INTRODUCTION

Many Bursaphelenchus nematodes belonging to the Bursaphelenchus xylophilus group sensu Braasch et al. (2009) are associated with cerambycid beetles in the tribe Lamiini. The pinewood nematode B. xylophilus (Steiner & Buhrer) Nickle, the causal agent of pine wilt disease (Kiyohara & Tokushige, 1971), and its closest relative, Bursaphelenchus mucronatus Mamiya and Enda are vectored by Monochamus cerambycid beetles (Linit, 1988; Mamiya & Enda, 1972, 1979; Morimoto & Iwasaki, 1972; Penas et al., 2006; Sousa et al., 2001, 2002; Tomminen, 1990). Also, nematode/vector combinations of B. xylophilus group species include, Bursaphelenchus conicaudatus Kanzaki, Tsuda & Futai/Psacothea hilaris (Pascoe) (Kanzaki et al., 2000); Bursaphelenchus luxuriosae Kanzaki & Futai and Bursaphelenchus acaloleptae Kanzaki, Ekino, Maehara, Aikawa, & Giblin-Davis/Acalolepta luxuriosa (Bates) (Kanzaki et al., 2020; Kanzaki & Futai, 2003a); and Bursaphelenchus firmae Kanzaki, Maehara, Aikawa, & Matsumoto/Monochamus grandis Waterhouse (Kanzaki et al., 2012). In contrast, Bursaphelenchus doui Braasch, Gu, Burgermeister, & Zhang is found in association with several species of cerambycid beetles, that is, Acalolepta fraudatrix (Bates) (Kanzaki et al., 2013), Acalolepta sejuncta (Bates) (Aikawa

Abstract

To show the importance of vector switching of nematodes in the evolution of the Bursaphelenchus xylophilus group, we tested a hypothesis that “Bursaphelenchus doui (or its ancestor) was transferred by Acalolepta fraudatrix, Acalolepta sejuncta, and/or Monochamus subfasciatus (or their ancestral species) from broad-leaved trees to conifers, switched vectors from these cerambycid beetles to Monochamus beetles in conifers, and then evolved into the common ancestor of Bursaphelenchus mucronatus and B. xylophilus.” We used a simple nematode-loading method to beetles and produced 20 binary combinations of five B. xylophilus group species and four cerambycid beetle species in the tribe Lamiini. The affinity of the nematodes for the beetles was examined based on phoretic stage formation of the nematodes. Phoretic stages of B. doui appeared in all beetle species examined, namely Acalolepta luxuriosa, Psacothea hilaris, A. fraudatrix, and Monochamus alternatus, although the affinity of the nematode for M. alternatus was weak. This finding indicates that B. doui could switch vectors to conifer-using Monochamus beetles after transfer by A. fraudatrix from broad-leaved trees to conifers. We conclude that vector switching of nematodes could have potentially happened during the evolutionary history of the B. xylophilus group.

KEYWORDS

Acalolepta fraudatrix, broad-leaved tree, Bursaphelenchus doui, conifer, phoretic stage
et al., 2020), Monochamus subfasciatus (Bates) (Kanzaki et al., 2008), and Monochamus saltuarius (Gebler) (Aikawa et al., 2020).

The habitats for the above nematodes are determined by their vector beetles. Therefore, B. conicaudatus and B. luxuriosae are found in broad-leaved trees, and B. xylophilus, B. mucronatus, and B. firmae inhabit conifers. By contrast, B. doui is present in both broad-leaved trees (Han et al., 2009) and conifers (Kanzaki et al., 2008) because vectors for this species, A. fraudatrix, A. sejuncta, and M. subfasciatus, use both. Monochamus saltuarius is also a vector but inhabits only coniferous species.

Kanzaki and Futai (2002a) proposed that the ancestral species of B. xylophilus group, which had originated in the Eurasian Continent, obtained the ability to use tree species of family Pinaceae instead of broad-leaved ones and expanded their distribution throughout the coniferous forests ranging widely in the ancient Eurasia-North America continent. Molecular phylogenetic analyses inferred from rRNA gene segments D2-D3 LSU in Figure 3 of Kanzaki et al. (2012) showed that nematodes in conifers evolved from nematodes in broad-leaved trees. The higher genetic diversity of B. mucronatus could be the result of an earlier origin in Eurasia, and B. xylophilus could have evolved recently from a B. mucronatus population in North America through geographical or reproductive isolation (Pereira et al., 2013). For this evolutionary process, cerambycid beetles must have transferred nematodes from broad-leaved trees to conifers. We hypothesized that "B. doui, or its ancestor, was transferred by A. fraudatrix, A. sejuncta, and/or M. subfasciatus (or ancestral species of these beetles) from broad-leaved trees to conifers, switched vectors from these beetles to Monochamus beetles, that is, M. saltuarius, in conifers, and later evolved into the common ancestor of B. mucronatus and B. xylophilus."

The life cycle of B. xylophilus is divided into propagative and dispersal phases. The fourth-stage dispersal juvenile (dauer juvenile; JIV) of B. xylophilus is vital in the nematode life cycle as the phoretic stage carried by beetles. Bursaphelenchus xylophilus JIV develops when late pupae and callow adults of B. xylophilus infect the tracheae of the beetles. Phoretic stages of B. luxuriosae (Ekino et al., 2017; Kanzaki et al., 2009) and B. conicaudatus (Kanzaki & Futai, 2001) enter the tracheae of the beetles. Phoretic stages of B. mucronatus (Mamiya & Enda, 1979), B. conicaudatus (Kanzaki & Futai, 2001), and B. firmae (Kanzaki et al., 2012) are also JIV. Phoretic stages of B. luxuriosae (Ekino et al., 2017; Kanzaki et al., 2009) and B. acaloleptae (Kanzaki et al., 2020) are the phoretic adults (PA) and B. doui (Kanzaki et al., 2013) both JIV and PA. JIV of B. conicaudatus and PA of B. luxuriosae are also induced by their vector beetles (Maehara et al., 2013). Moreover, JIV of B. xylophilus is induced not only by its primary vector M. alternatus Hope but also by nonvector P. hilaris, although the numbers and the percentages of JIV are markedly higher in the former than in the latter (Maehara & Futai, 2001). The third-stage dispersal juveniles (JIII) of B. xylophilus molt into JIV in response to long-chain C16 and C18 fatty acid ethyl esters that are secreted from the body surface of M. alternatus, specifically during adult eclosion (Zhao et al., 2013, 2014). Thus, JIV and PA are specific and essential to vector association.

In the present study, our objective was to test the above hypothesis and demonstrate the importance of vector switching of nematodes in the evolution of the B. xylophilus group. We used a simple nematode-loading method to cerambycid beetles (Maehara & Kanzaki, 2016), which could be used to examine the affinity of nematodes for not only their vectors but also nonvectors, and produced 20 binary combinations of five B. xylophilus group species and four cerambycid beetle species in the tribe Lamini. These nematode/beetle combinations were examined for the effects of the vector and nonvector beetles on the formation of the nematode phoretic stages, that is, JIV and PA.

2 | MATERIALS AND METHODS

2.1 | Beetle cultures

Japanese black pine trees, Pinus thunbergii Parl., infested with M. alternatus larvae were felled and cut at the Chiyoda Experimental Station of the Forestry and Forest Products Research Institute (FFPRI), Kasumigaura, Ibaraki, Japan in Spring 2009. Logs were placed in a screen cage at the FFPRI, Tsukuba, Ibaraki, Japan, and adults of M. alternatus that emerged from the logs in May to June 2009 were collected. Adults of A. luxuriosa were captured in May to July 2009 from Aralia elata (Miquel) Seemann trees in experimental fields at the FFPRI, Tsukuba and at the Tohoku Research Center, FFPR, Morioka, Iwate, Japan. Adults of P. hilaris were collected in May and June 2009 from a fig tree, Ficus carica L., planted in an experimental field at the FFPRI, Tsukuba. Monochamus alternatus, A. luxuriosa, and P. hilaris were allowed to oviposit on P. densiflora Sieb. and Zucc. logs cut about 1 week prior, fresh hand-rolled leaves of A. elata (Akutsu, 1985), and fresh hand-rolled leaves of Morus bombycis Koidzumi (Maehara et al., 2013), respectively. Eggs of M. alternatus were harvested from the logs by a chisel, and those of A. luxuriosa and P. hilaris were collected by opening the hand-rolled leaves. These eggs were put on wet filter paper with distilled water at 25°C in the dark until they hatched. Artificial diets were modified from the diet for M. alternatus proposed by Kosaka and Ogura (1990) and Kosaka and Enda (1991). Diet for M. alternatus was composed of 8 g of the current and 1-year-old needles of P. densiflora dried at 90°C for 1 day and milled into powder, 26.8 g of artificial silkworm diet (Silkmate 2M powder, Nosan Corporation, Kanagawa, Japan), 3.2 g of dried yeast (EBIOS, Asahi Group Foods, Ltd., Tokyo, Japan), and 62 ml of distilled water. For A. luxuriosa (Maehara et al., 2013), diet consisted of 8 g of leaves of A. elata dried at 70°C for 1 day and milled into powder, 26.8 g of Silkmate 2M powder, 3.2 g of dried yeast, and 62 ml of distilled water. For P. hilaris (Maehara & Kanzaki, 2016), diet contained 8 g of leaves of M. bombycis dried at 70°C for 1 day and milled into powder, 26.8 g of Silkmate 2M powder, 3.2 g of dried yeast, and 62 ml of distilled water. Approximately 20 g of each diet was placed into 50-ml Erlenmeyer flasks. Flasks were plugged with a silicone-rubber stopper (Silicosen, Shin-Etsu Polymer Co., Ltd.,...
Tokyo, Japan) and autoclaved at 121°C for 20 min. A hatched larva of *M. alternatus*, *A. luxuriosa*, or *P. hilaris* was placed into each flask. Larvae were reared at 25°C in the dark for 3–5 months. When mature, larvae were incubated at 10°C in the dark for 9 months. Larvae were subsequently removed from the flasks, rinsed in distilled water, dipped in 70% ethanol for 5 s, and then rinsed again in distilled water. The larvae for use in the first experiment were placed on wet filter paper with distilled water at 25°C in the dark until they pupated. Beetles for the second experiment were reared for one more generation in the same manner.

Mature larvae of *A. fraudatrix* were collected in April and May 2010 from *P. thunbergii* logs in Fukaura, Aomori, Japan and kept at 10°C in the dark in 15-ml centrifuge tubes with wet filter paper. Most of them pupated at 10°C. Remaining larvae pupated only after incubation at 25°C in the dark in the first experiment. For the second experiment, in summer 2010, some adults of *A. fraudatrix* reared from the larvae were allowed to oviposit on *Larix kaempferi* (Lamb.) Carrière logs that were cut about 1 month prior. After the frass of beetles was found on the logs, larvae were collected and placed into flasks with the artificial diet for *M. alternatus*. Larvae were reared at 25°C in the dark for 6–8 months and, when mature, were incubated at 10°C in the dark for 5–7 months. Larvae were subsequently treated in the same manner as larvae of *M. alternatus*, *A. luxuriosa*, and *P. hilaris* until they pupated.

### 2.2 Nematode cultures

Four species of *Bursaphelenchus* were used in the first experiment: a virulent isolate (T-4) of *B. xylophilus* isolated from a dead *P. densiflora* tree in Ichinoseki, Iwate, Japan in 1992 by T. Kiyohara (Aikawa et al., 2003); *B. luxuriosa* obtained from *A. luxuriosa* in Gose, Nara, Japan (Kanzaki & Futai, 2003a); *B. conicaudatus* isolated from *P. hilaris* in Kyoto, Kyoto, Japan (Kanzaki et al., 2000); and *B. doui* obtained from *M. subfasciatus* found at the Tama Forest Science Garden, FFPRI, Hachioji, Tokyo, Japan (Kanzaki et al., 2008). In the second experiment, two species of *Bursaphelenchus* were used: *B. xylophilus* (T-4) and *B. mucronatus* subsp. *kolymensis* (Braasch et al., 2011) obtained from *M. saltuarius* in Kyoto, Kyoto, Japan (Hosoda, 1973).

Nematodes were reared on *Botrytis cinerea* Pers. grown on autoclaved barley grains at 20°C in the dark for 9–16 days in the first experiment and at 25°C in the dark for 15 days in the second experiment, and were isolated aseptically from the culture using the Baermann funnel technique (Hooper, 1986). A nematode inoculum was prepared with 500 nematodes/30 μl suspension.

### 2.3 Loading beetles with nematodes on fungal plates

Mycelial disks (4 mm in diam.) of *Nectria viridescens* Booth, cut from fungal colonies growing on potato dextrose agar (Difco), were placed on malt extract agar (Difco) (5% agar) in 9 cm diam. Petri dishes. These dishes were incubated at 25°C in the dark for 20 days. A 30 μl nematode suspension (= 500 mixed-stage nematodes) was inoculated into each dish and incubated at 20°C in the dark for 11 days and subsequently at 25°C in the dark for 22 days in the first experiment, and at 25°C in the dark for 15–20 days in the second experiment. In both experiments, a final incubation at 10°C in the dark continued until larvae of cerambycid beetles pupated. After the pupation, one pupa was placed onto each dish (Figure 1). Control dishes received no pupae. Dishes were wrapped in Parafilm M® (Bemis Flexible Packaging, Wisconsin, USA) and incubated at 25°C in the dark.

The development of pupae was observed daily. Eight days after adult eclosion, adults of the beetles were removed from the dishes. After removal, each beetle was rinsed with distilled water, ground for 10 s using a blender in 40 ml of distilled water, and placed in a Baermann funnel overnight to extract the nematodes in the body. To determine the number of nematodes that were unable to enter beetle tracheae, rinse water from beetles and agar medium were placed in another Baermann funnel overnight. Harvested nematodes were then counted using a stereomicroscope, and $J_{hi}$, $J_{ip}$ PA, and all other developmental stages (propagative juveniles and adults) were recorded for each sample. When nematodes were too abundant to count, the suspension was diluted, and the numbers of nematodes were estimated. In the first experiment, we used 16 combinations of four nematode and four beetle species along with four controls.
with only nematodes. In the second experiment, eight combinations of two nematode and four beetle species were used along with two controls with only nematodes.

2.4 | Statistical analyses

All analyses were conducted using JMP® 11 (SAS Institute Inc., Cary, NC, USA). The total numbers of nematodes, J IV, and PA represent those carried internally by a beetle, and those on the surface of the beetle and remaining in the agar. Two-way analysis of variance (ANOVA) was used to analyse differences in the total numbers of nematodes, J IV, and J IV + PA; the numbers of J IV and J IV + PA carried by a beetle; and the percentages of total J IV and total J IV + PA to total nematodes among beetle treatments. For ANOVA, the numbers of nematodes were log_{10}-transformed, and the percentages of J IV and J IV + PA were arcsine transformed (Yonezawa et al., 1988).

3 | RESULTS

3.1 | Loading beetles with nematodes on fungal plates in the first experiment

Table 1 shows phoretic stage formation of four species of B. xylophilus group nematodes in four species of cerambycid beetles, and transfer of the nematodes to the beetles in the first experiment. The mean total nematode numbers in fungal plates with and without beetles were greater than 10,000, although the numbers varied widely among treatments and within each treatment (p < .0001 for nematodes, beetles, and nematodes × beetles interaction). Phoretic stages of B. conicaudatus and B. xylophilus were J IV, and the stage of B. luxuriosae was PA. Bursaphelenchus doui had both J IV and PA as its phoretic stages. More than 500 nematodes (J IV + PA) in the mean transferred to beetles: B. luxuriosae to both A. luxuriosa and A. fraudatrix; B. conicaudatus to P. hilaris; B. doui to A. luxuriosa, P. hilaris, and A. fraudatrix; and B. xylophilus to M. alternatus (p < .0001 for nematodes, beetles, and nematodes × beetles interaction). On the other hand, few numbers of B. luxuriosae and B. conicaudatus transferred to M. alternatus. The total numbers of J IV + PA (p < .0001 for nematodes, beetles, and nematodes × beetles interaction) and the percentages of total J IV + PA to total nematodes (p < .0001 for nematodes, beetles, and nematodes × beetles interaction) showed similar trends to the numbers of J IV + PA carried by a beetle among the plates with beetles. PA of B. luxuriosae, J IV of B. conicaudatus, and both PA and J IV of B. doui did not appear and all nematodes were propagative juveniles and adults in controls without beetles. In contrast, only a few J IV of B. xylophilus appeared in controls. Only in B. doui, the percentages of beetles carrying nematodes to total beetles were 100% for all four species of beetles, and those in the other nematodes were not always 100% for all beetles.

3.2 | Loading beetles with nematodes on fungal plates in the second experiment

Table 2 indicates phoretic stage formation data for B. m. kolymensis and B. xylophilus in four species of cerambycid beetles, and transfer of the nematodes to the beetles in the second experiment. Means of total nematode numbers in fungal plates with and without beetles were more than 10,000, although the numbers of B. xylophilus were higher than those of B. mucronatus (p = .0027 for nematodes, p = .8229 for beetles, and p = .0641 for nematodes × beetles interaction). Phoretic stages of B. m. kolymensis and B. xylophilus were J IV. Large numbers (mean > 1,000) of B. xylophilus transferred to M. alternatus, but such large numbers of B. m. kolymensis did not transfer to any beetle species (p < .0001 for nematodes, beetles, and nematodes × beetles interaction). The total numbers of J IV (p = .002 for nematodes, p < .0001 for beetles, and p = .0004 for nematodes × beetles interaction) and the percentages of total J IV to total nematodes (p = .0156 for nematodes and p < .0001 for beetles and nematodes × beetles interaction) indicated similar trends to the numbers of J IV carried by a beetle among the plates with beetles. J IV of B. m. kolymensis was induced in higher percentage by P. hilaris (4.4%) than by M. alternatus (1.79%). J IV of B. m. kolymensis did not appear and all nematodes were propagative juveniles and adults in controls without beetles. By contrast, only a few J IV of B. xylophilus appeared in controls. The percentages of beetles carrying nematodes to total beetles were 100% for P. hilaris in B. m. kolymensis and for P. hilaris and M. alternatus in B. xylophilus.

3.3 | Affinity of nematodes for beetles

The affinity of five species of B. xylophilus group nematodes for four species of cerambycid beetles was based on phoretic stage formation of the nematodes (= the percentage of total J IV + PA to total nematodes) in both the first and the second experiments (Table 3). The phoretic stages of B. luxuriosae and B. conicaudatus, PA and J IV, respectively, were induced by A. luxuriosa, P. hilaris, and A. fraudatrix; these stages were nearly absent in M. alternatus. PA and J IV of B. doui appeared in all species of beetles examined although the affinity of the nematode for M. alternatus was weak. J IV of B. m. kolymensis developed in the presence of every species of beetles used; however, the affinity was moderate or weak. In contrast, J IV of B. xylophilus appeared in high percentages, 20.1 ± 8.7 (mean ± SD) in the first experiment (Table 1) and 19.3 ± 13.1 in the second experiment (Table 2) in M. alternatus although the affinity for the other beetles was moderate or weak.

4 | DISCUSSION

To be carried by vector beetles, nematodes need to develop into the phoretic stages, because propagative juveniles and adults cannot transfer to beetles even if they are around pupal chambers of
| Treatment | No. of observations | Total no. of nematodes | No. of JIV carried by a beetle | No. of PA carried by a beetle | No. of JIV + PA carried by a beetle |
|-----------|---------------------|------------------------|--------------------------------|-----------------------------|-----------------------------------|
| B. luxuriosae + A. luxuriosa | 6 | 23,930 ± 9,150 | 0 ± 0 | 2,792 ± 2,363 | 2,792 ± 2,363 |
| B. luxuriosae + P. hilaris | 7 | 33,449 ± 21,263 | 0 ± 0 | 119 ± 152 | 119 ± 152 |
| B. luxuriosae + A. fraudatrix | 8 | 23,333 ± 10,888 | 0 ± 0 | 1,721 ± 1,712 | 1,721 ± 1,712 |
| B. luxuriosae + M. alternatus | 11 | 72,060 ± 13,960 | 0 ± 0 | 0.5 ± 0.5 | 0.5 ± 0.5 |
| B. luxuriosae | 10 | 86,980 ± 21,889 | – | – | – |
| B. conicaudatus + A. luxuriosa | 5 | 36,405 ± 25,171 | 23 ± 13 | 0 ± 0 | 23 ± 13 |
| B. conicaudatus + P. hilaris | 4 | 13,773 ± 13,580 | 513 ± 633 | 0 ± 0 | 513 ± 633 |
| B. conicaudatus + A. fraudatrix | 10 | 39,125 ± 21,266 | 46 ± 59 | 0 ± 0 | 46 ± 59 |
| B. conicaudatus + M. alternatus | 11 | 55,429 ± 18,223 | 0.09 ± 0.30 | 0 ± 0 | 0.09 ± 0.30 |
| B. conicaudatus | 10 | 103,840 ± 74,538 | – | – | – |
| B. doui + A. luxuriosa | 7 | 34,215 ± 12,166 | 2,159 ± 2,153 | 189 ± 281 | 2,348 ± 2,364 |
| B. doui + P. hilaris | 8 | 15,737 ± 7,752 | 2,649 ± 3,296 | 276 ± 368 | 2,925 ± 3,654 |
| B. doui + A. fraudatrix | 8 | 62,877 ± 26,080 | 3,244 ± 3,774 | 138 ± 215 | 3,381 ± 3,932 |
| B. doui + M. alternatus | 11 | 98,981 ± 11,920 | 3,193 ± 2,109 | 3 ± 3 | 112 ± 88 |
| B. doui | 11 | 105,156 ± 31,458 | – | – | – |
| B. xylophilus + A. luxuriosa | 5 | 29,593 ± 22,634 | 24 ± 35 | 0 ± 0 | 24 ± 35 |
| B. xylophilus + P. hilaris | 8 | 15,847 ± 11,040 | 51 ± 86 | 0 ± 0 | 51 ± 86 |
| B. xylophilus + A. fraudatrix | 9 | 13,443 ± 9,984 | 3 ± 5 | 0 ± 0 | 3 ± 5 |
| B. xylophilus + M. alternatus | 10 | 16,808 ± 3,778 | 1,161 ± 863 | 0 ± 0 | 1,161 ± 863 |
| B. xylophilus | 5 | 37,224 ± 9,282 | – | – | – |

| Treatment | No. of observations | Total no. of JIV | Total no. of PA | Total no. of JIV + PA | % total JIV + PA to total nematodes | % beetles carrying nematodes to total beetles |
|-----------|---------------------|-----------------|-----------------|---------------------|-----------------------------------|---------------------------------------------|
| B. luxuriosae + A. luxuriosa | 6 | 0 ± 0 | 3,625 ± 2,246 | 3,625 ± 2,246 | 16.1 ± 10.5 | 100.0 |
| B. luxuriosae + P. hilaris | 7 | 0 ± 0 | 738 ± 806 | 738 ± 806 | 2.3 ± 1.7 | 100.0 |
| B. luxuriosae + A. fraudatrix | 8 | 0 ± 0 | 3,193 ± 2,109 | 3,193 ± 2,109 | 14.6 ± 8.6 | 100.0 |
| B. luxuriosae + M. alternatus | 11 | 0 ± 0 | 2 ± 6 | 2 ± 6 | 0.03 ± 0.009 | 45.5 |
| B. luxuriosae | 10 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | – |
| B. conicaudatus + A. luxuriosa | 5 | 543 ± 617 | 0 ± 0 | 543 ± 617 | 3.1 ± 4.5 | 100.0 |
| B. conicaudatus + P. hilaris | 4 | 2,363 ± 1,028 | 0 ± 0 | 2,363 ± 1,028 | 29.1 ± 23.0 | 100.0 |
the beetles. Therefore, the affinity between nematodes and beetles can be examined by the induction of the phoretic stages in the presence of the beetles. Our simple nematode-loading method to beetles (Maehara & Kanzaki, 2016) can be used to examine not only the nematodes’ affinity for the vector beetles but also the potential affinity for the nonvectors which do not meet the nematodes in the field. Based on the potential affinity, we discussed vector switching of nematodes during the evolutionary history of the B. xylophilus group.

The main reason why the mean total numbers of nematodes in the experimental units with and without four species of beetles varied widely among treatments and within each treatment in the first experiment (Table 1) was that the days when the larvae of each beetle species pupated were varied. The nematode populations could have decreased in some dishes where the larvae pupated late. Some beetles were considered to have fed on agar media together with nematodes and to have killed a number of nematodes in the first experiment. Therefore, there were lower total numbers of nematodes in the units with beetles than in the corresponding control units without beetles (Table 1).

More than 500 nematodes in the mean numbers of B. luxuriosae, B. conicaudatus, B. doui, and B. xylophilus transferred to their vector beetles, that is, A. luxuriosa, P. hilaris, A. fraudatrix (Table 1), and M. alternatus (Tables 1 and 2). In contrast, small numbers of B. m. kolymensis transfer to M. alternatus (Table 2), because the natural vectors of B. m. kolymensis are not M. alternatus but M. saltuarius (Jikumaru & Togashi, 1995), M. nitens (Bates) (Kanzaki & Akiba, 2014), and M. rosenmuelleri (Cederhjelm) = M. urussovi (Fischer) (Togashi et al., 2008), although the primary vector beetle for B. m. mucronatus is M. alternatus (Mamiya & Enda, 1979). Several studies reported that JIV of B. xylophilus was induced by its vector beetles, M. alternatus (Maehara & Futai, 1996, 2001; Ogura & Nakashima, 2002) and M. carolinensis (Necibi & Linit, 1998). JIV of B. xylophilus molt into JIV in response to long-chain C16 and C18 fatty acid ethyl esters that are secreted from the body surface of M. alternatus specifically during adult eclosion (Zhao et al., 2013, 2014). JIV of B. conicaudatus and PA of B. luxuriosae are also induced by their vectors, P. hilaris and A. luxuriosae, respectively (Maehara et al., 2013). Moreover, JIV of B. xylophilus is induced not only by its vector M. alternatus but also by nonvector P. hilaris that inhabits not conifers but broad-leaved trees, although the numbers and the percentages of JIV are higher in the former species (Maehara & Futai, 2001). In the present study, PA of B. luxuriosae and both JIV and PA of B. doui were equally induced by nonvectors, that is, A. fraudatrix, and both A. luxuriosae and P. hilaris, respectively (Table 1). In the other combinations of five nematode and four nonvector beetle species, the phoretic stages appeared to some extent with the exception of B. luxuriosae and B. conicaudatus in M. alternatus (Tables 1 and 2). Few PA and JIV were recovered from these two combinations. Chemical signals were not identified for induction of the phoretic stages by vectors and nonvectors, except for B. xylophilus JIV induction by M. alternatus described above (Zhao et al., 2013, 2014). PA of B. luxuriosae, JIV of B. conicaudatus and B. m. kolymensis, and both PA and JIV of B. doui did not appear in controls without beetles, while only a few JIV of B. xylophilus appeared in controls (Tables 1 and 2). Maehara et al. (2018) also reported appearance of a few JIV of B. xylophilus

| Treatment                        | No. of observations | Total no. of JIV | Total no. of PA | Total no. of JIV + PA | % total JIV + PA to total nematodes | % beetles carrying nematodes to total beetles |
|----------------------------------|---------------------|-----------------|-----------------|----------------------|-----------------------------------|---------------------------------------------|
| B. conicaudatus + A. fraudatrix  | 10                  | 151 ± 154       | 0 ± 0           | 151 ± 154            | 0.36 ± 0.35                       | 80.0                                        |
| B. conicaudatus + M. alternatus  | 11                  | 0.09 ± 0.30     | 0 ± 0           | 0.09 ± 0.30          | 0.0002 ± 0.0007                  | 9.1                                         |
| B. doui + A. luxuriosa           | 7                   | 3,842 ± 2,322   | 480 ± 258       | 4,322 ± 2,439        | 13.0 ± 6.3                       | 100.0                                      |
| B. doui + P. hilaris             | 8                   | 4,750 ± 3,847   | 647 ± 493       | 5,397 ± 4,235        | 30.8 ± 15.1                      | 100.0                                      |
| B. doui + A. fraudatrix          | 8                   | 4,489 ± 4,570   | 220 ± 292       | 4,709 ± 4,750        | 11.1 ± 15.6                      | 100.0                                      |
| B. doui + M. alternatus          | 11                  | 640 ± 436       | 3 ± 3           | 643 ± 437            | 0.67 ± 0.49                      | 100.0                                      |
| B. xylophilus + A. luxuriosa     | 5                   | 108 ± 161       | 0 ± 0           | 108 ± 161            | 2.5 ± 5.3                        | 100.0                                      |
| B. xylophilus + P. hilaris       | 8                   | 408 ± 272       | 0 ± 0           | 408 ± 272            | 4.3 ± 6.0                        | 100.0                                      |
| B. xylophilus + A. fraudatrix    | 9                   | 39 ± 52         | 0 ± 0           | 39 ± 52              | 0.80 ± 1.32                      | 55.6                                       |
| B. xylophilus + M. alternatus    | 10                  | 3,241 ± 1,416   | 0 ± 0           | 3,241 ± 1,416        | 20.1 ± 8.7                       | 100.0                                      |
| B. xylophilus                    | 5                   | 24 ± 43         | 0 ± 0           | 24 ± 43              | 0.07 ± 0.11                      | -                                          |

Note: Values are means ± SD. JIV and PA represent the fourth-stage dispersal juveniles and the phoretic adults, respectively. Underlines indicate the combinations which occur under natural conditions.
TABLE 2 Effects of four cerambycid beetle species in the tribe Lamiini on phoretic stage formation of Bursaphelenchus mucronatus kolymensis and B. xylophilus, and transfer of the nematodes to the beetles in the second experiment

| Treatment | No. of observations | Total no. of nematodes | No. of J IV carried by a beetle | Total no. of J IV | % total J IV to total nematodes | % beetles carrying nematodes to total beetles |
|-----------|---------------------|------------------------|--------------------------------|------------------|-------------------------------|-----------------------------------------------|
| B. m. kolymensis + A. luxuriosa | 3 | 29,726 ± 31,354 | 2 ± 4 | 169 ± 212 | 0.67 ± 1.03 | 33.3 |
| B. m. kolymensis + P. hilaris | 9 | 16,753 ± 12,777 | 113 ± 133 | 724 ± 671 | 4.4 ± 2.7 | 100.0 |
| B. m. kolymensis + A. fraudatrix | 6 | 22,795 ± 6,179 | 4 ± 5 | 204 ± 168 | 0.95 ± 0.75 | 66.7 |
| B. m. kolymensis + M. alternatus | 13 | 17,804 ± 6,842 | 11 ± 32 | 322 ± 271 | 1.79 ± 1.50 | 69.2 |
| B. m. kolymensis | 10 | 11,140 ± 4,652 | – | 0 ± 0 | 0 ± 0 | – |
| B. xylophilus + A. luxuriosa | 2 | 35,629 ± 35,208 | 13 ± 18 | 125 ± 141 | 1.1 ± 1.5 | 50.0 |
| B. xylophilus + P. hilaris | 10 | 31,044 ± 18,219 | 64 ± 78 | 419 ± 543 | 2.0 ± 2.7 | 100.0 |
| B. xylophilus + A. fraudatrix | 6 | 27,185 ± 12,883 | 36 ± 48 | 344 ± 276 | 1.2 ± 1.0 | 83.3 |
| B. xylophilus + M. alternatus | 14 | 22,788 ± 11,133 | 1,945 ± 1,631 | 3,559 ± 2,233 | 19.3 ± 13.1 | 100.0 |
| B. xylophilus | 9 | 38,507 ± 14,455 | – | 40 ± 37 | 0.11 ± 0.10 | – |

Note: Values are means ± SD. J IV represents the fourth-stage dispersal juveniles. An underline indicates the combination which occurs under natural conditions.

TABLE 3 Affinity of five species of Bursaphelenchus xylophilus group nematodes for four cerambycid beetle species in the tribe Lamini based on the phoretic stage formation of the nematodes in the first and the second experiments

|                  | A. luxuriosa | P. hilaris | A. fraudatrix | M. alternatus |
|------------------|--------------|------------|---------------|---------------|
| B. luxuriosae    | +++          | ++         | +++           | ±             |
| B. conicaudatus  | ++           | +++        | +             |               |
| B. doui          | +++          | +++        | +++           | +             |
| B. mucronatus    | +            | ++         | + or ++       | ++            |
| kolymensis       | +            | ++         | + or ++       | +++           |

Note: +++ (strong), the percentage of total J IV or PA to total nematodes was more than 10%; ++ (moderate), 1%-10%; + (weak), 0.1%-1%; ± (almost no), less than 0.1%.

without beetles. Factors involved in the appearance of J IV are not known.

The evolution of the B. xylophilus group nematodes from broad-leaved tree species to species in conifers is indicated by molecular phylogenetic analyses in Figure 3 of Kanzaki et al. (2012). This evolution required cerambycid beetles to transfer nematodes from broad-leaved trees to conifers. Our hypothesis was “B. doui (or its ancestor) was transferred by A. fraudatrix, A. sejuncta, and/or M. subfasciatus (or their ancestral species) from broad-leaved trees to conifers, switched vectors from these beetles to Monochamus beetles, e.g., M. saltuarius, in conifers, and then evolved into the common ancestor of B. mucronatus and B. xylophilus.” We selected A. fraudatrix in the present study because the larvae of this beetle are often found in dead pine trees. PA of B. luxuriosae and J IV of B. conicaudatus were induced by A. luxuriosae, P. hilaris, and A. fraudatrix, but their stages were almost absent in M. alternatus (Table 3). This finding indicates that B. luxuriosae and B. conicaudatus cannot switch their vectors to Monochamus beetles. In contrast, PA and J IV of B. doui appeared with all four species of beetles examined, although its affinity for M. alternatus was weak (Table 3). Moreover, only in B. doui, the percentages of beetles carrying nematodes to total beetles were 100% for all four species of beetles. Therefore, B. doui (or its ancestor) could switch vectors from A. fraudatrix (or its ancestor) to conifer-using Monochamus beetles, e.g., M. saltuarius, after transfer by A. fraudatrix from broad-leaved trees to conifers. This idea receives support by the observation that M. saltuarius is an actual vector of B. doui (Aikawa et al., 2020). The affinity of B. m. kolymensis and B. xylophilus for M. alternatus was stronger than that of B. doui for the beetle species (Table 3). Moreover, J IV of the former two species also developed in A. luxuriosae, P. hilaris, and A. fraudatrix, but their affinity for these beetle species was weaker than that of B. doui. This observation may reflect vestigial characters from the evolutionary
process. These results support our hypothesis that the common ancestor of *B. m. kolymensis* and *B. xylophilus* evolved from *B. doui* (or its ancestor) that switched vectors to *Monochamus* beetles and completed its life cycle in conifers. In addition, *B. m. kolymensis* was induced in higher percentage by *P. hilaris* than by *M. alternatus*, although these percentages were not so high because both beetles are nonvectors of this nematode (Table 2).

Toki and Kubota (2010) determined the molecular phylogeny of cerambycid beetles in the tribe Lamini (25 species and 3 additional subspecies in 12 genera) in Japan based on mitochondrial 16S rRNA and cytochrome oxidase subunit I. Kanzaki et al. (2012) developed the molecular phylogenetic analyses of the *B. xylophilus* group inferred from rRNA gene segments D2-D3 LSU. In addition, Kanzaki and Futai (2002b, 2003b) reported cospeciation between *B. conicaudatus* and its vector beetle, *P. hilaris*. However, relationships between *B. doui* and its four species of vector beetles, *A. fraudatrix*, *A. sejuncta*, *M. subfasciatus*, and *M. saltuarius*, cannot be explained by cospeciation between the nematode and the vectors. We can understand relationships between *B. doui* and its vectors when we consider vector switching of the nematode species based on the wide-range of affinity of the nematode for cerambycid beetles.

The four species of beetles used in the present study inhabit East Asia, including Japan (Iwata, 1992; Makihara, 1992; Ohbayashi, 1992). Before the Japanese archipelago was separated from the Eurasian Continent in the Miocene (about 20 million years ago) (Santos & Senshu, 2011), the evolution of *Bursaphelenchus* nematodes through vector switching could have occurred in this continent. Vector switching of *B. xylophilus* actually occurred from *Monochamus* beetles in North America to *M. alternatus* in Japan, and then to *M. galloprovincialis* (Olivier) in Portugal (Abkulsul & Stamps, 2012; Ryss et al., 2011). We conclude that vector switching of nematodes could have potentially happened during the evolutionary history of the *B. xylophilus* group.

ACKNOWLEDGMENTS
We sincerely thank Ms. S. Matsuzawa and Ms. N. Kawamura, Tohoku Research Center, FFPRI, for their assistance in rearing beetles and collecting references. This work was supported in part by Grants-in-Aid for Scientific Research (B) (No. 23380092 and JP20H03038) and Scientific Research (C) (No. JP17K07860) from the Japan Society for the Promotion of Science.

CONFLICT OF INTEREST
None declared.

AUTHOR CONTRIBUTIONS
Noritoshi Maehara: Conceptualization (lead); data curation (lead); formal analysis (lead); funding acquisition (lead); investigation (lead); methodology (lead); project administration (lead); resources (equal); writing-original draft (lead); writing-review & editing (equal). Natsumi Kanzaki: Conceptualization (supporting); data curation (supporting); funding acquisition (supporting); investigation (supporting); methodology (supporting); project administration (supporting); resources (equal); writing-review & editing (equal). Takuya Aikawa: Funding acquisition (supporting); investigation (supporting); methodology (supporting); project administration (supporting); resources (equal); writing-review & editing (equal). Katsunori Nakamura: Funding acquisition (supporting); investigation (supporting); methodology (supporting); project administration (supporting); resources (equal); writing-review & editing (equal).

DATA AVAILABILITY STATEMENT
The data used in this paper are deposited in Dryad (https://doi.org/10.5061/dryad.5qfttdz3g).

ORCID
Noritoshi Maehara https://orcid.org/0000-0003-4689-3038
Natsumi Kanzaki https://orcid.org/0000-0001-8752-1674

REFERENCES
Aikawa, T., Kikuchi, T., & Kosaka, H. (2003). Demonstration of interbreeding between virulent and avirulent populations of *Bursaphelenchus xylophilus* (Nematoda: *Aphelenchoidei*) by PCR-RFLP method. *Applied Entomology and Zoology, 3*, 565–569. https://doi.org/10.1303/Aez.2003.565
Aikawa, T., Ozawa, S., Maehara, N., Masuya, H., Nakamura, K., & Kanzaki, N. (2020). Discovery of a phoretic association between *Bursaphelenchus doui* (Nematoda: *Aphelenchoidei*) and *Monochamus saltuarius* and *Acalolepta sejuncta* (Coleoptera: Cerambycidae). *Nematology*, 22, 713–722. https://doi.org/10.1163/156585411X685411
Abkulsul, S., & Stamps, W. T. (2012). Insect vectors of the pine-wood nematode: A review of the biology and ecology of *Monochamus* species. *Forest Pathology, 42*, 89–99. https://doi.org/10.1111/j.1439-0329.2011.00733.x
Akutsu, K. (1985). Studies on biology and control of udo longicorn beetle (*Acalolepta luxuriosa* Bates). *Bulletin of the Tokyo-to Agricultural Experiment Station, 18*, 1–72. (In Japanese with English abstract).
Braasch, H., Burgermeister, W., & Gu, J. (2009). Revised intra-generic grouping of *Bursaphelenchus* Fuchs, 1937 (Nematoda: *Aphelenchoidei*). *Journal of Nematode Morphology and Systematics*, 12, 65–88.
Braasch, H., Gu, J., & Burgermeister, W. (2011). *Bursaphelenchus mucronatus* kolymensis comb. n. – new definition of the “European type” of *B. mucronatus*. *Journal of Nematode Morphology and Systematics*, 14, 77–90.
Ekino, T., Yoshiga, T., Takeuchi-Kaneko, Y., & Kanzaki, N. (2017). Transmission electron microscopic observation of body cuticle structures of phoretic and parasitic stages of Parasitaphelenchinae nematodes. *PLoS One, 12*(6), e0179465. https://doi.org/10.1371/journal.pone.0179465
Han, H., Chung, Y.-J., & Shin, S.-C. (2009). First report of *Bursaphelenchus doui* on tulip tree (*Liriodendron tulipifera*) in Korea. *Plant Disease, 93*, 1221. https://doi.org/10.1094/PDIS-93-11-1221C
Hooper, D. J. (1986). Extraction of nematodes from plant material. In J. F. Southey (Ed.), *Laboratory methods for work with plant and soil nematodes* (pp. 51–58). Her Majesty’s Stationery Office.
Hosoda, R. (1973). Comparison between *Bursaphelenchus xylophilus* and the closely related species, *Bursaphelenchus* sp. No. 5. *Transactions of Annual Meeting of Kansai Branch of the Japanese Forestry Society*, 24, 177–180. (In Japanese).
Iwata, R. (1992). Genus *Monochamus* Guérin-Méneville. In N. Ohbayashi, M. Sato, & K. Kojima (Eds.), *An illustrated guide to identification of longicorn beetles of Japan* (pp. 579–583). Tokai University Press (In Japanese).
Kanzaki, N., & Futai, K. (2002a). A PCR primer set for determination of *Bursaphelenchus acaloleptae* n. sp. sharing tree and beetle carrier hosts with *Bursaphelenchus xylophilus* (Nematoda: Aphelenchoididae). *Nematology*, 3, 473–479. https://doi.org/10.1163/15685410175325089

Kanzaki, N., & Futai, K. (2001). Life history of *Bursaphelenchus conicaudatus* (Nematoda: Apehelenchoidae) in relation to the yellow-spotted longicorn beetle, *Psafeothela hilaris* (Coleoptera: Cerambycidae). *Nematology*, 3, 473–479. https://doi.org/10.1163/15685410175325089

Kanzaki, N., & Futai, K. (2002b). Phylogenetic analysis of the phoretic association between *Bursaphelenchus conicaudatus* (Nematoda: Apehelenchoidae) and *Psacothea hilaris* (Coleoptera: Cerambycidae). *Nematology*, 4, 759–771. https://doi.org/10.1163/15685410276039566

Kanzaki, N., & Futai, K. (2003a). Description and phylogeny of *Bursaphelenchus luxuriosus* n. sp. (Nematoda: Apehelenchoidae) isolated from *Acaloleta luxuriosa* (Coleoptera: Cerambycidae). *Nematology*, 5, 565–572. https://doi.org/10.1163/15685410332268328

Kanzaki, N., & Futai, K. (2003b). Application of molecular phylogenetic analysis to the evolution and co-speciation of entomophilic nematodes. *Russian Journal of Nematology*, 11, 107–117.

Kanzaki, N., Maehara, N., Aikawa, T., & Matsumoto, K. (2012). *Bursaphelenchus firmae* n. sp. (Nematoda: Apehelenchoidae), isolated from *Monochamus grandis* Waterhouse that emerged from dead firs, *Abies firma* Sieb. et Zucc. *Nematology*, 14, 395–404. https://doi.org/10.1163/156854111X602974

Kanzaki, N., Maehara, N., Aikawa, T., & Nakamura, K. (2013). An entomoparasitic adult form in *Bursaphelenchus doui* (Nematoda: Tylenchomorpha) associated with *Acaloleta fraudatrix*. *Journal of Parasitology*, 99, 803–815. https://doi.org/10.1645/GE-3253.1

Kanzaki, N., Tsuda, K., & Futai, K. (2000). Description of *Bursaphelenchus conicaudatus* n. sp. (Nematoda: Apehelenchoidae), isolated from the yellow-spotted longicorn beetle, *Psacothea hilaris* (Coleoptera: Cerambycidae) and fig trees, *Ficus carica*. *Nematology*, 2, 165–168. https://doi.org/10.1163/156854100509051

Kiyohara, T., & Tokushige, Y. (1971). Inoculation experiments of a nematode, *Bursaphelenchus sp.*, onto pine trees. *Journal of the Japanese Forestry Society*, 53, 210–218. https://doi.org/10.11159/jdfs1953.53.7.210 (In Japanese with English abstract).

Kosaka, H., & Enda, N. (1991). Simple rearing method of larvae of the Japanese pine sawyer, *Monochamus alternatus* (Coleoptera: Cerambycidae), on artificial diets. *Forest Pests*, 40, 183–187 (In Japanese).

Kosaka, H., & Ogura, N. (1990). Rearing of the Japanese pine sawyer, *Monochamus alternatus* (Coleoptera: Cerambycidae), on artificial diets. *Applied Entomology and Zoology*, 25, 532–534. https://doi.org/10.1303/aez.25.532

Linit, M. J. (1988). Nematode-vector relationships in the pine wilt disease system. *Journal of Nematology*, 20, 227–235.

Maehara, N., & Futai, K. (1996). Factors affecting both the numbers of the pinewood nematode, *Bursaphelenchus xylophilus* (Nematoda: Apehelenchoidae), carried by the Japanese pine sawyer, *Monochamus alternatus* (Coleoptera: Cerambycidae), and the nematode’s life history. *Applied Entomology and Zoology*, 31, 443–452. https://doi.org/10.1303/aez.31.443

Maehara, N., & Futai, K. (2001). Presence of the cerambycid beetles *Psacothea hilaris* and *Monochamus alternatus* affecting the life cycle strategy of *Bursaphelenchus xylophilus*. *Nematology*, 3, 455–461. https://doi.org/10.1163/15685410175325078

Maehara, N., & Kanzaki, N. (2016). Transfer of *Monochamus okinawaensis* (Nematoda: Apehelenchoidae) associated with *Monochamus maruokai* (Coleoptera: Cerambycidae) into *M. alternatus* (Coleoptera: Cerambycidae) and *Psacothea hilaris* (Coleoptera: Cerambycidae). *Nematology*, 18, 679–685. https://doi.org/10.1163/156854111-00002983

Maehara, N., Kanzaki, N., Aikawa, T., & Nakamura, K. (2013). Effects of two species of cerambycid beetles, tribe Lamini (Coleoptera: Cerambycidae), on the phoretic stage formation of two species of nematodes, genus *Bursaphelenchus* (Nematoda: Apehelenchoidae). *Nematological Research*, 43, 9–13. https://doi.org/10.3725/jnr.43.9

Maehara, N., Kanzaki, N., Aikawa, T., & Nakamura, K. (2018). Effect of *Monochamus grandis* (Coleoptera: Cerambycidae) on phoretic stage formation of *Bursaphelenchus xylophilus* (Nematoda: Apehelenchoidae) and the transfer of nematodes to the beetle. *Nematology*, 20, 43–48. https://doi.org/10.1163/156854111-00003123

Makihara, H. (1992). Genus *Acaloleta* Pascoe, 1858. In N. Ohbayashi, M. Sato, & K. Kojima (Eds.), *An illustrated guide to identification of longicorn beetles of Japan* (pp. 584–591). Tokai University Press (In Japanese).

Mamiya, Y., & Enda, N. (1972). Transmission of *Bursaphelenchus lignatus* (Nematoda: Apehelenchoidae) by *Monochamus alternatus* (Coleoptera: Cerambycidae). *Nematologica*, 18, 159–162. https://doi.org/10.1163/187529272X00395

Mamiya, Y., & Enda, N. (1979). *Bursaphelenchus mucronatus* n. sp. (Nematoda: Apehelenchoidae) from pine wood and its biology and pathogenicity to pine trees. *Nematologica*, 25, 353–361. https://doi.org/10.1163/187529279X00091

Morimoto, K., & Iwasaki, A. (1972). Role of *Monochamus alternatus* (Coleoptera: Cerambycidae) as a vector of *Bursaphelenchus lignatus* (Nematoda: Apehelenchoidae). *Journal of the Japanese Forestry Society*, 54, 177–183. https://doi.org/10.11159/jdfs1953.54.6.177 (In Japanese with English abstract)

Morimoto, K., & Iwasaki, A. (1973). Studies on the pine sawyer (IV). Biology of the pine sawyer and the pine wood nematode in the pupal cell. *Transactions of Annual Meeting of Kyushu Branch of the Japanese Forestry Society*, 26, 199–200. (In Japanese).

Necibi, S., & Linit, M. J. (1998). Effect of *Monochamus carolinensis* on *Bursaphelenchus xylophilus* dispersal stage formation. *Journal of Nematology*, 30, 246–254.

Ogura, N., & Nakashima, T. (2002). In vitro occurrence of dispersal fourth stage juveniles in *Bursaphelenchus xylophilus* co-incubated with *Monochamus alternatus*. *Japanese Journal of Nematology*, 32, 53–59. https://doi.org/10.3725/jjn.42.9
Ohbayashi, N. (1992). Genus Psacothea Gahan, 1888. In N. Ohbayashi, M. Sato, & K. Kojima (Eds.), An illustrated guide to identification of longicorn beetles of Japan (pp. 593–594). Tokai University Press (In Japanese).

Penas, A. C., Bravo, M. A., Naves, P., Bonifácio, L., Sousa, E., & Mota, M. (2006). Species of Bursaphelenchus Fuchs, 1937 (Nematoda: Parasitaphelenchidae) and other nematode genera associated with insects from Pinus pinaster in Portugal. *Annals of Applied Biology*, 148, 121–131. https://doi.org/10.1111/j.1744-7348.2006.00042.x

Pereira, F., Moreira, C., Fonseca, L., van Asch, B., Mota, M., Abrantes, I., & Amorim, A. (2013). New insights into the phylogeny and world wide dispersion of two closely related nematode species, *Bursaphelenchus xylophilus* and *Bursaphelenchus mucronatus*. *PLoS One*, 8(2), e56288. https://doi.org/10.1371/journal.pone.0056288

Ryss, A. Y., Kulinich, O. A., & Sutherland, J. R. (2011). Pine wilt disease: A short review of worldwide research. *Forestry Studies in China*, 13, 132–138. https://doi.org/10.1007/s11632-011-0205-8

Santosh, M., & Senshu, H. (2011). History of supercontinents and its relation to the origin of Japanese islands. *Journal of Geography*, 120, 100–114. https://doi.org/10.5026/jgeography.120.100 (In Japanese with English abstract)

Sousa, E., Bravo, M. A., Pires, J., Naves, P., Penas, A. C., Bonifácio, L., & Mota, M. M. (2001). *Bursaphelenchus xylophilus* (Nematoda: Aphelenchoidea) associated with *Monochamus galeraprovincialis* (Coleoptera; Cerambycidae) in Portugal. *Nematology*, 3, 89–91. https://doi.org/10.1163/156854101300106937

Sousa, E., Naves, P., Bonifácio, L., Bravo, M. A., Penas, A. C., Pires, J., & Serrão, M. (2002). Preliminary survey for insects associated with *Bursaphelenchus xylophilus* in Portugal. *Bulletin OEPP/EPPO Bulletin*, 32, 499–502. https://doi.org/10.1046/j.1365-2338.2002.00597.x

Togashi, K., Taga, Y., Iguchi, K., & Aikawa, T. (2008). *Bursaphelenchus mucronatus* (Nematoda: Aphelenchoidea) vectored by *Monochamus urussovi* (Coleoptera: Cerambycidae) in Hokkaido, Japan. *Journal of Forest Research*, 13, 127–131. https://doi.org/10.1007/s10310-007-0057-1

Toki, W., & Kubota, K. (2010). Molecular phylogeny based on mitochondrial genes and evolution of host plant use in the long-horned beetle tribe Lamiini (Coleoptera: Cerambycidae) in Japan. *Environmental Entomology*, 39, 1336–1343. https://doi.org/10.1603/EN09347

Tomminen, J. (1990). Presence of *Bursaphelenchus mucronatus* (Nematoda: Aphelenchoidea) fourth dispersal stages in selected conifer beetles in Finland. *Silva Fennica*, 24, 273–278. https://doi.org/10.14214/sf.a15582

Yonezawa, K., Sasaki, Y., Imanishi, S., & Fujii, K. (1988). Biometry (212 pp). Asakura Press. (In Japanese).

Zhao, L., Mota, M., Vieira, P., Butcher, R. A., & Sun, J. (2014). Interspecific communication between pinewood nematode, its insect vector, and associated microbes. *Trends in Parasitology*, 30, 299–308. https://doi.org/10.1016/j.pt.2014.04.007

Zhao, L., Zhang, S., Wei, W., Hao, H., Zhang, B., Butcher, R. A., & Sun, J. (2013). Chemical signals synchronize the life cycles of plant-parasitic nematode and its vector beetle. *Current Biology*, 23, 2038–2043. https://doi.org/10.1016/j.cub.2013.08.041

---

**How to cite this article:** Maehara N, Kanzaki N, Aikawa T, Nakamura K. Potential vector switching in the evolution of *Bursaphelenchus xylophilus* group nematodes (Nematoda: Aphelenchoidea). *Ecol Evol*. 2020;10:14320–14329. https://doi.org/10.1002/ece3.7033