

Application of molecular phylogenetic analysis to the evolution and co-speciation of entomophilic nematodes

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Summary. Molecular phylogenetic analysis was applied to two evolutionary aspects of the genus *Bursaphelenchus*. For the first, the morphological and biological features of the six species of the genus *Bursaphelenchus* were compared with their molecular phylogenetic relationship, and then the evolutionary sequences of these features were determined. For the second, phylogenetic correspondence between *B. conicaudatus* and its phoretic vector, *Psacotheta hilaris*, was analyzed molecular biologically, and co-speciation between them was affirmed. The molecular analysis also suggested that the paleogeographical events must be determinant of the co-speciation. The present results ascertained 1) the effectiveness of the molecular phylogenetic analysis in the evolutionary studies of nematodes, and 2) the validity of *Bursaphelenchus* species in the *xylophilus* group as the model organisms of entomophilic nematodes in the study of the molecular co-speciation.

Key words: biological features, co-speciation, evolution, molecular phylogeny, morphology, *xylophilus* group.

Most plant parasitic and free living nematodes are very tiny and so are hardly identified morphologically without microscopes. On the other hand, molecular analytical identification is applicable to even individual nematode, and has become convenient with the aid of commercially available kits. Molecular methods have been applied also to phylogenetic analysis in many biological taxa, including nematodes. Thus, the molecular methods will be used in various aspects of nematology now on.

One of the most prospective fields for molecular phylogenetic method is that of the evolutionary ecology. In this paper, we would like to introduce our studies on the morphological and ecological evolution of *Bursaphelenchus* species in the *xylophilus* group and their co-speciation with its vector insect.

For the reasons of plant quarantine, the *Bursaphelenchus* species of the *xylophilus* group are ones of the most important nematodes. Further, the *xylophilus* group could be a good model for the study on morphological and ecological evolution of entomophilic nematodes and that on co-speciation of entomophilic nematode with its phoretic

host, because of following reasons. 1) A lot of information on the morphological and ecological features have been accumulated for the *xylophilus* group species due to its importance in plant quarantine. 2) Whenever needed, we can get a good number of nematodes for the *xylophilus* group because these species can be easily reared on various fungi. 3) Their vector beetles, cerambycid ones, can be reared on the artificial diets, thus the nematode-vector relationship can be analyzed experimentally.

Evolution of the *xylophilus* group

Six species have been placed in the *xylophilus* group so far (Giblin & Kaya, 1983; Kanzaki & Futai, 2002a; 2003), namely, *B. conicaudatus* Kanzaki, Tsuda & Futai, 2000, *B. fraudulentus* Rühm, 1956 (Goodey, 1960), *B. kolyomensis* Korenchenko, 1980, *B. luxuriosae* Kanzaki & Futai 2003, *B. mucronatus* Mamiya & Enda, 1979 and *B. xylophilus* (Steiner & Buhner, 1934) Nickle, 1970. Within these six species, *B. kolyomensis* is assumed to be the European morphotype of *B. mucronatus* (Magnusson & Kulinich, 1996).

These six species are closely related to each other, however, are distinguishable with its morphology and biological features. Comparing the morphology and the biological features of these six species with their molecular phylogenetic relationship, the evolutionary sequences of morphological and biological features can be assumed.

Morphological and biological features. These six species can be distinguished by the body shape and shape of female tail (Table 1). In the body shape, males and females of *B. conicaudatus*, *B. fraudulentus*, and *B. luxuriosae* have stout body, *i.e.*, small *a* value (*ca.* 30), while *B. kolymensis*, *B. mucronatus*, and *B. xylophilus* have slender body, *i.e.*, large *a* value (*ca.* 40) (Table 1). As for the shapes of tail, females of *B. luxuriosae* have tapered tail with irregular end and rough dorsal surface (Kanzaki & Futai, 2003), those of *B. conicaudatus* have tapered and mucronated tail (Kanzaki *et al.*, 2000), those of *B. fraudulentus*, *B. kolymensis*, and *B. mucronatus* have broad and mucronated tail (Rühm, 1956; Korentchenko, 1980; Mamiya & Enda, 1979), and those of *B. xylophilus* have broad and rounded tail (Steiner & Buhrer, 1934) (Fig. 1).

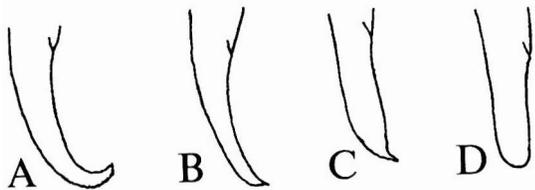


Fig. 1. Morphologies of *Bursaphelenchus* female tail in the *xylophilus* group: A: *B. luxuriosae*; B: *B. conicaudatus*; C: *B. fraudulentus*, *B. kolymensis*, and *B. mucronatus*; D: *B. xylophilus*.

These six species also differ in their biological features, *i.e.*, vector beetle species, host tree species, and distribution, *i.e.*, reported location. Table 2 summarizes these biological features of the six *Bursaphelenchus* species.

Generally, the nematodes of this group have been isolated from the xylem of woody plants in the northern hemisphere (*e.g.*, Braasch, 2002), and except *B. fraudulentus*, are in close phoretic association with the longicorn beetles of the tribe Lamiini (*e.g.*, Kanzaki & Futai, 2002a). The vector of *B. fraudulentus* is unknown.

The vector beetle and host tree species of these

nematodes are as follows: *B. luxuriosae* is isolated from a species of cerambycid beetle, *Acalolepta luxuriosa*, and its host trees, trees of family Araliaceae (Kanzaki & Futai, 2003); *B. conicaudatus* is isolated from yellow-spotted longicorn beetle, *Psa-cothea hilaris*, and its host trees, mulberry and fig trees (family Moraceae) (Kanzaki *et al.*, 2000); *B. fraudulentus* is mainly isolated from several species of broad-leaved trees (*e.g.*, families of Rosaceae and Fagaceae) (Rühm, 1956; Braasch, 2002), though the vector beetle species of this nematode has not identified so far; the other three species, *B. kolymensis*, *B. mucronatus*, and *B. xylophilus* have been isolated from the beetles of the genus *Mono-chamus* and trees of family Pinaceae, (Korentchenko, 1980; Mamiya, 1984; Rutherford & Webster, 1987; Braasch *et al.*, 2001; Braasch, 2002) (Table 2).

Although many more information are needed, the geographical distribution of these six species are considered from the locations reported as follows: *B. conicaudatus* distributes Japan Islands, probably Taiwan Islands, and the continental China (Kanzaki *et al.*, 2000; Kanzaki & Futai 2002b); *B. fraudulentus* is mainly isolated from the European countries (Rühm, 1956; Schauer-Blume, 1990; Braasch, 2002); *B. luxuriosae* distributes Japan Islands and probably distributes the Korean Peninsula (Kanzaki & Futai, 2003); *B. mucronatus* (*B. kolymensis*) has been found widely from the Eurasian continent (Europe, Russia, East Asia) (Korentchenko, 1980; Braasch *et al.*, 2001; Braasch, 2002); *B. xylophilus* originated in North America (Mamiya, 1984; Rutherford & Webster, 1987).

Molecular phylogeny of the group. Five species of the *xylophilus* group nematode, *B. conicaudatus*, *B. fraudulentus*, *B. luxuriosae*, *B. mucronatus* and *B. xylophilus*, and two out group species, *B. abruptus* and *Aphelenchoides fragariae* were used for the molecular phylogenetic analysis (see Kanzaki & Futai 2002a; 2003). *B. kolymensis* was unavailable for this analysis, and considered as the variety of *B. mucronatus* in this analysis.

Based on molecular phylogenetic tree generated from DNA base sequences of the partial mitochondrial cytochrome oxidase subunit I (mtCOI) gene, monophyly was strongly supported for these five species (Fig. 2), *i.e.*, these species are considered to have evolved from common ancestor.

The phylogenetic tree indicates that, *B. luxuriosae*, *B. conicaudatus*, *B. fraudulentus*, and then the others have branched off from the ancestor in this order (Fig. 2).

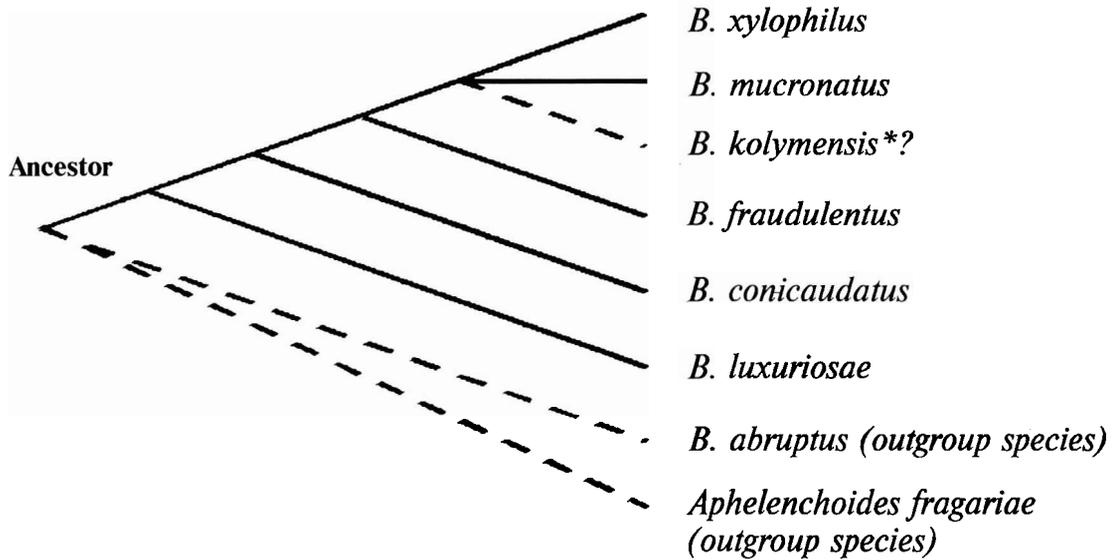


Fig. 2. Phylogenetic relationships of the *xylophilus* group of *Bursaphelenchus* species. Phylogenetic tree was generated based on the Neighbor Joining analysis. * unavailable for the analysis (see text).

Morphology, biological features, and molecular phylogeny. Based on the morphology, bionomics, reported location and molecular phylogeny, the evolutionary history of the *xylophilus* group is considered as follows (Fig. 3). The ancestor of the group had occurred in the central or eastern Eurasian continent, and the species had been in the phoretic association with the ancestor of the beetle tribe Lamiini. This ancestor species must have small “*a*” value, tapered female tail, and have habitat preference for broad-leaved trees (Kanzaki & Futai, 2002a). Through the process of the expansion from the eastern Eurasian continent to the western Europe, at first, *B. luxuriosae*, *B. conicaudatus* and then *B. fraudulentus* had branched off from the ancestor, orderly (Kanzaki & Futai, 2002a; 2003). In this process, the female tail became broader, and mucron occurred at the tip of female tail. While the original ancestor became thinner in their body shape, *i.e.*, larger “*a*” value, and changed habitat preference from broad-leaved trees to conifers, thereby spread through the coniferous forest of ancient Eurasia-North America continent (Kanzaki & Futai, 2002a).

B. mucronatus and *B. xylophilus* had branched from each other at the time of continental vicariance into the Eurasian continent and the North America by the Oligocene (De Guiran & Bruguier, 1989).

Based on the molecular phylogenetic analysis,

the evolutionary history of the *xylophilus* group nematodes was postulated. To evaluate the above-mentioned evolutionary hypotheses on the biological features of nematodes, many more related nematodes and their vector beetles are necessary to be found, described, and studied ecologically, and then, the molecular phylogeny of these species must be analyzed in the future.

Co-speciation of *Bursaphelenchus conicaudatus* and its vector beetle *Psacotheta hilaris*

There are many instances of co-evolution and co-speciation between two or more species included in endo-symbiosis and/or mutualistic relationship (*e.g.*, Yamamura, 1995). As for a commensalisms such as phoresis, however, co-speciation has not been studied so far.

The entomophilic (phoretic) nematodes vectored by insects cannot disperse without vector insects. Therefore the speciation of nematodes must be influenced by the speciation of its vector insect, *i.e.*, co-speciation might have occurred between an entomophilic nematode and its vector insect. In order to examine 1) whether or not the co-speciation occurs in a phoretic relationship, and 2) which factors influence the co-speciation, molecular phylogenetic analysis on *Bursaphelenchus conicaudatus*, and that on its phoretic host, *Psaco-*

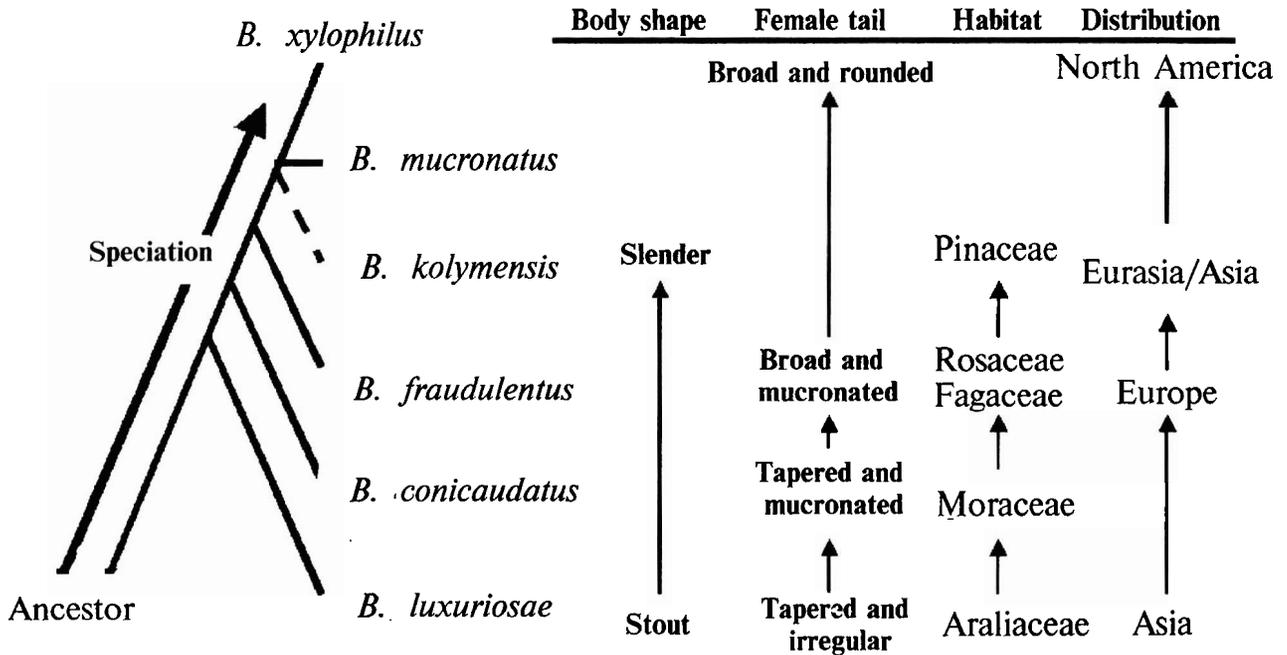


Fig. 3. Evolutional history of the *xylophilus* group nematodes in their morphology and biological features.

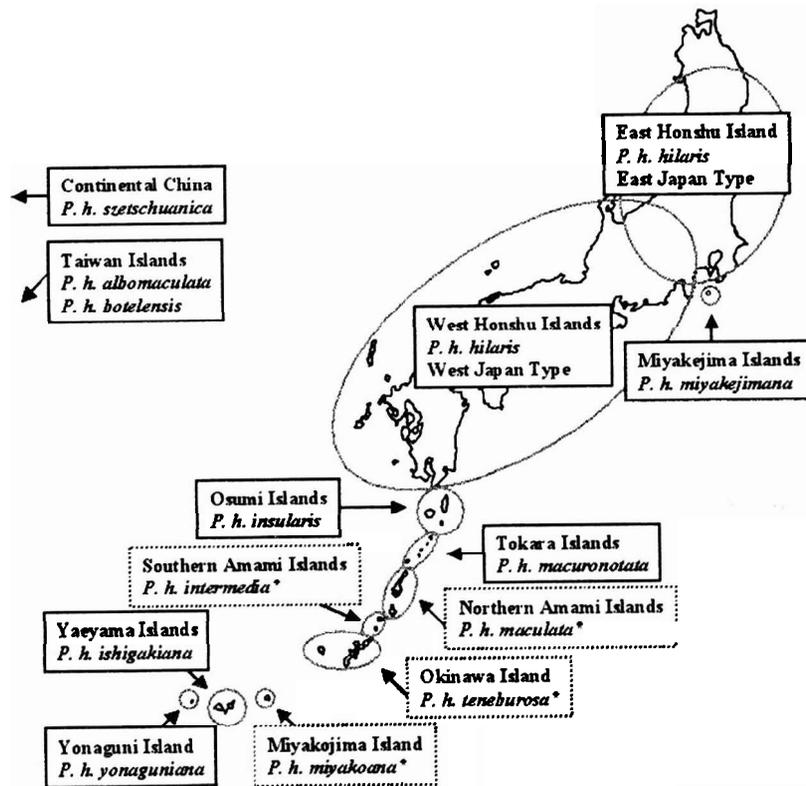


Fig. 4. Subspecies of *Psacothea hilaris* and its distribution. Details are shown in the text.

Table 1. Morphological divergence of the *xylophilus* group nematodes.

Species	Value "a"	Shape of female tail	Tip of female tail
<i>B. luxuriosae</i>	ca. 30	Tapered	irregular
<i>B. conicaudatus</i>	ca. 30	Tapered	with small mucro
<i>B. fraudulentus</i>	ca. 30	Broad	mucronated
<i>B. kolymensis</i>	ca. 40	Broad	mucronated
<i>B. mucronatus</i>	ca. 40	Broad	mucronated
<i>B. xylophilus</i>	ca. 40	Broad	rounded

Table 2. Biological features of the *xylophilus* group nematodes.

Species	Vector beetle	Host tree	Reported location
<i>B. luxuriosae</i>	<i>Acalolepta luxuriosa</i>	Araria elata	Asia
<i>B. conicaudatus</i>	<i>Psacotheta hilaris</i>	Moraceae	Asia
<i>B. fraudulentus</i>	unknown	Broad-leaved trees*	Europe*
<i>B. kolymensis</i>	<i>Monochamus</i> spp.	Pinaceae	Russia
<i>B. mucronatus</i>	<i>Monochamus</i> spp.	Pinaceae	Asia/Europe
<i>B. xylophilus</i>	<i>Monochamus</i> spp.	Pinaceae	North America

*Mainly isolated from broad-leaved trees in European countries, however, a case of isolation from dead pine in North America has been reported (Braasch, 2002).

Table 3. Average numbers of nematodes carried by an individual beetle.

Subspecies/type	n	No. of nematode/beetle average \pm SD (range)	% of beetles infested with nematode
West Japan type*	123	80 \pm 283 (0-2755)	97
East Japan type*	41	274 \pm 459 (0-2650)	98
<i>P. miyakejimana</i>	4	71 \pm 58 (25-80)	100
<i>P. insularis</i>	41	987 \pm 1592 (6-6510)	100
<i>P. macronotata</i>	54	207 \pm 637 (0-4260)	71
<i>P. maculata</i>	46	289 \pm 574 (0-5520)	74
<i>P. intermedia</i>	53	122 \pm 199 (0-760)	85
<i>P. tenebrosa</i>	32	160 \pm 363 (0-1740)	88
<i>P. miyakoana</i>	31	30 \pm 45 (0-181)	71
<i>P. ishigakiensis</i>	63	136 \pm 343 (0-2040)	84
<i>P. yonaguniana</i>	21	167 \pm 233 (0-580)	86

**Psacotheta hilaris hilaris*.

thea hilaris were conducted. The yellow-spotted longicorn beetle, *P. hilaris*, has many subspecies within Japan, and assumed to be suitable for the materials of this analysis. The details of this analysis are found in Kanzaki & Futai (2002b).

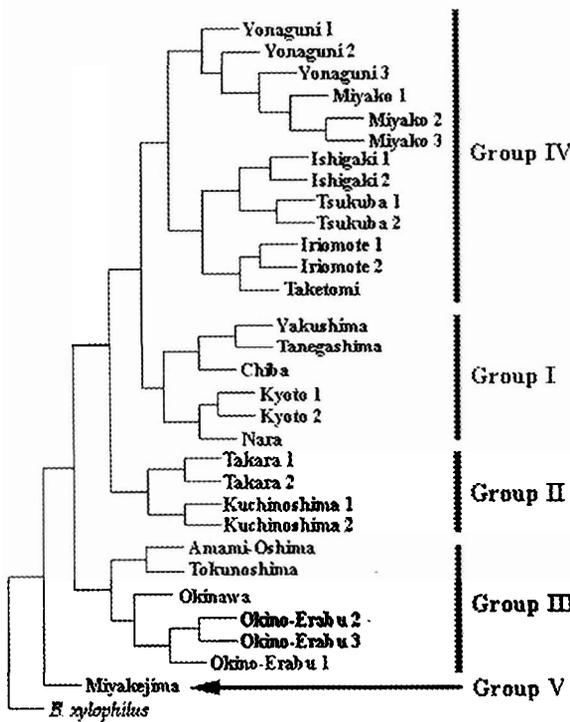
Life history of the *Bursaphelenchus conicaudatus* in relation to its vector beetle, *Psacotheta hilaris*. The yellow-spotted longicorn beetle, the vector of the nematode, is an important pest for the trees of the family Moraceae, e.g., figs and mulberries (e.g., Sakakibara, 1992a). The phoretic association between the nematode and the beetle is as follows.

In early summer, the adult beetles, which keeps the dispersal fourth stage juvenile nematodes in their tracheal system, emerges from the dead portion of a weakened host trees (Kanzaki & Futai, 2001). Then the beetles emerged feed on the leaves

of the host trees as its maturation feeding (Sakakibara, 1992a). The maturation feeding continues one to two weeks, to mature their reproductive organs (Sakakibara, 1992a).

Then the beetles mate and the females oviposit their eggs on the dead or weakened portion of living host trees (Sakakibara, 1992a). The nematodes enter their host trees through the oviposition marks of the vectors (Kanzaki & Futai, 2001). The beetle larvae hatch about one week after oviposition, and start to grow feeding on the inner bark and xylem tissues (Sakakibara, 1992a). The nematodes maintain their population feeding on many species of fungi, which invade the xylem tissues killed by the feeding behavior of beetle larvae (Kanzaki & Futai, 2001). The beetle larvae keep on growing until next summer, with the exception of winter season, feeding on the xylem

Bursaphelenchus conicaudatus



Psacotha hilaris

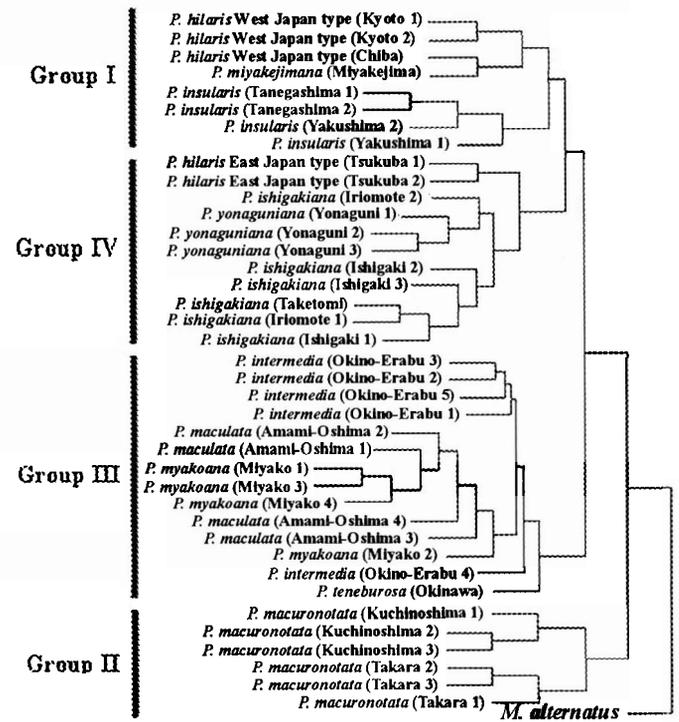


Fig. 5. Phylogenetic relationships of nematode isolates and corresponding beetle subspecies. Phylogenetic trees were generated based on the MP analysis

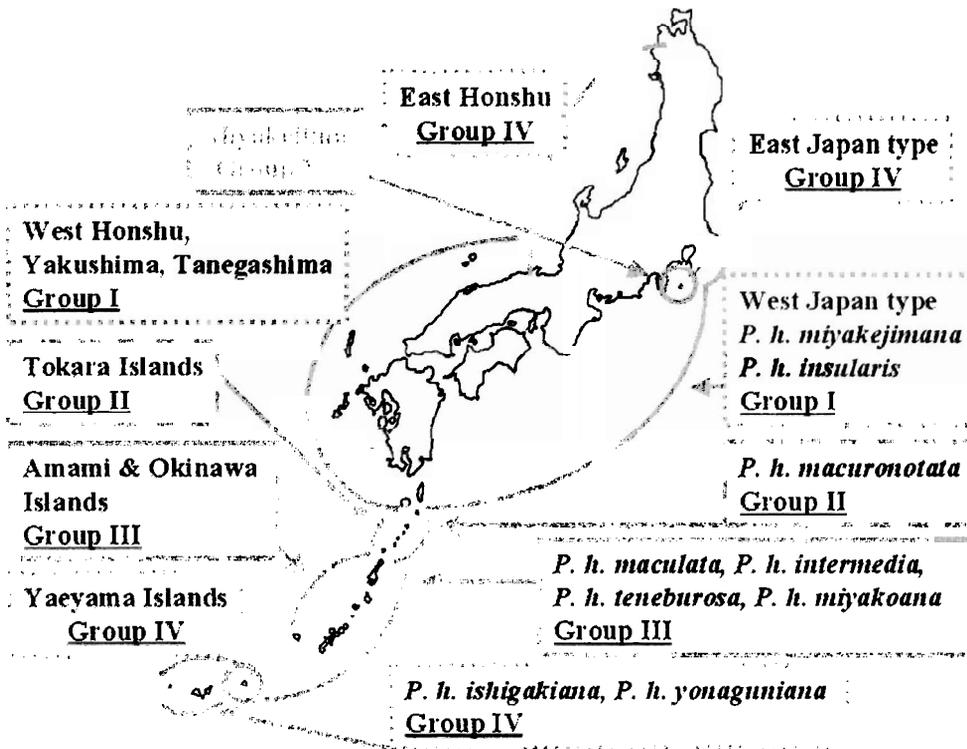


Fig. 6. Distribution of the phylogenetic groups of nematode isolates and corresponding beetle subspecies.

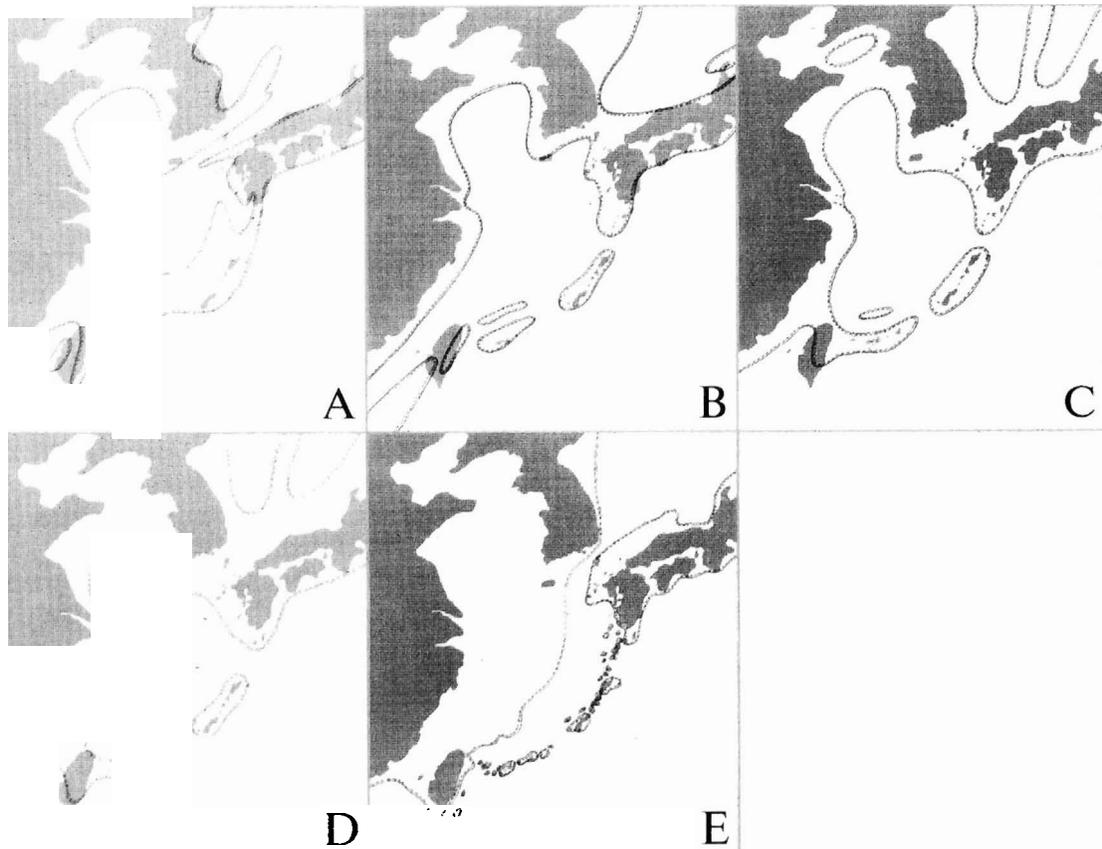


Fig. 7. Palaeogeography of the Japan Islands. A: 10M years ago; B: 5M years ago; C: 1.5M years ago; D: 1M years ago; E: 20 thousands years ago.

tissues, and the nematodes maintain their population feeding on the fungi, around the beetle tunnels (Sakakibara, 1992a; Kanzaki & Futai, 2001).

Therefore, the nematode depends its dispersal and food resources on its vector beetle, *i.e.*, the presence of the vector beetle is essential for the nematode life cycle.

Distribution and intraspecific divergence of *Psa-cothea hilaris*. The yellow-spotted longicorn beetle has been divided into 13 subspecies including 14 morphotypes (Makihara, 1986; Ohbayashi, 1992; Fig. 4). In Japan, 10 subspecies (11 morphotypes) are reported (Makihara, 1986), and the distribution ranges of these subspecies are as follows. *P. hilaris hilaris* East Japan type: the eastern Honshu Island; *P. h. hiliaris* west Japan type: the western Honshu, Shikoku, and Kyusyu Island; *P. h. miyakejimana*: Izu Islands; *P. h. insularis*: Osumi Is-

lands; *P. h. macuronotata*: Tokara Islands; *P. h. maculata*: the Northern Amami Islands; *P. h. intermedia*: the Southern Amami Islands; *P. h. teneburosa*: Okinawa Islands; *P. h. miyakoana*: Miyakojima Island; *P. h. ishigakiana*: Yae-yama Islands; *P. h. Yonaguniana*: Yonagunijima Island (Ohbayashi, 1992). Each of these subspecies is distinguished from the others by its body color, color and shapes of spots, and shapes of elytra (Ohbayashi, 1992). Further, the following four subspecies, which have brown bodies, *P. h. maculata*, *P. h. intermedia*, *P. h. teneburosa*, and *P. h. miyakoana*, used to be recorded as another species, *P. teneburosa*, while the others have black bodies (Ohbayashi, 1992).

Within these 10 subspecies (11 types), two morphotypes of *P. h. hiliaris* are considered to be immigrant populations (Fig. 4), and several hypotheses are proposed for their geographical origins. They are considered to be to have been in-

roduced into Japan from Taiwan or the Northern China (East Japan type) (Makihara, 1986), and Tsushima Island or the Northern China (West Japan type) (Makihara, 1986; Sakakibara, 1992b).

Molecular phylogeny of the beetle subspecies and corresponding nematode isolates. In order to examine whether the co-speciation has taken place between *Psacotha* beetle and *B. conicaudatus* nematodes, we analyzed the phylogenetic among beetle subspecies and that among nematode isolates obtained from the beetles, based on the molecular base sequences of partial mtCOI genes. The average number of the nematodes carried by individual beetle were also examined and compared among beetle subspecies.

In total, 459 individual beetles were collected from various regions (48 sites from 15 islands) within Japan. The beetles collected were dissected and examined the internal nematodes. The nematodes obtained from beetles were reared on the lawn of *Botrytis cinerea*, and the total nematode DNA was extracted. The beetle DNA was extracted from abdomen flight muscle obtained at the dissection procedure. The DNA sequences of the beetles and nematodes were determined by the methods of PCR direct sequencing, using the primers of Lunt *et al.* (1996) for beetles and those of Kanzaki & Futai (2002) for nematodes. In total, 39 beetle individuals and 30 nematode isolates were available.

The nematodes were isolated from all 10 subspecies (11 morphotypes) of beetles examined. The average numbers of nematodes kept in an individual beetle varied ranging *ca.* 30 to 1000, and *ca.* 70 to 100% of beetles were infested with nematodes (Kanzaki & Futai, 2002b; Table 3). The universality of the phoretic association was suggested (Kanzaki & Futai, 2002b; Table 3).

The phylogenetic analysis divided the beetles into four subspecies groups, and nematode isolates into five isolate groups (Fig. 5). The phylogenetic status of the beetles of east Japan morphotype and that of nematode isolate obtained from the beetle, on which there have been many hypotheses, were close to those of Yae-yama Islands (Fig. 5).

Nematodes of *B. conicaudatus* and its vector *Psacotha hilaris* phylogenetically corresponded to each other between the former's isolates and the later's subspecies. Thus, a kind of co-speciation between the nematode and the vector beetle was suggested (Fig. 5).

However, there were two discrepancies in this corresponding relationships (Fig. 6). The beetles of Miyakejima Island belonged to the group I, close to the beetles of west Honshu and Osumi Islands,

while the nematode isolate obtained from the beetles belonged to the group V, close to the root of the phylogenetic tree, and apart from the other isolates (Fig. 6). In the Miyakojima Island, the beetles belonged to group III, close to the beetles of Amami and Okinawa Islands, while the nematode of the island belonged to the group IV, close to the nematodes of Yaeyama Island and east Honshu area (Fig. 6).

These discrepancies were assumed to have occurred by the palaeogeographical events, because the Japan Island has very complicated geographical history (Kizaki & Oshiro, 1980; Nakamura *et al.*, 1996; Hikida & Ota, 1997; Ota, 1998).

Palaeogeographical implication on the co-speciation. The intraspecific divergence of *P. hilaris* is considered to have occurred concomitant to the palaeogeographical vicariance of the Ryukyu Archperago (Makihara, 1986). The present Japan Islands is a cascade of islands expanded from Hokkaido to the Ryukyu Archperago. In 10 million years ago, however, the Japan Islands was a part of a large landbridge expanded from the Eurasian Continent. The ancestor of the beetle, infested by the ancestor of the nematode, assumed to have immigrated into the landbridge (Kizaki & Oshiro, 1980; Fig. 7A). This landbridge submerged partially, and divided into several landmasses by five million years ago (Kizaki & Oshiro, 1980; Fig. 7B). One of these landmasses, the present Amami and Okinawa Islands, was isolated from the other landmasses and the continent, and the beetles of group III is assumed to have established in this period. Then, these landmasses, excepting one, "the landmass of group III", were connected to the continent 1.5 million years ago, due to the emergence of the land area (Kizaki & Oshiro, 1980; Fig. 7C). After this period, the sea level increased gradually and the Yaeyama Islands and Honshu Islands were isolated from the continent (Hikida & Ota, 1997; Ota, 1998; Fig. 7D), and the present Japan Islands has been formed in about 20 thousand years ago (Hikida & Ota, 1997; Ota, 1998; Fig. 7E). The Tokara Islands, locating between Osumi and Amami Islands, was formed by volcanic activities by 150 thousand years ago.

The most part of Miyakojima Island, where the nematode of group IV was obtained from the beetle of group III, is assumed to have submerged once about one million years ago (Hikida & Ota, 1997; Ota, 1998; Fig. 7D), and the original populations of the beetles and nematodes extinguished in this period. Then the island emerged again until 20 thousands years ago (Hikida & Ota, 1997; Ota,

1998; Fig. 7E). Thus the present beetle subspecies and nematode isolate of the Miyakojima Island are the immigrants, and the origins of the beetles and the nematodes could be Okinawa and Yae-yama Islands, respectively.

The Miyakejima Island, where the group V nematode was obtained from the group I beetle, is a volcanic island formed in 150 thousand years ago, and has never been connected to the other islands or continent (Taira, 1990; Ohmori, 1998). Thus the entire terrestrial fauna of this island is immigrant, and the origin of the beetle population is provably the west Honshu Island. But the origin of the nematode is unknown, because the nematode of Miyakejima Island is apart from the other isolates genetically. Further samplings in wide geographical ranges, e.g., continental China, are needed to clarify the origin of the nematode of Miyakejima Island.

The east Japan type beetle and the nematode population isolated from the beetle were close to those of Yaeyama Islands, where geographically apart from the east Japan. The beetle of the east Honshu morphotype is assumed to be immigrant, transported artificially from continental China or Taiwan Islands. These results do not conflict to each other, because the Yaeyama Islands is palaeogeographically close to the Taiwan Islands and continental China, i.e., the Yaeyama Islands was separated from the Taiwan Islands and continental China in the last stage of the vicariance procedure of the Japan Islands. Thus the Yaeyama populations of the beetles and nematodes are assumed to be close to those of Taiwan Islands and/or the continental China. However, to confirm this hypothesis, further studies are needed on the beetles of Taiwan Islands and the continental China, and if these beetles kept the nematode, nematodes are also to be examined.

Concluding remarks

In this paper, we introduced two instances of applications of molecular phylogenetic analysis to the evolutionary ecology of entomophilic nematodes and its co-speciation with vector beetle, using the *xylophilus* group species of the genus *Bursaphelenchus*. In the first instance, evolutionary sequence of morphological and biological features were determined, and in the second instance, the co-speciation between a species of *Bursaphelenchus* nematode and its vector beetle, *Psacotha hilaris* was disclosed, and factors influencing the co-speciation was considered. Through these two instances, we suggested availability of the *xylophilus*

group of the genus *Bursaphelenchus* as a prospective model system of the entomophilic nematodes.

The present paper focused its interests on the aspects of evolutionary events. However, the molecular physiological studies on these nematodes could be interesting and possible fields. For example, *B. xylophilus* never becomes the dispersal fourth-stage juveniles without the presence of the vector beetles (e.g., Maehara & Futai, 1996; 1997), but molecular physiological studies on this phenomenon has not yet been conducted so far.

There are many interesting themes also in the relationship between host plants and *Bursaphelenchus* nematode. The nematodes of the *xylophilus* group inhabit the xylem of the woody plants, which is severely resource-limited habitat, and the exploitation pattern of the habitat resources differs from species to species in the *xylophilus* group. For example, *B. conicaudatus* does not have the pathogenicity to the host trees, and uses weakened or dead portion of living trees for long period (Kanzaki & Futai, 2001), while an adventive pathogen, *B. xylophilus* kills its Asian pine trees and thus obtains its habitat and food resources (e.g., Kishi, 1995). The molecular genetic and physiological analyses on the establishment of those life strategies remain as the important themes to be solved.

The biological interactions of the *xylophilus* group nematodes with other organisms could provide many interesting issues, and the evolutionary, ecological and molecular physiological analyses on these issues would open up the prospect of nematology in the future.

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Kanzaki N., Futai K. Применение молекулярного филогенетического анализа для изучения эволюции и параллельного видообразования у энтомофильных нематод.

Резюме. Методами молекулярного филогенетического анализа исследовали два аспекта эволюционной истории нематод рода *Bursaphelenchus*. Во-первых, было проведено сравнение морфологических и биологических особенностей шести видов рода *Bursaphelenchus* с их положением на молекулярном филогенетическом древе. Затем были определены эволюционные ряды изменений этих признаков. Во-вторых, были исследованы сходные особенности филогении нематод вида *B. conicaudatus* и их переносчиков *Psacotha hilaris*. Молекулярно-филогенетический анализ выявил признаки параллельного видообразования у этих групп, а также возможную роль палеогеографических событий, как основных детерминант этого процесса. В целом, полученные результаты показывают эффективность методов молекулярно-филогенетического анализа для изучения эволюции нематод и указывают на возможность использования нематод рода *Bursaphelenchus* группы видов *xylophilus* как модельных объектов для молекулярного изучения параллельного видообразования у энтомофильных нематод.
