
B. tiliae
idius
B. masseyi
B. trypophloei
trypophloei
B. paraburgeri
B. saudi
B. burgermeisteri
B. obeche
B. africanus rossicus
B. africanus africanus
africanus
B. okinawaensis
okinawaensis
B. kevini
B. debrae
B. anatolius
kevini
Sheraphelenchus parabrevigulonis
Sheraphelenchus sucus
Sheraphelenchus
Clade III
B. kesiyae
B. parathailandae
B. thailandae
B. kiyoharai
B. penai
B. braaschae
B. tadamiensis
B. willibaldi
B. sycophilus
B. arthuri
B. arthuroides
B. fungivorus
B. seani
B. posterovulvus
fungivorus
Parasitaphelenchussp. ex. Alniphagus costatus
Parasitaphelenchus sp. ex. Scolytus fronralis
Parasitaphelenchus
B. platzeri
B. cocophilus
cocophilus
Clade II
B. andrassyi
B. vallesianus
B. sexdentati
B. borealis
B. poligraphi
B. crenati
sexdentati
B. eggersi
B. hildegardae
B. tusciae
eggersi
B. eremus
B. clavicauda
B. yongensis
eremus
B. paracorneolus
B. rufipennis
hofmanni (3)
B. abietinus
B. hellenicus
B. rainulfi
abietinus
B. gerberae
B. sakishimanus
B. hylobianum
B. niphades
B. chengi
B. antoniae
B. parantoniae
hylobianum
Clade I
B. ulmophilus
B. hofmanni
B. chitwoodi
B. parapinasteri
B. anamurius
B. mazandaranense
hofmanni (1. hofmanni)
Ruehmaphelenchus
R. digitulus
R. asiaticus
R. formosanus
Ruehmaphelenchus sp. NK202
Ruehmaphelenchus juliae
B. wuae
B. parvispicularis
B. paraparvispicularis
B. corneolus taiwanensis
hofmanni (2. corneolus)
B. sinensis
aberrans
Aphelenchoides stammeri
Bursaphelenchus abruptus
Clade 0: two new genera
Aphelenchoides fragariae
Pseudaphelenchus vindai
Outgroup
10% difference within ca 2.7 kb of aligned near-full-length of SSU and D2-D3 LSU

◀ **Fig. 2** Molecular phylogenetic relationships among Parasitaphelenchinae (*Parasitaphelenchus*, *Bursaphelenchus*, *Ruehmaphelenchus*, and *Sheraphelenchus*) species. *Pseudaphelenchus vindai* and *Aphelenchoides fragariae* were employed as the outgroup species. A Bayesian tree was inferred from near full length of SSU and D2–D3 LSU under GTR + I + G model. lnL = 14,594.6387; freqA = 0.2636; freqC = 0.1739; freqG = 0.2529; freqT = 0.3096; *R* (a) = 1.3683; *R* (b) = 2.9705; *R* (c) = 1.3798; *R* (d) = 0.6676; *R* (e) = 5.4051; *R* (f) = 1; Pinva = 0.2724; Shape = 0.3578 for near-full-SSU; lnL = 19,766.9844; freqA = 0.1784; freqC = 0.1679; freqG = 0.3165; freqT = 0.3372; *R* (a) = 0.5418; *R* (b) = 3.183; *R* (c) = 1.1367; *R* (d) = 0.6894; *R* (e) = 5.1676; *R* (f) = 1; Pinva = 0.2337; Shape = 0.6559 for D2-D3 LSU. Posterior probability values exceeding 50% are given on appropriate clades. The compared sequences are provided as the supplementary materials (Suppl. 5, 6)

economic damage under field conditions, including natural forests or plantations [10, 11, 16, 24–29, 69–72] (Table 1). In addition, *B. sycophilus* is an obligate parasite of live syconia of a fig species, *Ficus variegata* [36]. The most important life history characters and the plant pathogenicity (or parasitism) of these species are summarized as follows.

Bursaphelenchus cocophilus

The biological characters and pathogenicity, including disease symptoms, host range, and distribution of *B. cocophilus*, have been reviewed in many sources [15, 16, 73]. Therefore, only some important characters will be summarized here.

The nematode was first recognized as the pathogen of red ring disease in the early twentieth century in the Central and South American tropics [74, 75]. The symptom is typically a wilt of various species of palms, especially of three introduced palm species: coconut palm, *Cocos nucifera* L.; African oil palm, *Elaeis guineensis* Jacq.; and Canary Island date palm, *Phoenix canariensis* Chabaud which are economically important [1]. The disease is characterized by a reddish-brown ring (= red ring) in the cross section of the stem of dead and dying trees, but symptoms can be highly variable with different species and cultivars of host palm under different environmental conditions [76–78]. This red ring is considered to be the result of a plant resistance response, e.g., accumulation of phenolic compounds of host palms, but the detailed mechanism of the pathogenicity is not understood [79]. The pathogenic nematode is vectored by several species of weevils, primarily the South American palm weevil, *R. palmarum* [80]. The third-stage dispersal (dauer or insect-parasitic) juveniles of the nematode enter the body cavity of an adult host weevil, are transported to a healthy tree, and infest it concurrently with the weevils' oviposition [16]. Nematodes feed on plant tissue, propagate, and spread within the tree to cause wilting symptoms [16, 81]. Because the nematode is associated with American native weevils, and does not cause severe damage on native host palms, the nematode is considered a native species of Central and South America [16]. Thus, the disease could be regarded as a typical pattern of a native parasite (weak pathogen) switching its host to naïve introduced susceptible plants to become an important pathogen.

Bursaphelenchus xylophilus

As with *B. cocophilus*, detailed biological characters and disease symptoms of *B. xylophilus* have been previously well summarized [11, 12••, 82, 83••, 84••, 85–94]. The nematode causes typical wilting symptoms on susceptible pine species [13]. The nematode is vectored by longhorn beetles, primarily *Monochamus* spp., i.e., *M. alternatus* in Asia and *M. galloprovincialis* in European countries [22, 23]. Pine

Table 1 Plant (pine) pathogenicity of *xylophilus* group nematodes and feeding and oviposition habits of their vector beetles

Nematode species	Pathogenicity to pine trees	Vector	Natural host (habitat) tree	Vectors' feeding and oviposition
B. xylophilus	Weak-moderate (native range) Moderate-strong (invasive range)	*Monochamus* spp.	Pinaceae	Feeds on live bark[a] and oviposits into the trunk of dead trees
B. mucronatus	Weak-moderate	*Monochamus* spp.	Pinaceae	Feeds on live bark[a] and oviposits into the trunk of dead trees
B. firmae	Weak	*M. grandis*	Pinaceae	Feeds on live bark[a] and oviposits into the trunk of dead trees
B. luxuriosae	Weak	*Acalolepta luxuriosa*	Araliaceae	Feeds on fresh leaves, bark[a], and shoots[a] and oviposits into the trunk of healthy trees[a]
B. doui	Not detected	*A. fraudatrix*, *M. subfasciatus*	Wide range of broad-leaved and coniferous trees	Feeds on dead bark and twigs and oviposits into the trunk of dead trees
B. conicaudatus	Not detected	*Psacothea hilaris*	Moraceae	Feeds on fresh leaves and oviposits into the dead/weakened part of the trunk of live trees

[a] Nematodes have the opportunity to enter healthy tissues of plants

wood nematode is naturally distributed in the central and eastern part of the North American continent and associated with native *Monochamus* spp. and pines without causing significant disease [20]. Thus, the disease is regarded as a typical invasive pest introduced from North America to Asian and European countries. To be vectored by the beetles, nematodes develop to the dispersal third-stage juveniles and to the fourth-stage (dauer) juveniles that are induced by chemical signals released by vector pupae (Fig. 1) [85, 86]. Thus, this two-step dauer formation could affect its vector specificity. The juveniles enter the tracheal system of the teneral adult beetles in their pupal chamber and are then transported to healthy host trees [22, 23]. After emergence, vector beetles feed on the twigs (bark) of healthy host trees, and the nematodes invade the host tree through the feeding wounds (primary infection) [20, 22, 23]. In addition, after the trees are killed by nematode infection, the vector beetles come back to the dead tree and oviposit in the trunk, where the nematodes enter the dead tree through the oviposition marks (secondary infection) [20, 22, 23]. After the primary infection, nematodes molt to the adult, start propagation, and spread throughout the tree to cause wilting symptoms [87]. The detailed mechanism of pathogenicity has not been clarified yet. However, a hypersensitive reaction occurs in susceptible pine tissue, especially related to water conductance, which is an important factor in the etiology of the disease [13].

Bursaphelenchus mucronatus

Bursaphelenchus mucronatus is the putative sister of *B. xylophilus* [88–90]. This species is distributed widely in the Eurasian continent and separated into two subspecies, *B. m. mucronatus* and *B. m. kolymensis* [89]. The species is also associated with *Monochamus* spp. and pine species, i.e., the replacement species of *B. xylophilus* in the Eurasian continent [91, 92]. Thus, the biological nature of this species is very similar to that of *B. xylophilus*, although it scarcely induces disease symptoms under natural conditions [92]. The entrance to and exit from the vector beetle is basically the same as those of *B. xylophilus*, i.e., the dauer juveniles of the nematode enter the tracheal system of teneral adult beetles and enter the host tree through the maturation feeding wound (primary infection to live hosts) and oviposition marks (secondary infection to dead host) [92]. These two subspecies, *B. m. mucronatus* and *B. m. kolymensis*, were formerly regarded as Asian and European types, respectively, i.e., considered to be segregated from each other by their geographical distribution ranges [89]. However, according to recent isolation records in Japan, these subspecies seem to prefer different vector beetles, i.e., *B. m. mucronatus* is often isolated from *M. alternatus* primarily distributed in lowland or warmer areas, whereas *B. m. kolymensis* is isolated from various *Monochamus* spp. distributed in highland or cooler areas [93, 94].

The pathogenicity of *B. mucronatus* has been examined by many inoculation experiments in several countries using native pine trees. Kanzaki and Futai [22] demonstrated that *B. m. kolymensis* killed 65% of potted seedlings of *Pinus densiflora* Siebold & Zucc. under shaded (35% relative illumination) conditions, and the nematode was recovered from all hosts that survived at 6 months after inoculation. Further, Kanzaki et al. [71] conducted an inoculation experiment using naturally shaded *P. densiflora* and *Pinus thunbergii* Parl. in two stands in an experimental field. The experiment confirmed that the nematode killed 17% of lightly shaded (36–38% relative illumination) *P. thunbergii* and 100% of strongly shaded (3–6% relative illumination) *P. densiflora* and *P. thunbergii*, and those hosts that survived became symptomless carriers from which the nematode was re-isolated more than 6 months after inoculation. In both experiments, none of the unshaded trees were killed by the nematode.

Braasch et al. [24] and Braasch [25] demonstrated that drought and high temperatures enhanced the pathogenicity of *B. m. kolymensis* to potted seedlings of *Pinus sylvestris* L. in a greenhouse experiment. The nematode killed inoculated trees under high temperature (20–90% at 25 °C, 30–100% at 30 °C) or drought conditions (60–100%). However, the nematode did not kill the host trees under lower temperature (less than 20 °C). Tomminen [95] also suggested that the nematode is not pathogenic under low-temperature conditions.

Akbulut et al. [28] and Dayi and Akbulut [69] conducted inoculation experiments using four European pine species in Turkey. They inoculated the nematode on the 2- or 3-year-old potted seedlings and demonstrated that the nematode killed 7, 60, 47–73, and 83–87% of the seedlings of *Pinus brutia* Tenore, *Pinus pinea* L., *Pinus nigra* J.F. Arnold, and *P. sylvestris*, respectively. Those experiments were conducted in a greenhouse under relatively high-temperature conditions (25–28 °C), and the nematode pathogenicity seemed enhanced by the temperature.

These experiments suggest that the pathogenicity of *B. mucronatus* is weak to moderate, i.e., the nematode kills its natural host trees when the host is weakened by water stress, shading, high temperature, or other environmental factors.

The pathogenicity of *B. xylophilus* against its native host, e.g., *Pinus taeda* L. and *Pinus rigida* Mill., seems similar to that of *B. mucronatus* to its native host [96, 97].

Bursaphelenchus firmae

Bursaphelenchus firmae has been isolated from the tracheal system of *Monochamus grandis* Waterhouse (Coleoptera:

Cerambycidae) from Japan [90]. As with *B. mucronatus*, this species is also phylogenetically very close to *B. xylophilus* [90]. The detailed life history of *B. firmae* has not been closely examined, but because its vector beetle, *M. grandis*, has a similar life history to other pine-associated *Monochamus* spp. [98, 99], the transportation mode of the species is thought to be similar to that of *B. xylophilus* and *B. mucronatus*, i.e., the dauer juveniles enter the tracheal system of the teneral adult vector and are inoculated into live and dead trees through feeding wounds and oviposition marks, respectively. The vector beetle, *M. grandis*, primarily uses fir species (*Abies* spp.) [98, 99], and the nematode and the vector are hypothesized to be segregated from *B. mucronatus* by their vectors' host preferences.

The pathogenicity of *B. firmae* has been examined with an inoculation test, using 3-year-old seedlings of Japanese black and red pines, *P. thunbergii* and *P. densiflora*, respectively. The nematode showed weak pathogenicity to *P. thunbergii*, but not to *P. densiflora*, i.e., the nematode caused damage to the water-conductance tissues of *P. thunbergii* without visual symptoms to the host plant [100].

Bursaphelenchus luxuriosae

The "parasitic adult form," which was recently hypothesized as a phoretic form of *Bursaphelenchus luxuriosae*, was isolated from the body cavity and tracheal system of a species of longhorn beetle, *Acalolepta luxuriosa* (Bates) (Coleoptera: Cerambycidae) [101–103]. The beetle is associated with broad-leaved trees belonging to the Araliacea, especially *Aralia elata* (Miq.) Seem. [98, 99]. In contrast to *Monochamus* spp., adults of *A. luxuriosa* feed on leaves and at the base of young shoots for maturation, and oviposit on the live shoots of host trees [98, 99]. Therefore, although the host tree species (conifers vs. broad-leaved trees) and phoretic stage (dauer juvenile vs. phoretic adults) are clearly different from *B. xylophilus*, *B. mucronatus*, and *B. firmae* [85, 90, 92], the vectoring is assumed to be similar to those three species, i.e., the nematodes enter the host tree through the vectors' feeding wounds and oviposition marks.

The pathogenicity of *B. luxuriosae* has been examined in several inoculation tests using *P. thunbergii*. The nematode caused tissue damage in 3-year-old seedlings similar to *B. firmae*, and survived for more than 6 months in live 10-year-old pine trees [100, 104]. Thus, *B. luxuriosae* is thought to have weak pathogenicity to pine species and can maintain its population in live pines without causing any visual symptoms.

Bursaphelenchus sexdentati

Bursaphelenchus sexdentati was originally described from a bark beetle species, *Ips sexdentatus* De Geer (Coleoptera: Curculionidae) from Germany [105], and subsequently isolated from dead conifers mostly from European countries [34, 106]. The vector bark beetle, *I. sexdentatus*, usually enters the inner bark of pine trees in various conditions including weakened and stressed live hosts, where they mate, construct galleries, and oviposit [107]. Thus, as with other bark beetle-associated *Bursaphelenchus* species, the nematode is hypothesized to be transported as dauers while sticking to the bottom surface of the elytra and beneath the scutellum of its vector beetle, and enters the host tree concurrently with its vector [32, 33].

Inoculation tests with *B. sexdentati* have been conducted several times in European countries and showed moderate pathogenicity, i.e., although histological analysis was not conducted, the nematode showed relatively high mortality to potted seedlings of *P. sylvestris* (80–100%), *Pinus pinaster* Aiton (53–100%), *P. nigra* (7–100%), *P. brutia* (40%), and *P. pinea* (80%) [24, 26, 29, 69].

Bursaphelenchus leoni

Bursaphelenchus leoni was originally reported from the dead wood of *P. pinaster* from France [108], and then repeatedly isolated from the dead wood of several European pine species [34, 106] and, once, a species of bark beetle, *Dryocoetes autographus* (Ratzeburg) (Coleoptera: Curculionidae), generally known as a secondary pest of pine logs [109]. Thus, although the detailed life cycle of this nematode has not been examined, it is expected to be the same as that of other bark beetle-associated *Bursaphelenchus* species.

An inoculation experiment with *B. leoni* was conducted using potted seedlings of several European pine species. The nematode showed no symptoms on *P. nigra* [24], but showed moderate pathogenicity to *Pinus halepensis* Mill., where the nematode killed 55% of inoculated plants [26].

Bursaphelenchus hellenicus

Bursaphelenchus hellenicus was described from *Tomicus piniperda* (L.) (Coleoptera: Curculionidae) and dead *P. brutia* from Greece [110]. It has been isolated from the dead logs of several European pine species [34, 106] and three bark beetle species, *T. piniperda*, *Hylurgus ligniperda* Fabricius (Coleoptera: Curculionidae), and *I. sexdentatus* [110, 111], which are primary and secondary pests of conifers [107, 109]. As with *B. leoni*, the life cycle of this species is expected to be the same as that of other bark beetle-associated *Bursaphelenchus* species.

The pathogenicity of the species was examined once, using potted seedlings of several pine species, and showed weak pathogenicity by killing *P. halepensis* (35%) and *P. brutia* (0–10%) [24, 26]. However, the species did not cause visual symptoms on *P. sylvestris* or *P. pinaster* [24].

Bursaphelenchus anamurius

This nematode species was described from dead *P. brutia* from Turkey [112]. Thus, the phoretic host insect has not been confirmed.

The pathogenicity of the species was examined twice in inoculation experiments where the nematode killed potted 2–3-year-old seedlings of *P. nigra* (0–73%), *P. pinaster* (46–80%), *P. sylvestris* (13–46%), *P. brutia* (26%), and *P. pinea* (46%) [29, 69].

Bursaphelenchus vallesianus

Bursaphelenchus vallesianus was described from dead *P. sylvestris* from Switzerland [113], and subsequently recovered from dead *P. sylvestris* in Turkey during *Bursaphelenchus* diversity surveys [106, 114].

The pathogenicity of this species was examined together with that of *B. anamurius*, and *B. vallesianus* killed potted 2- to 3-year-old seedlings of *P. nigra* (0–60%), *P. pinaster* (10–20%), *P. sylvestris* (0–13%), *P. brutia* (53%), and *P. pinea* (67%) [29, 69].

Bursaphelenchus yongensis

Bursaphelenchus yongensis was first described from dead *Pinus massoniana* Lamb. from China [115], and subsequently isolated from dead *P. thunbergii* and the bark beetle, *Cryphalus fulvus* Niijima (Coleoptera: Curculionidae), which is known as a secondary pest of *Pinus* spp., emerging from *P. densiflora* from Japan and dead *P. rigida* from Korea [115, 116]. Han [72] examined its pathogenicity to several conifers, namely *Abies* sp., *Larix kaempferi* (Lamb.) Carr., *Pinus koraiensis* Siebold & Zucc., *Pinus strobus* L., and *P. thunbergii*. Although the species caused wilt and mortality on *L. kaempferi*, none of the other tree species showed any wilting symptoms.

Bursphelenchus kiyoharai

Bursaphelenchus kiyoharai was isolated from an ambrosia beetle species, *Xyleborus seriatus* Blandford (Coleoptera: Curculionidae) from two localities in Japan [50]. The vector beetle mostly utilizes newly dead broad-leaved trees, e.g., Fagaceae, but sometimes bores into conifers [117]. Although *X. seriatus* belongs to a group of ambrosia beetles, the species utilizes the inner bark, not sapwood [117]. The nematode was isolated and cultured from the dissected body of adult beetles [50]. However, the detailed manner of insect host usage, i.e., the organ of insect harborage and transport of the nematode, and the presence or absence of insect parasitism were not determined.

A single inoculation test was conducted using 3-year-old Japanese black pine. The nematode showed weak pathogenicity, causing tissue damage similar to that of *B. firmae* and *B. luxuriosae* [100, 104].

Bursaphelenchus sycophilus

Currently, two species, *B. cocophilus* and *B. sycophilus*, are known to be obligate plant parasites, i.e., these two species have not been successfully cultured on any fungal cultures [36]. However, their biological characters and phylogenetic status are clearly different from each other. *B. sycophilus*, a parasite of live fig (*Ficus*) syconia, is structurally (morphologically) adapted to the plant-parasitic life history. The most obvious character is its stylet morphology. The stylet of *B. sycophilus* has a long conus, thick and stout shaft, and extremely well-developed basal swellings [36], which are common to the other three aphelenchoidid fig parasites, *Schistonchus*, *Martininema*, and *Ficophagus* [118], i.e., these four phylogenetically separated genera share convergent morphology in their stylets. During a fig-nematode survey in Japan where more than 10 species of native figs were examined for their nematode associations, *B. sycophilus* was isolated exclusively from *Ficus variegata* (Blume) [36], i.e., the species is highly host specific within the *Ficus* subgenus/subsection *Sycomorus*, and the host specificity is likely determined by its vector fig wasp, *Ceratosolen appendiculatus* (Mayr) (Hymenoptera: Agaonidae). In addition to *B. sycophilus*, undescribed *Bursaphelenchus* spp. have been recovered from the syconia of several African fig species in the subgenus/subsection *Sycomorus* [119].

Other Species Tested for Plant Pathogenicity

Currently, inoculation tests have been conducted for several other *Bursaphelenchus* species, namely *Bursaphelenchus doui*, *Bursaphelenchus conicaudatus*, *Bursaphelenchus niphades*, *B. okinawaensis*, *Bursaphelenchus poligraphi*, *B. yongensis*, *B. hofmanni*, and *Bursaphelenchus tusciae* [24, 100, 104]. However, none of them showed clear pathogenicity to pine (and several other conifer) species.

Interestingly, *B. doui* and *B. conicaudatus* are close relatives of *B. xylophilus*, *B. mucronatus*, *B. firmae*, and *B. luxuriosae* [73, 100, 104], and *B. poligraphi* is one of the closest relatives of *B. sexdentati* [73, 100]. These negative results suggest that the occurrence of pathogenicity is not clearly correlated with their phylogenetic status.

Origin of Pathogenicity in Relation to the Ecology of Vectors

Pathogenicity of Pine Wood Nematode and Its Close Relatives

The pathogenicity of *Bursaphelenchus* spp. is not closely correlated with their phylogenetic status. Thus, as an alternative hypothesis, the manner of nematode infection, i.e., behavioral characters of vector beetles and their associated nematodes, and their plant pathogenicity, was compared focusing on the *xylophilus* group, the close relatives of *B. xylophilus*. In the animal parasites, the manner of infection and transmission mode are known to affect virulence [120, 121].

Currently, the *xylophilus* group contains more than 10 species [90, 122], six of which—*B. xylophilus*, *B. mucronatus*, *B. firmae*, *B. luxuriosae*, *B. doui*, and *B. conicaudatus*—have been examined for their plant pathogenicity and vector relationships (Table 1). The former four species show weak to strong pathogenicity as introduced above, and the latter two did not show any internal or external symptoms on pine tree species.

The life histories of the latter two species are as follows.

Bursaphelenchus doui was first described from packing materials from Taiwan and Korea to China [123], and then several different isolates were isolated from dead *P. densiflora* and the tracheal system of two longhorn beetle species, *Monochamus subfasciatus* (Bates) (Coleoptera: Cerambycidae) and *Acalolepta fraudatrix fraudatrix* (Bates) (Coleoptera: Cerambycidae) from Japan [58, 124]. The nematode has two different phoretic stages, dauer juveniles and phoretic adults, and both occurred on a single individual of *A. f. fraudatrix* [58], i.e., intermediate between *B. luxuriosae* and the other *xylophilus* group species [125]. The adults of both vector species feed and oviposit on dead broad-leaved and coniferous trees [98, 99]. Thus, the nematodes' entry into the host tree is hypothesized to occur during oviposition of the vectors, and the nematode seems to utilize many different groups of trees as its host (habitat).

Bursaphelenchus conicaudatus has been isolated almost exclusively from the yellow-spotted longhorn beetle, *Psacothea hilaris* (Pascoe) (Coleoptera: Cerambycidae) [56, 126, 127]. The vector beetle is separated into more than 10 geographic subspecies, and the nematode has been isolated from all those examined [98, 99, 127]. The beetles feed on the leaves of Moraceae trees, e.g., figs (*Ficus* spp.) and mulberry (*Morus* spp.), and oviposit in the shoots of a newly dead tree or the dead/weakened parts of a live tree [56]. Thus, the nematodes' entrance into the host (habitat) trees occurs only concurrently with the vectors' oviposition.

The plant and insect associations of the above-mentioned six *Bursaphelenchus* spp. are summarized in Table 2. Interestingly, the presence and absence of pathogenicity are not clearly correlated with the vector beetle genera (*Monochamus* or others), the nematodes' phoretic stage (dauer juvenile or phoretic adult), or the host tree group (Pinaceae or broad-leaved tree family), but are seemingly correlated with the condition of the host tree tissue which nematodes encounter, i.e., dead/weakened or healthy.

Bursaphelenchus xylophilus and three other *xylophilus* group species which were more or less pathogenic under experimental conditions, i.e., *B. mucronatus*, *B. firmae*, and *B. luxuriosae*, have an opportunity to invade and propagate in the live tissue of the trees, i.e., *B. xylophilus* and *B. mucronatus* are experimentally confirmed to invade live tissue during primary infection [92, 128]; the vector of *B. firmae* has a similar feeding and oviposition manner as the vectors of the former two species [90], suggesting that *B. firmae* has the same invasion opportunity as the former two species, and *A. luxuriosa* adults feed and oviposit on the healthy tissue of their host trees [101]. Contrastingly, the two avirulent species do not have an opportunity to invade live (healthy) tissue of their host trees. For example, the distribution of *B. conicaudatus* in its host tree is clearly limited to the dead part of the wood, and the nematode was not recovered from the healthy tissue neighboring the dead part [56]. Thus, the nematodes' exposure and ability to enter live tissue are likely an important factor for their successful infection.

The host plants usually have two types of resistance against pathogens and parasites: static and dynamic. Static resistance is expressed constantly regardless of infection via the physical structure and chemicals accumulated in the plant tissues [128], whereas dynamic resistance occurs in response to the infection of pathogens with the reaction being manifested as an alteration of the tissues, synthesis of defensive chemicals, and hypersensitive reactions [129].

Therefore, to survive and establish a population in a host tree, nematodes must be tolerant of or avoid static resistance during primary infection. The pathogenic species including *B. xylophilus* seemingly have the ability to colonize live (healthy) tissue of host trees, and this survivability could be a pre-requirement (pre-adaptation) for causing lethal plant disease such as pine wilt. Thereafter, to cause tissue and systemic symptoms, nematodes must disperse and propagate, overcoming or avoiding dynamic resistance in the host. The resistant reactions of pine trees have been examined with many inoculation experiments. For example, emission of ethylene and increase of tannins have been observed prior to expression of the wilting symptoms [13, 130–133], and thus, the hypersensitive reaction of a pine host hypothetically involves wilting symptoms [132, 134, 135]. Molecularly, the expression of resistance-related genes, e.g., PR-proteins, expansins, and pectate lyase, has been observed a couple of weeks after the inoculation [136•, 137]. Interestingly, these resistance reactions occur clearly in the inoculation treatments with virulent nematodes and susceptible hosts, whereas the reactions

Table 2 Pathogenicity to pine trees of *Bursaphelenchus* (*xylophilus*) group nematodes and feeding and oviposition habits of their vector beetles

Nematode species	Pathogenicity to pine trees	Vector[a]	Natural host (habitat) tree	Vectors' feeding and oviposition	Nematodes' phoretic stage
B. xylophilus	Weak-moderate (native range)Moderate-strong (invasive range)	*Monochamus* spp.	Pinaceae	Feeds on live bark[b] and oviposits into the trunk of dead trees	Fourth-stage dispersal (dauer) juvenile
B. mucronatus	Weak-moderate	*Monochamus* spp.	Pinaceae	Feeds on live bark[b] and oviposits into the trunk of dead trees	Fourth stage dispersal (dauer) juvenile
B. firmae	Weak	*M. grandis*	Pinaceae	Feeds on live bark[b] and oviposits into the trunk of dead trees	Fourth-stage dispersal (dauer) juvenile
B. luxuriosae	Weak	*Acalolepta luxuriosa*	Araliaceae	Feeds on fresh leaves, bark[b] and shoots[b], and oviposits into the trunk of healthy trees[b]	Phoretic adult form
B. doui	Not detected	*A. fraudatrix*, *M. subfasciatus*	Wide range of broad-leaved and coniferous trees	Feeds on dead bark and twigs, and oviposits into the trunk of dead trees	Fourth-stage dispersal (dauer) juvenile and phoretic adult form
B. conicaudatus	Not detected	*Psacothea hilaris*	Moraceae	Feeds on fresh leaves and oviposits into the dead/weakened part of trunk of live trees	Fourth-stage dispersal (dauer) juvenile

[a] All species belong to Coleoptera: Cerambycidae: Lamiinae: Lamiini

[b] Nematodes have the opportunity to enter healthy tissues of plants

are much weaker in the treatment with an avirulent nematode and a susceptible host or a virulent nematode and a resistant host [136•, 137]. This suggests that the defense reactions (represented by a hypersensitive reaction) of susceptible trees do not cease, but expand within the tree, i.e., the defense reactions do not work well to inactivate the nematodes. Contrastingly, resistant trees show only weak reactions [136•, 137], suggesting that the nematodes do not propagate or disperse widely within the tree even if the nematode succeeds during the primary infection. Thus, the weak and moderate pathogens which cause only localized tissue damage are seemingly able to overcome static resistance, but are eliminated or kept to a local infection by dynamic resistance of the host tree.

Although the detailed mechanism of the disease is not understood, the nematode is not likely to produce a specialized phytotoxin. So far, regardless of many detailed chemical analyses [138, 139], a nematode-derived phytotoxin has not been identified. Thus, the nematode activities, e.g., feeding, surface materials, and/or secreted enzymes, are hypothesized to be the elicitor(s) of the reactions. More experimental work is needed to understand the mechanisms of the pathogenicity.

Pathogenicity of Other *Bursaphelenchus* Nematodes

Similar to more or less pathogenic *xylophilus* group species, the other *Bursaphelenchus* spp. showing pathogenicity under field and/or experimental conditions—*B. cocophilus*, *B. sexdentati*, *B. hellenicus*, *B. leoni*, *B. hellenicus*, *B. vallesianus*, *B. anamurius*, and *B. kiyoharai*—have an opportunity to encounter live tissues of the host plants. The biological characters of their vector insects are summarized above and in Table 3.

Thus, these nematodes, with the exception of *B. leoni*, appear to have an opportunity to encounter healthy tissues of their associated host plants [16, 73, 107, 109, 117, 140, 141].

Other *Bursaphelenchus* spp. examined for their pathogenicity include *B. niphades*, *B. okinawaensis*, *B. poligraphi*, *B. yongensis*, *B. hofmanni*, and *B. tusciae* isolated from *Niphades variegatus* (Roelofs) (Coleoptera: Curculionidae), *M. maruokai*, *Polygraphus poligraphus* (L.) (Coleoptera: Curculionidae), *C. fulvus*, *Pityokteines curvidens* (Germar) (Coleoptera: Curculionidae), and *H. ligniperda*, respectively. Except for *P. poligraphus*, a primary and secondary pest of spruce (*Picea* spp.) [98, 107, 109, 141], these vector beetles utilize dead or dying trees, i.e., *N. variegatus* oviposit on dead Pinaceae trees [98]; *C. fulvus* and *P. curvidens* enter dead or dying Pinaceae trees [98]; and *M. maruokai* feeds and oviposits on dead broad-leaved trees [98, 99] (Table 3).

Currently, available information about inoculation experiments of *Bursaphelenchus* spp. and their biological characters suggests that nematode species associated with more aggressive vector species generally have higher pathogenicity. However, the life histories of most *Bursaphelenchus* nematodes have not been sufficiently examined. For example, several *Bursaphelenchus* spp. have been described from *Dendroctonus* spp., one of the most aggressive genera of bark beetles from North America [33]. However, their detailed biological characters and potential risk have not been examined sufficiently. Furthermore, intensive field surveys have not been conducted in the Americas, the origin of

Table 3 Pathogenicity to pine trees of other *Bursaphelenchus* nematodes and feeding and oviposition habits of their vector beetles

Nematode species	Pathogenicity to host plant	Vector[a]	Natural host (habitat) tree	Vectors' feeding and oviposition	Nematodes' phoretic stage
B. cocophilus	Weak (native host)Moderate-strong (introduced host)	Weevils, primarily *Rhynchophorus palmarum* (Coleoptera Curculionidae)	Arecaceae	Bores into live trees[b] (to feed and oviposit)	Third-stage dispersal (dauer or parasitic) juvenile
B. sexdentati	Weak-moderate	*Ips sexdentatus*	Pinaceae	Bores into newly dead or live trees[b] (to feed and oviposit)	Third-stage dispersal (dauer) juvenile
B. hellenicus	Weak	*Tomicus piniperdae*, *Hylurgus ligniperda*, *I. sexdentatus*	Pinaceae	Bores into newly dead or live trees[b] (to feed and oviposit)	Third-stage dispersal (dauer) juvenile
B. leoni	Weak	*Dryocoetes autographus*	Pinaceae	Bores into newly dead trees (to feed and oviposit)	Third-stage dispersal (dauer) juvenile
B. vallesianus	Weak	Unknown	Pinaceae	?	Hypothesized to be stage dispersal (dauer) juvenile
B. anamurius	Weak	Unknown	Pinaceae	?	Hypothesized to be stage dispersal (dauer) juvenile
B. kiyoharai	Weak	*Xyleborus seriatus*	Various broad-leaved and coniferous trees	Bores into newly dead or live trees[b] (to feed and oviposit)	Third-stage dispersal (dauer) juvenile
B. niphades	Not detected	*Niphades variegatus* (Coleoptera: Curculionidae)	Pinaceae	Bores into newly dead trees (to feed and oviposit)	Third-stage dispersal (dauer) juvenile
B. okinawaensis	Not detected	*Monochamus maruokai* (Coleoptera: Cerambycidae)	Various broad-leaved trees	Feeds on dead twigs and oviposits on newly dead trees	Third-stage dispersal (dauer) juvenile
B. poligraphi	Not detected	*Polygraphus poligraphus*	Pinaceae	Bores into newly dead or live trees[b] (to feed and oviposit)	Third-stage dispersal (dauer) juvenile
B. yongensis	Not detected	*Cryphalus fulvus*	Pinaceae	Bores into newly dead trees (to feed and oviposit)	Third-stage dispersal (dauer) juvenile
B. hofmanni	Not detected	*Pityokteines curvidens*	Pinaceae	Bores into newly dead trees (to feed and oviposit)	Third-stage dispersal (dauer) juvenile
B. tusciae	Not detected	*H. ligniperda*	Pinaceae	Bores into newly dead trees (to feed and oviposit)	Third-stage dispersal (dauer) juvenile

[a] All species belong to Coleoptera: Curculionidae: Scolytinae; otherwise, the order and family are suggested after the species names

[b] Nematodes have the opportunity to enter healthy tissues of plants

two of the most devastating *Bursaphelenchus* spp., since Massey [33]. More field surveys and experimental studies are needed to understand the pathogenicity of other *Bursaphelenchus* spp. in relation to the biological characters of their insect vectors and host plants.

Ecological Function of Pathogenicity

The relationship between *B. xylophilus* and *Monochamus* beetles in invaded areas is regarded as a population mutualism, where the vector beetle provides transport for the nematodes and expansion of their distribution while the nematode provides oviposition resources for the beetles by killing the tree [15, 73]. Alternatively, nematodes with weak pathogenicity in their native range will also benefit from associations with their native vectors. Thus, weakly pathogenic nematodes can keep their populations in host trees without causing symptoms [27, 71, 103], but can kill the host when it is weakened by environmental conditions (or other pathogens) [27, 71]. This suggests that the nematode can propagate in and occupy the dead (killed) host trees prior to the colonizing of other nematode species as many endophytic (weakly pathogenic) fungi do [142]. Furthermore, nematodes can increase their vector hosts' oviposition resources when a disease cycle occurs under stressful environmental conditions for normally asymptomatic host plants.

In the case of bark beetle-associated species, the nematodes enter concurrently with their vector beetles [24, 32, 42]. When the beetles enter the dying or newly dead trees, nematodes probably act as simple phoretic associates. However, when the vector beetle attacks live trees, a nematodes' inherent pathogenicity, which causes tissue damage to the tree [24, 26, 29, 69], may positively work to assist a vectors' invasion, i.e., nematodes cause tissue damage that suppresses the hosts' defense response to the beetles. However, the methodology for artificial culturing of bark beetles is not well established, and it is difficult to conduct co-inoculation experiments using the nematodes and beetles to elucidate the ecological function and inherent pathogenicity of these nematodes. Thus, more field surveys and epidemiological analyses are needed.

Concluding Remarks

The genus *Bursaphelenchus* is a highly divergent and interesting group of nematodes. The genus comprises a mostly fungal-feeding clade which has been examined for its genus-wide phylogeny and bionomics, including vector and host relationships. Based upon these comparisons, the genus as currently comprised is hypothesized to be derived from a fungal-feeding and insect-phoretic species inhabiting a relatively humid environment. Furthermore, genome- and gene-expression-based analyses were conducted for *B. xylophilus* [143–146, 147•, 148, 149], and similar analyses are ongoing for several other species in the genus. Thus, the diversity in their biological characters, e.g., occurrence of hermaphroditism and insect and plant parasitism, makes them an interesting system for genome-based evolutionary studies on sex determination, biological interactions, and phenotypic plasticity of fungal-feeding nematodes [36, 61, 147•].

Economically, the genus includes demonstrated and potential plant pathogens. In inoculation experiments to evaluate pathogenicity, potted seedlings are generally less tolerant to nematode infection than those planted in the ground [27, 71]. Thus, some of the experiments evaluated in this review may overestimate the pathogenicity of inoculated species. However, many species of *Bursaphelenchus*, more than expected, were considered more or less pathogenic to their native hosts.

Based upon the expansion of international trade, the risk of invasion of nonnative nematodes is increasing [31, 150–152]. The ecological and economical risks of each species need to be evaluated to protect global forest resources. In most inoculation experiments, many species that are not causing disease under natural field conditions are considered weak to moderate pathogens, probably because of the experimental conditions for host testing. For example, small seedlings are not attacked by vector bark beetles, and potted seedlings usually receive higher temperatures and drought stresses than ground-planted trees. These results suggest that currently avirulent species could become pathogens depending upon changes in environmental conditions. In the present review, the pathogenicity of *Bursaphelenchus* spp. is tentatively hypothesized to be related to the bionomics of vector insects, i.e., the nematodes tend to express pathogenicity when they have more opportunities for exposure to healthy plant tissue. Therefore, to evaluate the potential risks of *Bursaphelenchus* spp., more biological characterization of each species is proposed.

Currently, regardless of many experimental studies, the biological information about *Bursaphelenchus* spp. is still insufficient for strong predictive purposes. More detailed field surveys are needed utilizing the genus as an experimental model and to evaluate its potential risks in a variety of global introduction scenarios.

Compliance with Ethical Standards

Conflict of Interest Drs Kanzaki and Giblin-Davis declare no conflicts of interests.

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