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Survey of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) in Japan

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Summary – A survey was conducted for entomopathogenic nematodes in the five climatic regions of Japan: subtropical islands, warm temperate coastal region along the Pacific, central mountainous region, temperate region, and cool temperate region. Over 1400 soil samples from 266 sites were tested for the presence of nematodes using the *Galleria* baiting technique. One hundred and twenty steinernematids and 29 heterorhabditids were detected in 62 sites and 24 sites, respectively. Eight steinernematid species were recognized: seven undescribed species and *Steinernema kushidai*. Each species had a characteristic distribution: three wide-spread common, one coastal, one subtropical, and three mountainous species. In contrast, the heterorhabditids were identified as *Heterorhabditis indica* and *H. megidis*. The former was widely found in the coastal region from the subtropical to the warm temperate zone while the latter was found mainly in the eastern part of the warm temperate coastal region. © Elsevier - ORSTOM

Résumé – ***Enquête sur les nématodes entomopathogènes (Rhabditida: Steinernematidae et Heterorhabditidae) du Japon*** – Une enquête a été menée concernant les nématodes entomopathogènes dans les cinq régions climatiques du Japon : les îles subtropicales, la région tempérée chaude des côtes du Pacifique, la région montagneuse australe, la région tempérée et la région tempérée froide. Plus de 1400 échantillons de sol provenant de 266 sites ont été testés pour la présence de nématodes à l'aide de *Galleria*-pièges. Cent vingt-et-un Steinernematides et 29 Heterorhabditides ont été détectés dans, respectivement, 62 et 24 sites. Huit espèces de Steinernematides ont été reconnues : sept espèces non décrites et *Steinernema kushidai*. Chacune des espèces montre une répartition particulière : trois sont très répandues, une est côtière, une subtropicale et les trois autres montagnardes. En contraste, les Heterorhabditidae ont été identifiés comme *Heterorhabiditis indica* et *H. megidis*. La première espèce est largement répandue dans la région côtière depuis la zone subtropicale jusqu'à la zone tempérée chaude, tandis que la seconde est surtout rencontrée dans la partie est de la région côtière tempérée. © Elsevier - ORSTOM

Keywords: distribution, entomopathogenic nematodes, *Heterorhabditis*, Japan, species diversity, *Steinernema*, survey.

Nematodes of the families Steinernematidae and Heterorhabditidae have characteristics that make them extremely useful as biological control agents. Numerous surveys have been conducted world-wide, resulting in the isolation of many species and strains: Australia (Akhurst & Bedding, 1986), North America (Akhurst & Brooks, 1984; Poinar *et al.*, 1987; Mráček & Webster, 1993; Rueda *et al.*, 1993; Nguyen & Smart, 1994), Puerto Rico (Roman & Beavers, 1982), South America (Doucet, 1986; Doucet & Doucet, 1990, Nguyen & Smart, 1990; Stock, 1995), Hawaii (Hara *et al.*, 1991), Europe (Mráček, 1980; Deseo *et al.*, 1984; Husberg *et al.*, 1988; Hominick & Briscoe, 1990; Griffin *et al.*, 1991), Africa (Shamseldean & Abd-Elgawad, 1994), India (Poinar *et al.*, 1992), Sri Lanka (Amarasinghe *et al.*, 1994), Asia (Zhang *et al.*, 1992; Choo *et al.*, 1995). In Japan, *Steinernema kushidai* Mamiya, 1988 was first isolated in Shizuoka from a tree nursery where an outbreak and an infestation of white grubs had occurred (Kushida *et al.*, 1987). Later surveys conducted in eastern Japan (Mamiya & Ogura, 1989) and Kyushu district (Kimura & Ishibashi, 1991) revealed the presence of other steinernematids.

The present study describes a survey conducted between 1991 and 1994 in five climatic regions, from subtropical to cool temperate regions, mostly in major forests and ranging in elevation from near sea level to about 1600 m. This work was aimed at collecting indigenous entomopathogenic nematodes adapted to the various climates for possible use in biological control regimens.

Materials and methods

The present survey was conducted from the south part of Japan in a north-east direction along the Pacific coast to collect nematodes adapted to dry, hot, and warm environments, then from the south to the north of the central part (Izu Peninsula - Nagano Pref.) to collect nematodes adapted to cool or cold mountainous environments. Moreover, some other

regions sampled between 1991 and 1994 were added to the present study.

A total of 1416 soil samples was collected from 276 sites, which represented the five climatic regions (Fig. 1) in the present study as follows: *i*) subtropical islands, four islands in Okinawa Pref. (Nansei-shoto Isls.), 309 samples from 81 sites; *ii*) warm temperate coastal region along the Pacific coast, the warm region affected by the warm current "Kuroshio", from Cape Sata-misaki (south end of Kyushu, Kagoshima Pref.) to Cape Inubo-zaki (Chiba Pref.), 732 samples from 116 sites; *iii*) central mountainous region, the cool highland at more than 500m elevation from Izu Peninsula (Shizuoka Pref.) to Niigata Pref., 165 samples from 31 sites; *iv*) temperate region, Oki Isls. (Shimane Pref.) and some regions in the eastern and northern part of Japan (Chiba, Ibaraki and Miyagi Prefs), 129 samples from 26 sites; *v*) cool temperate region (Hokkaido Pref.), 44 samples from twelve sites. The climatic division was determined based on vegetation and elevation, referring to Maekawa (1977) and Nakamura *et al.* (1996). Samples were collected from November to May before the luxuriant growth of weeds and bushes, with the exception of the Okinawa Is. where samples were taken in June after the rainy season. The number of samples taken from each site was usually five (ranging from three to thirty samples/site) with approximately 600-700 g soil collected for each sample to a depth of 5-20 cm.

Approximately 500 cm^3 of soil of each sample was placed in a polythene cup (110 mm diam., 50 mm depth) covered with a lid and three to five final instar larvae of *Galleria mellonella* were added (Bedding & Akhurst 1975). Each cup was incubated at room temperature (20-25°C) for 2 weeks. Each container was checked for larval mortality every 2-3 days from 5 or 6 days after setting the *Galleria* trap up to 14 days. Dead larvae were placed individually on a polyurethane sponge (20 mm length, 15 mm width and less than 10 mm thickness) in each cell of a twelve-well plate with water. The plates were kept at room temperature (20-25°C) and periodically examined for presence of nematodes. Cadaver from which infective juveniles began to emerge were individually transferred to a plastic cup (60 mm diam., 35 mm depth) with the polyurethane sponge and distilled water. In the present study, an "isolate" represented the nematodes obtained from a single dead *Galleria* larva and not from one soil sample. Infective juveniles isolated in this manner were rinsed in distilled water several times in the cup and stored at 5-7°C (*Steinernema* spp. except *S. kushidai*) or 10-12°C (*S. kushidai* and *Heterorhabditis* spp.).

Identification of nematode isolates was based on morphometrics of infective juveniles and morphology of 1st generation males under light microscopy and also by PCR-RFLP analysis of the internal transcribed spacer region from the ribosomal DNA repeat unit. Specimens for morphological study were prepared according to Minagawa and Mizukubo (1994). The extraction of genomic DNA from a single nematode was conducted according to Joyce *et al.* (1994*c*) and Reid *et al.* (1997). The ITS region was amplified by PCR with the 18S and 26S primer set (Vrain *et al.*, 1992). Subsequent RFLP analysis was performed as per Reid *et al.* (1997). Each isolate was assigned to an RFLP type based on the restriction fragment patterns yielded by *Alu* I and *Hinf* I (*Steinernema* spp.) or *Alu* I and *Rsa* I (*Heterorhabditis* spp.). Seventeen restriction enzymes (*Alu* I, *Bst*O I, *Dde* I, *Eco*R I, *Hae* III, *Hha* I, *Hin*d III, *Hinf* I, *Hpa* II, *Kpn* I, *Pst* I, *Pvu* II, *Rsa* I, *Sal* I, *Sau*3A I, *Sau*96 I, *Xba* I) were used for the detailed RFLP analysis of each representative of the RFLP types (Reid *et al.*, 1997). The detailed RFLP profiles were compared with the RFLP database of the nematodes at CABI International Institute of Parasitology, UK.

Results

Positive soil samples with entomopathogenic nematodes were obtained from all sampling regions (Fig. 1), and represented 10.0% of the total number of soil samples (142/1416) or 28.2% of the geographical sites (75/266). Steinernematids were recovered in 8.5% of the soil samples (120/1416), from 23.3% of the sites (62/266), and heterorhabditids in 2.0% of the soil samples (29/1416), from 9.0% of the sites (24/266). The incidence and species diversity of the nematodes in the four main sampling regions were obviously different. A much higher prevalence was noted in the central mountainous region (64.5% of the sites [20/31]) than in the subtropical islands (8.6% [7/81]), the temperate region (23.1% [6/26]) and the warm temperate coastal region (33.6% [39/116]). Eighty-two steinernematid isolates from 47 positive sites were classified into eight species: seven undescribed species and *S. kushidai*, by means of morphology and/or RFLP analysis. The undescribed *Steinernema* species are herein referred as MY1, MY2, MY3, MY4, MY5, MY6, and MY7, based on RFLP profiles (Fig. 2). The incidence and species diversity of steinernematids increased as follows: subtropical islands (1.2% of the sites [1/81], *Steinernema* sp. MY4) < temperate region (23.1% [6/26], *Steinernema* spp. MY1, MY3, and unidentified isolates) < warm temperate coastal region (27.6% [32/116], *Steinernema* spp. MY1, MY2, MY3, *S. kushidai*, and unidentified isolates) < central mountainous region (64.5% [20/31], *Steinernema* spp. MY1, MY3, MY5, MY6, MY7, *S. kushidai*, and unidentified isolates) (Fig. 3). Twenty-eight heterorhabditid isolates from 23 positive sites were identified as *H. indica* Poinar,

Fig. 1. *Localities and regions where samplings for heterorhabditids and steinernematids were conducted. Figures indicated latitude (N) and longitude (E). Each symbol often represents more than one site.*

Karunakar & David, 1992 and *H. megidis* Poinar, Jakson & Klein, 1987. The RFLP profiles of the Japanese isolates of *H. megidis* showed the same pattern as that of the Ohio isolate (original strain) of the three RFLP types of *H. megidis* (Ohio, North West European [NWE], and Irish types; Hominick, *et al.*, 1996).

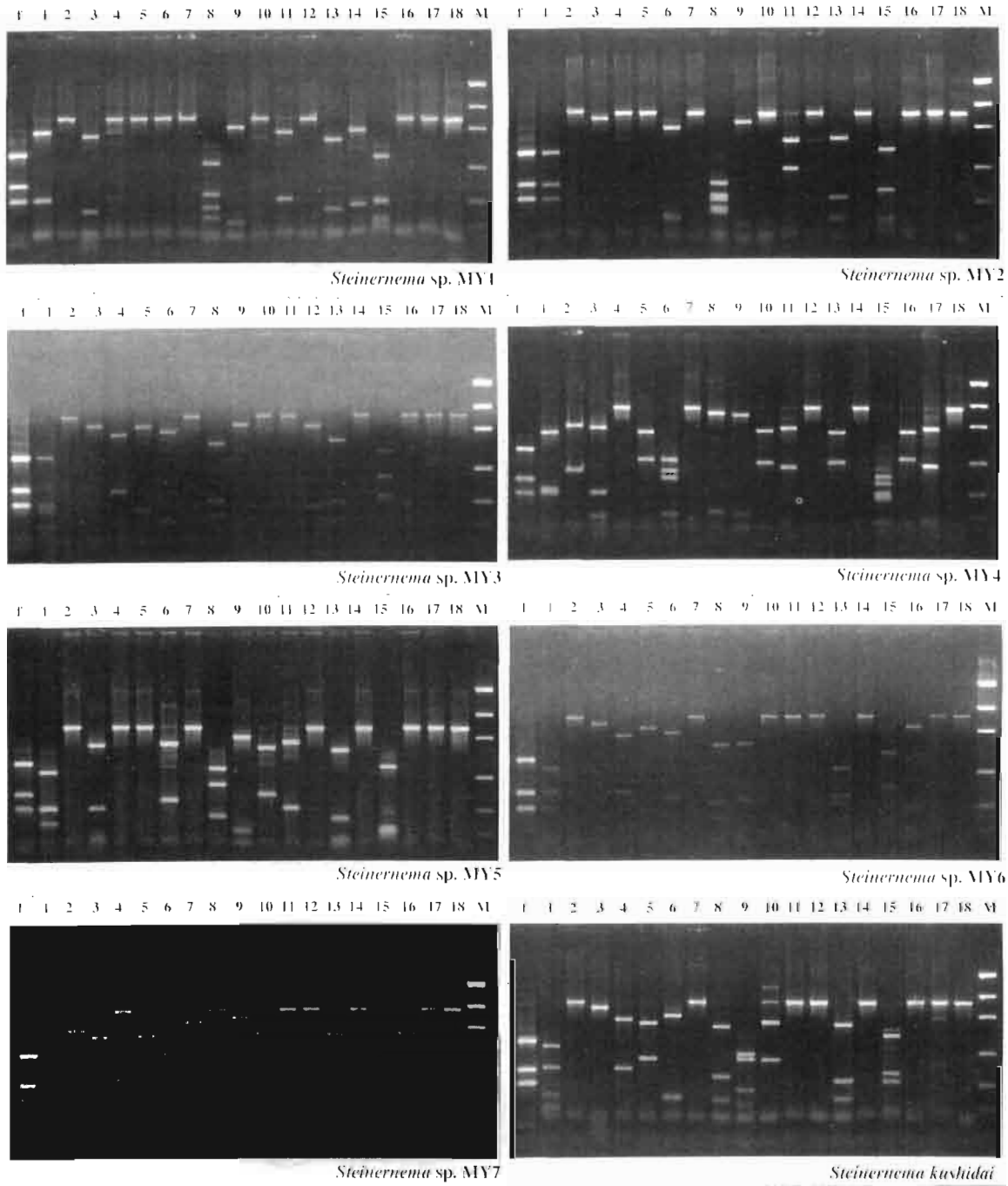

Fig. 2. *Restriction Fragment Length Polymorphism profiles of seven undescribed* Steinernema *sp. and* S. kushidai. *Ethidium bromide stained 1.5 % agarose gel. Lane 18: PCR amplified fragment of Internal Transcribed Spacer region from rDNA with 18s and 26s primer set. Lanes 1- 17: restriction digestion pattern (1:* Alu *I, 2:* BstO *I, 3:* Dde *I, 4:* EcoR *I, 5:* Hae *III, 6:* Hha *I, 7:* Hind *III, 8:* Hinf *I, 9:* Hpa *II, 10:* Kpn *I, 11:* Pst *I, 12:* Pvu *II, 13:* Rsa *I, 14:* Sal *I, 15:* Sau*3A I, 16:* Sau*96 I, 17:* Xba *I). Lane f: Positive control (Alu I restriction digest of ITS region from* S. feltiae *site 76 strain UK). Lane M: DNA size marker (from top to bottom: 2, 1.2, 0.8, 0.4, 0.2, 0.1kbp). Each isolates of* Steinernema *sp. from the following locality (see Table 1), MY1: Sata; MY2: Atsumi; MY3: Omaezaki; MY4: Yonaguni Is.; MY5: Enzan; MY6: Matsumoto; MY7: Omachi;* S. kushidai*: Toyo.*

However, another European study (Smits *et al.*, 1991; Dix *et al.*, 1992; Joyce *et al.*, 1994*a, b, c*) suggested that the Irish group was not conspecific with the NWE/Ohio groups. Accordingly, in the present study, the authors treated the three groups showing closely related RFLP pattern of ITS (Reid, 1994) as the *H. megidis* species complex. The incidence and species diversity of heterorhabditids increased as follows: the temperate region (0% of the sites [0/26]) < the central mountainous region (6.5% [2/31], *H. megidis*) < the subtropical islands (7.4% [6/81], *H. indica*) < the warm temperate coastal region along the Pacific (13.8% [16/116], both species and an unidentified isolate) (Fig. 4).

A total of 39 isolates (38 steinernematids and one heterorhabditid) could not be identified, because they did not provide living infective juveniles from infected wax moth larvae or were lost during culture storage

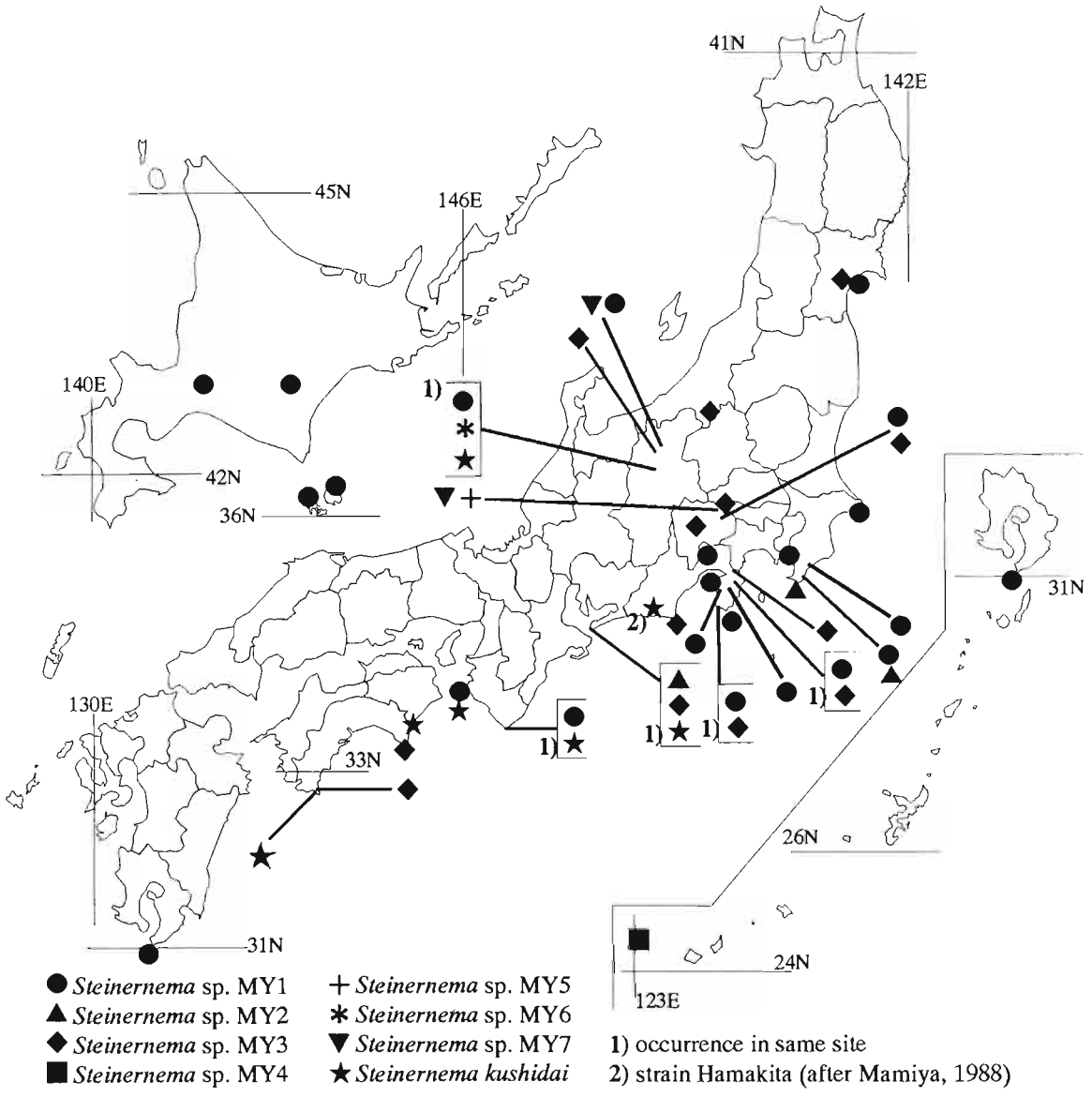

Fig. 3. *Distribution of* Steinernema *spp. from a survey of Japan. Latitude (N) and longitude (E) are indicated. Each symbol often represents more than one site.*

manipulation. In 1993, five steinernematid isolates (obtained from five of ten samples) were recovered from Mihama (Wakayama Pref.). They yielded no infective juveniles and several 1st generation adults obtained from some dead *Galleria* larvae indicated unfavourable conditions for propagation of steinernematids. These isolates could clearly be separated from all other Japanese isolates by the spicule shape. In the following year, the same site was sampled and the isolates obtained on this occasion (three positive samples/ten samples) showed the same spicule shape and the same RFLPs pattern as those of *Steinernema* sp. MY1. Accordingly, the first isolates from Mihama were not included in this grouping.

Environmental characters and geographical features of the sites with nematodes are indicated in Table 1. The geographical distribution of steinernematids and heterorhabditids identified to species level is summarized in Figs 3 and 4. *Steinernema* sp. MY1 was isolated from 28 sites in Kyushu, Honshu, Hokkaido, and Oki Isls, from coastal to mountainous regions. *Steinernema* sp. MY2 was isolated from three coastal sites, in the eastern part of the warm temperate coastal region. *Steinernema* sp. MY3 was isolated from thirteen sites in Shikoku and Honshu, from coastal to mountain regions. *Steinernema* sp. MY4 was isolated only from one inland site in Yonaguni Is. in the subtropical region. *Steinernema* spp. MY5, MY6 and MY7 were isolated from one, one, and two sites, respectively, in the central mountainous region. *S. kushidai* was isolated from five sites in the warm temperate coastal region and one site in the central mountainous region. *H. indica* was isolated from fourteen sites in the subtropical to warm temperate coastal regions. *H. megidis* was isolated from seven sites in the eastern part of the warm temperate coastal region and two sites in the central mountainous region. The steinernematid distribution was very broad, ranging from beach sites to inland sites and from subtropical to cool temperate regions, while most of the heterorhabditids occurred in the coastal area from the warm region (Fig. 1).

The vertical distribution of the steinernematids ranged from beaches to mountains about 1400 m in

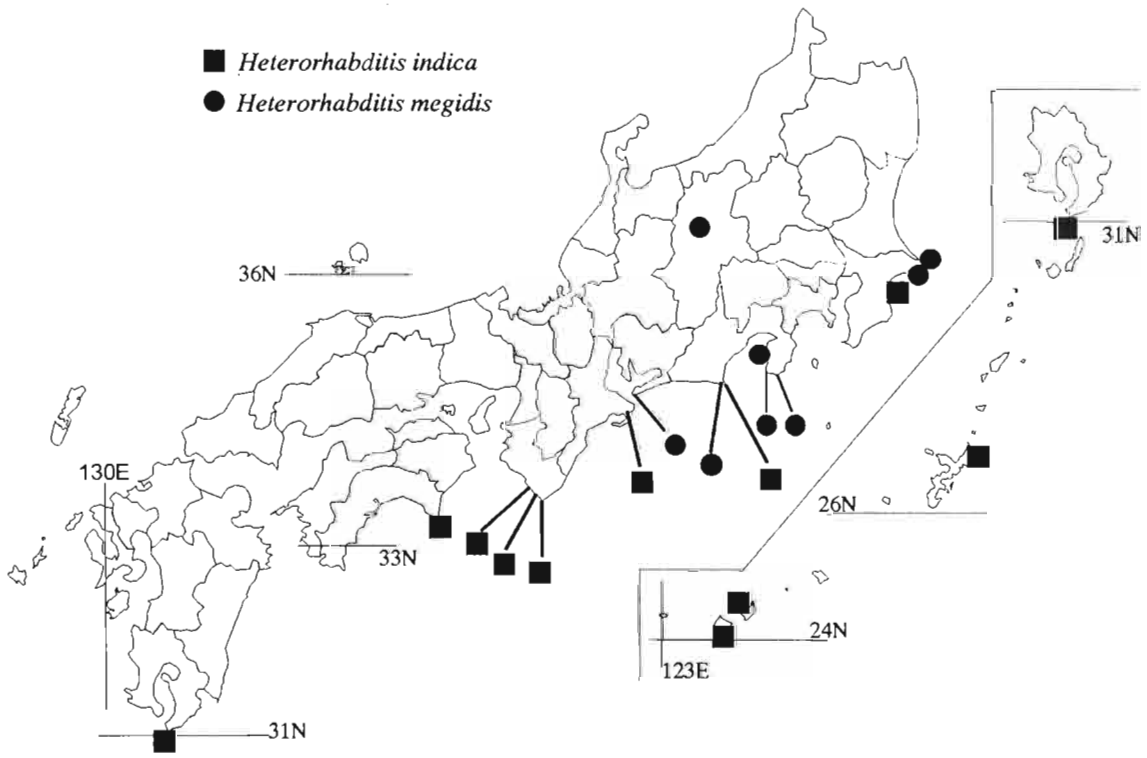

Fig. 4. *Distribution of* Heterorhabditis *spp. from a survey of Japan. Latitude (N) and longitude (E) are indicated. Each symbol often represents more than one site.*

elevation. In the case of the survey at Enzan (Yamanashi Pref.) in the central mountainous region, 30 soil samples were taken from six sites (five samples/site) from two mountains (two sites about 1000 m and 1400 m in elevation from one mountain and two sites 1000 m to 1200 m, one site about 1400 m, and one site about 1600 m in elevation from a nearby mountain). Steinernematids were isolated from five of the six sites (1000-1400 m in elevation). No steinernematids were isolated from the site at about 1600 m. *Steinernema* spp. MY5 (one isolate at about 1000 m) and MY7 (one isolate at about 1400 m) were isolated from the first mountain and *Steinernema* spp. MY1 (five isolates at about 1000-1400 m) and MY3 (one isolate at about 1000-1200 m) from the second mountain. There was some frozen soil under the surface in the two sites at 1400 and 1600 m in the second mountain, when the sampling was conducted (April). *Steinernema* sp. MY1 was isolated from the soil surrounded by frozen soil in the former site. *Steinernema* sp. MY1 was found from the coast close to sea level to mountains about 1400 m in elevation. *Steinernema* sp. MY2 was isolated only near sea level. *Steinernema* sp. MY3 was found from the coast close to sea level to mountains about 1000-1200 m in elevation. *Steinernema* sp. MY4 was isolated at about 100 m. *Steinernema* sp. MY5 was isolated at about 1000 m, *Steinernema* sp. MY6 was isolated at about 700-800 m. *Steinernema* sp. MY7 was isolated at about 800-1400 m. *S. kushidai* was found from the coast nearly at sea level to a coastal hill at less than 100 m and in one mountain site at about 700-800 m (Table 1). The vertical distribution of heterorhabditids ranged from beaches to low mountains about 200 m in elevation, with the exception of two isolates (*H. megidis*) from mountains about 700-800 m in elevation (Table 1). To date, *S. kushidai* and *H. megidis* have not been isolated from the inland part between Shizuoka Pref. and Nagano Pref. (Figs 3, 4).

Steinernema spp. MY4, MY5, MY6, and MY7, *S. kushidai,* and *H. megidis* were isolated only from woodlands. *Steinernema* spp. MY1, MY2, and MY3 and *H. indica* were isolated from both grasslands and woodlands (Table 1). *Steinernema* spp. MY1 and MY3 were isolated from vegetation ranging from grasslands close to woodlands to well-developed forests (climax vegetation). *Steinernema* sp. MY2 and *H. indica* were isolated from vegetation ranging from grasslands far from woodlands to well-developed forests. *Steinernema* spp. MY5 and MY6 were isolated from sparse forests (secondary forests). *S. kushidai* and *H. megidis* were isolated from sparse to well-developed forests. *Steinernema* spp. MY4 and MY7 were isolated from well-developed forests.

Although in most of the positive sampling sites only one nematode species was recovered at a time, some species sympatrically occurred in fourteen sampling sites and steinernematids and heterorhabditids were isolated from the same soil samples in six of these fourteen sites (Table 2). However two or more congeneric species were never isolated from the same soil sample. *H. megidis* and the steinernematids were found sympatrically in eight of nine sites with *H. megidis*, while steinernematids were found in only three of fourteen sites with *H. indica*. The two heterorhabditid species were always isolated allopatrically. In the case of the adjacent occurrence of the two heterorhabditid species, the two sampling sites in Omaezaki (Shizuoka) were separated by less than 5 km and the site where *H. indica* occurred was closer to the Pacific coast than the site where *H. megidis* occurred.

Discussion

Although most of the soil samples were taken from woodlands, the area surveyed covered a wide range of climates ranging from subtropical to cool temperate regions and forests are the dominant habitat in Japan. Also, vertical distribution of nematodes was studied from sea level to high altitudes (up to 1600 m). Accordingly, the present results are an indication of the natural distribution of steinernematids and heterorhabditids in Japan, corresponding to the climatic and/or geographical variation. Steinernematids were more common than heterorhabditids, as is the case in most surveys except for North Carolina (Akhurst & Brooks, 1984), New Jersey (Stuart & Gaugler, 1994), Hawaii (Hara *et al.*, 1991), and Egypt (Shamseldean & Abd-Elgawad, 1994), in which heterorhabditids were more common. However, in the subtropical region (Nansei-shoto Isls.), heterorhabditids were more prevalent than steinernematids, although the overall nematode prevalence was much lower. Heterorhabditids occurred mostly in the coastal area, while steinernematids were found in only one inland site. The prevalence of the two families was similar to the Hawaiian survey (6.8% positive sites for entomopathogenic nematodes, 6.3% positive for heterorhabditids, and 0.6% positive for steinernematids; Hara *et al.*, 1991). In the Hawaiian survey, 95.5% (21/22) of isolates were collected from beach sites and one isolate at 61m elevation (Hara *et al.*, 1991). Conversely, in this survey only one isolate was collected from a beach site, while six were recovered from coastal forests or a grassland away from the beach (Table 1). This difference may be due to the different species involved (*H. hawaiiensis* Gardner, Stock & Kaya, 1994 from Hawaiian Isls., *H. indica* from Nansei-shoto Isls.) and their host insects, as Akhurst and Bedding (1986) mentioned. It should be also considered that differences in geological origin (Hawaiian Isls. are oceanic islands, Nansei-shoto isls. are continental

Table 1. *Habitat characteristics of positive sampling sites of* Steinernema *and* Heterorhabditis *species*

Species		Locality	Climatic region[1)]	Configuration of the site	Elevation (m)	Distance from sea(m)	Habitat[2)]	Vegetation
***Steinernema* species**								
kushidai	Kochi	Toyo	WTC	Coast	<10	<10	W:Roadside	*Conilignosa (Cryptomeria)*
	Kochi	Tosa-shimizu	WTC	Coastal Mountain	100	<30	W:Roadside	*Laurilignosa*
	Wakayama	Hidaka	WTC	Coastal Mountain	50-100	<100	W:Slope	*Laurilignosa*
	Wakayama	Kushimoto	WTC	Coast	70-80	<50	W:Beside park	*Laurilignosa*
	Aichi	Atsumi	WTC	Coast (Tip of Headland)	<10	<100	W	Pine, Evergreen
	Nagano	Matsumoto	CM	Mountain	700-800	Inland	W:Beside park	*Deciduilignosa* (Oak)
MY1	Kagoshima	Sata	WTC	Coast	5-10	100-200	W:Road-side	*Laurilignosa*
	Kagoshima	Sata	WTC	Coastal Mountain	<100	100-200	W	Evergreen *Cryptomeria*
	Shimane	Nishinoshima	T	Hill	200	Inland	W	*Conilignosa* (Pine)
	Shimane	Saigo	T	Coast (Tip of Cape)	100-200	<100	W+G	Pine, Evergreen, Glass
	Shimane	Fuse	T	Mountain	400-500	Inland	W	*Deciduilignosa*
	Shimane	Fuse	T	Mountain	300	Inland	W:Beside pass	*Deciduilignosa*
	Wakayama	Kushimoto	WTC	Coast	70-80	200	W	*Laurilignosa*
	Wakayama	Kushimoto	WTC	Coast	70-80	50-100	W:Beside park	*Laurilignosa*
	Wakayama	Kushimoto	WTC	Coast (Tip of Cape)	70-80	<20	W+G:Park	Pine, Evergreen, Turf
	Wakayama	Mihama	WTC	Coastal Mountain	200-300	500	W+G:Open-land(Park)	Pine, Broad-leaved, Grass
	Shizuoka	Fujinomiya	CM	Mountain	500-600	Inland	W:River-side	*Deciduilignosa*
	Shizuoka	Atami	CM	Mountain	534	Inland	W:Forest Park	*Deciduilignosa*
	Shizuoka	Syuzenji	CM	Mountain	500	Inland	W:Forest Park	*Deciduilignosa*, Bamboo grass
	Shizuoka	Toi	WTC	Coast (Tip of Cape)	40-50	<50	W	*Laurilignosa*, Bamboo grass
	Shizuoka	Nishi-Izu	WTC	Coastal Hill	10-20	500-600	W	*Laurilignosa*
	Shizuoka	Minami-Izu	WTC	Coastal Hill	200	500-600	W	*Laurilignosa*
	Shizuoka	Minami-Izu	WTC	Coast	50-60	<100	W:Beside park	*Laurilignosa*
	Yamanashi	Enzan	CM	Mountain	1100-1200	Inland	W	*Deciduilignosa*
	Yamanashi	Enzan	CM	Mountain	1400	Inland	W	*Deciduilignosa*, Bamboo grass
	Nagano	Matsumoto	CM	Mountain	700-800	Inland	W:Beside park	*Deciduilignosa* (Oak)
	Nagano	Omachi	CM	Mountain	800-900	Inland	W+G	Grass,Oak
	Chiba	Amatsu-Kominato	WTC	Coastal Mountain	100-200	50-100	W	*Laurilignosa*
	Chiba	Wada	WTC	Coast (Beach)	<5	<100	W:Shelter belt	Pine

To be continued next page

Table 1. *(cont.)*

Species		Locality	Climatic region[1)]	Configuration of the site	Elevation (m)	Distance from sea(m)	Habitat[2)]	Vegetation
	Chiba	Kyonan	WTC	Coastal Mountain	200	1.1-1.2k	W	*Laurilignosa*
	Chiba	Iioka	WTC	Coastal Hill	100-200	700-800	W	*Laurilignosa*
	Miyagi	Sendai	T	Plain	40	Inland	W:Coniferous plantation	*Cryptomeria*
	Hokkaido	Yubari	CT	Mountain	500-600	Inland	W	*Deciduilignosa*, Bamboo grass
	Hokkaido	Obihiro	CT	Plain	40	Inland	W	*Deciduilignosa*, Bamboo grass
MY2	Aichi	Atsumi	WTC	Coast (Tip of Headland)	<10	<50	W	Pine, Evergreen
	Chiba	Shirahama	WTC	Coast (Beach)	<5	10-50	G:Road-side	Bush, Grass
	Chiba	Wada	WTC	Coast (Beach)	<5	10-50	W:Shelter belt	Pine
MY3	Kochi	Muroto	WTC	Coastal Mountain	200	<1k	W	*Laurilignosa*
	Kochi	Tosa-shimizu	WTC	Mountain (Headland)	100-200	2k	W	*Laurilignosa*
	Aichi	Atsumi	WTC	Coast (Tip of Headland)	<10	<100	W	Pine, Evergreen
	Shizuoka	Omaezaki	WTC	Plain	20-30	1k	W	*Conilignosa*, Evergreen
	Shizuoka	Kan-nan	CM	Mountain	700	Inland	W	*Laurilignosa*
	Shizuoka	Atami	CM	Mountain	534	Inland	W:Forest Park	*Deciduilignosa*
	Shizuoka	Minami-Izu	WTC	Coastal Hill	200	500-600	W	*Laurilignosa*
	Yamanashi	Enzan	CM	Mountain	1000-1100	Inland	W	Pine, Bamboo grass
	Yamanashi	Nirasaki	CM	Mountain	500-600	Inland	W:Ruin of Castle	*Deciduilignosa*
	Nagano	Omachi	CM	Hill	800-900	Inland	G:Beside pine forest	Bush, Grass
	Niigata	Yuzawa	CM	Mountain	600-700	Inland	W:Coniferous plantation	*Cryptomeria*
	Tokyo	Okutama	CM	Mountain (Limestone area)	700-800	Inland	W:Beside pass	*Deciduilignosa*
	Miyagi	Sendai	T	Plain	40	Inland	W:Beside urban area	*Conilignosa*
MY4	Okinawa	Yonaguni Is.	SI	Mountain	100	Inland	W	*Laurilignosa*
MY5	Yamanashi	Enzan	CM	Mountain	1000	Inland	W	Pine, Bamboo grass
MY6	Nagano	Matsumoto	CM	Mountain	700-800	Inland	W:Beside park	*Deciduilignosa* (Oak)
MY7	Yamanashi	Enzan	CM	Mountain(Ridge)	1400	Inland	W	*Deciduilignosa*, Bamboo grass
	Nagano	Omachi	CM	Mountain	800-900	Inland	W:Coniferous plantation	*Cryptomeria*

To be continued next page

Table 1. (cont.)

Species		Locality	Climatic region[1)]	Configuration of the site	Elevation (m)	Distance from sea(m)	Habitat[2)]	Vegetation
***Heterorhabditis* species**								
indica	Okinawa	Ishigaki Is.	SI	Coast	30	300-400	G:Beside cropland	Grass (*Arundo donax*)
	Okinawa	Ishigaki Is.	SI	Coast (Beach)	1-2	10-20	W:Park	*Laurilignosa*
	Okinawa	Iriomote Is.	SI	Plain	20	<1k	W:Beside cropland	*Laurilignosa*
	Okinawa	Iriomote Is.	SI	Mountain	100-200	<2k[3)]	W:Natural botanical garden	*Laurilignosa*
	Okinawa	Kunigami	SI	Coast	<100	1k	W:Beside village	*Laurilignosa*
	Okinawa	Kunigami	SI	Coast	<100	<1k	W:Shelter belt	*Conilignosa* (Pine)
	Kagoshima	Sata	WTC	Coastal Mountain	<100	100-200	W	Evergree, *Cryptomeria*
	Kochi	Muroto	WTC	Coastal Mountain	200	<1k	W:Beside orchard	*Laurilignosa*
	Wakayama	Susami	WTC	Coastal Hill	100	100	W	*Laurilignosa*
	Wakayama	Susami	WTC	Small Island	<2	<5	W	*Laurilignosa*
	Wakayama	Kushimoto	WTC	Coast (Tip of Cape)	70-80	<20	W+G:Park	Pine, Evergreen, Turf
	Mie	Ago	WTC	Plain	<50	3-3.5k	G:Beside cropland	Herb, Grass
	Shizuoka	Omaezaki	WTC	Coast	50	100-200	W	*Laurilignosa*
	Chiba	Kujukuri	WTC	Coast (Beach)	<5	10-20	G:Beach	Grass
megidis	Aichi	Atsumi	WTC	Coast (Tip of Headland)	<10	<50	W	Pine, Evergreen
	Shizuoka	Omaezaki	WTC	Plain	20-30	1k	W:Forest of Shinto shrine	*Conilignosa*, Evergreen
	Shizuoka	Toi	WTC	Coast (Tip of Cape)	40-50	<50	W	*Laurilignosa*, Bamboo grass
	Shizuoka	Nishi-Izu	WTC	Coastal Hill	10-20	500-600	W	*Laurilignosa*
	Shizuoka	Minami-Izu	WTC	Coast	50-60	<100	W:Beside park	*Laurilignosa*
	Nagano	Matsumoto	CM	Mountain	700-800	Inland	W	*Deciduilignosa* (Oak)
	Nagano	Matsumoto	CM	Mountain	700-800	Inland	W:Beside park	*Deciduilignosa* (Oak)
	Chiba	Iioka	WTC	Coastal Hill	100-200	700-800	W	*Laurilignosa*
	Chiba	Cyoshi	WTC	Coast	<5	100-200	W	Evergreen, Pine, Grass

1) CM: Central Mountainous region; CT: Cool Temperate region; SI: Subtropical Islands; T: Temperate region; WTC: Warm Temperate Coastal region. 2) W: Woodland, G: Grassland. 3) The site is close to swamp land in brackish-water area.

islands) affected the formation of the endemic fauna and flora in the each island (Ito, 1995).

The incidence of heterorhabditids (9.7%) in the temperate zone including Kyushu to Hokkaido and Oki Isls. was comparable with the survey in Ireland (10.5%; Griffin *et al.*, 1994*b*). Both the Japanese and Irish surveys found heterorhabditids mainly in the coastal belt. However, the vegetation of the sites with heterorhabditids and the species constitution were different: in Ireland, heterorhabditid isolates (*H. megidis* species complex) were observed only from dunes or grassland (Griffin *et al.*, 1994*b*); during the present survey, *H. indica* was observed from grassland and woodland and *H. megidis* from woodland (Table 1).

Table 2. *Sympatric occurrence of steinernematids and heterorhabditids in some sites.*

Locality	Site No.	*Steinernema* spp.						*Heterorhabditis* spp.		Total
		kushidai	MY1	MY2	MY3	MY6	sp.?	*indica*	*megidis*	
Kagoshima	Sata 2		1/11					1/11		2/11
Wakayama	Kushimoto 3	1/10	1/10							2/10
	Kushimoto 4		2/10					2/10		3/10[1)]
Aichi	Atsumi 1	1/30		1/30	3/30				1/30	6/30
Shizuoka	Omaezaki 1				2/11				1/11	3/11
	Omaezaki 2						1/12	1/12		2/12
	Atami 1		3/7		1/7					4/7
	Toi 1		3/5						1/5	3/5[2)]
	Nishi-Izu 1		1/2				1/2		1/2	2/2[3)]
	Minami-Izu 1		1/5		1/5					2/5
	Minami-Izu 2		2/10						1/10	2/10[2)]
Nagano	Matsumoto 1	1/5	1/5			1/5			1/5	3/5[2)]
	Matsumoto 2						1/5		1/5	2/5
Chiba	Iioka 3		1/7						2/7	2/7[2)]

Each value indicates the no. positive samples / no. samples from a site.
sp. ?: unidentified isolate
1) *Steinernema* sp. MY1 and *Heterorhabditis indica* from a single sample.
2) *Steinernema* sp. MY1 and *H. megidis* from a single sample.
3) *Steinernema* sp.? and *H. megidis* from a single sample.

In the case of the American survey, *H. megidis* was found from grassland (golf course; Poinar *et al.*, 1987), while in the Canadian survey it was found only from orchards (Mráček & Webster, 1993). Other European studies (Smits *et al.*, 1991; Joyce *et al.*, 1994*a*; Hominick *et al.*, 1995; Miduturi *et al.*, 1996) recorded the *H. megidis* species complex from inland location. Accordingly, it seems that the occurrence of the *H. megidis* species complex is restricted neither by the vegetation nor by the configuration at the species group level, as these isolates are considered for the moment to be very closely related or conspecific. The different habitat preference among the isolates in these various countries is probably due to differences in the distribution and/or the species of suitable host insects, in the subspecies or strains of the nematode, and in the vegetation differences among these countries.

From the isolation pattern (Fig. 3, Table 1), the steinernematid species could be divided into four groups: three temperate widespread species (*Steinernema* spp. MY1 and MY3, and *S. kushidai*); three mountainous species (*Steinernema* spp. MY5, MY6, and MY7); one coastal species (*Steinernema* sp. MY2); and one subtropical species (*Steinernema* sp. MY4) (Fig. 3, Table 1). *Steinernema* sp. MY1 was the most prevalent species in Japan, which is similar to the dominance of *S. feltiae* in Ireland (Blackshaw, 1988) and Britain (Hominick & Briscoe, 1990). It occurred in various temperature regions from the hot environment of Cape Sata-misaki, at the south end of Kyushu, to the cold environment of Hokkaido and from the coast to mountains about 1400 m in elevation. *Steinernema* sp. MY3 was the second most prevalent species in the temperate zone and occurred from the coast to mountains about 1000-1100 m in elevation. Recently, this species was isolated from the mountain area of the northern part of Kyushu and Hokkaido (Yoshida unpubl.). The two species had almost the same distribution pattern and were isolated from similar environments (Table 1), but they occurred separately from each other except on two sites (Table 2). *S. kushidai* occurred widely in the warm temperate coastal region from Kochi to Shizuoka, especially in coastal forest. The species was first detected from an inland tree nursery (Hamakita City, Shizuoka Pref.), in the warm temperate region (Kushida *et al.*, 1987). It has been considered to be more adapted to warm environments because of their activity (Kushida *et al.*, 1987). However, this species was also isolated from the mountain region in a cool climate zone in this survey (Table 1).

In contrast with the former three wide-spread species, another five uncommon species were found only in specific regions (Fig. 3, Table 1) and this may reflect a greater adaptation to a specific habitat. *Steinernema* sp. MY4 is considered to be a subtropical species, found only in laurel forest. A subtropical island, Yonaguni Is. is close to Taiwan rather than the Japanese main land and the species was not isolated from any other subtropical islands in Japan. *Steinernema* sp. MY2 is considered to be a coastal species, found only in beach-side forest or grassland and sandy soil. Natural sandy beaches were well conserved in the three sites where this species was isolated. *Steinernema* spp. MY5, MY6, and MY7 were rare and isolated only from the forest at high elevation. These species may therefore be considered to prefer cool or cold mountain environment.

With respect to the heterorhabditids, it was considered that *H. indica* was a widespread, coastal species that was not restricted to beach sites and that *H. megidis* was a temperate widespread species. Moreover the two species also appear to differ in their vegetation preference range. *H. indica* occurred in various vegetation: laurel and pine forest, grassland beside cropland, a turf grass site in a park and a beach having only a few grasses and no trees (Table 1), from dry open land like a beach to wet, well-developed forest. By contrast, most of the Japanese isolates of *H. megidis* occurred only in woodland: laurel, deciduous and pine forest (Table 1). Accordingly, in Japan, it is considered that the occurrence of *H. indica* is restricted by the configuration (coastal site) and that the occurrence of *H. megidis* is restricted by the vegetation (forest site).

In previous surveys, one isolate of *S. carpocapsae* (= *S. feltiae*) and four isolates of *Steinernema* spp. were reported from the north part of Honshu (Mamiya & Ogura, 1989), and two isolates of *S. carpocapsae* and 22 isolates of *S. feltiae* from Kyushu (Kimura & Ishibashi, 1991). Although the present survey did not extensively sample those areas (only Kagoshima, Ibaraki, and Sendai overlapped with regions of the previous surveys; Fig. 1), neither *S. carpocapsae* nor *S. feltiae* were detected. There were no isolates having short infective juveniles like *S. carpocapsae*, other than *S. kushidai*. Many isolates examined morphologically had infective juveniles with some measurements that overlapped those of *S. feltiae*. However no isolates were identified as *S. feltiae* from either RFLP patterns or morphological observations. Moreover, a recent resampling conducted in Kyushu (including some sites where *S. carpocapsae* or *S. feltiae* had been detected) only found *Steinernema* spp. MY1 and MY3 (Yoshida unpubl.). *Steinernema* sp. MY4 was morphologically similar to *S. affine*, which to date has only been reported from northern Europe (Poinar, 1990), but the RFLP patterns were significantly different between these two species. Cross breeding tests have not been conducted yet, since *Steinernema* sp. MY4 was not able to reproduce in either *in vivo* or *in vitro* cultures (despite the nematode being isolated by using the *Galleria* trap method). The RFLP pattern of *Steinernema* sp. MY7 was similar to that of *S. intermedium*. However the morphometrics of infective juveniles were different.

The seven steinernematid species other than *S. kushidai* are new to science as indicated by RFLP analysis and/or morphological study. To date, these steinernematid species are indigenous to Japan. By contrast, all the heterorhabditid isolates were either *H. indica* or *H. megidis*. *H. indica* occurs widely in tropical zones in Egypt (Shamseldean & Abd-Elgawad, 1996), India (Poinar *et al.*, 1992), Sri Lanka, northern Australia (Hominick *et al.*, 1996), and Cuba (Joyce *et al.*, 1994*a*). Therefore, it could be that the distribution pattern of *H. indica* in Japan was influenced by the warm current "Kuroshio" which starts from the Philippine Sea, flows along Taiwan, Nanseishoto Isls., and the southern coast of Japan and departs from Japan off the east coast of the Boso Peninsula (Wadachi, 1987). It has been suggested that this warm current affects the distribution of a number of insects, especially wood-boring insects (Browne, 1961; Nobuchi & Makihara, 1987). Browne (1961) noted "Currents have also undoubtedly assisted in conveying elements of the Malaysian fauna to southern Japan." Moreover, Konishi (1991) suggested that some parasitic wasps of wood-boring insects enlarged their distribution area by means of driftwood floating on the ocean current with their hosts inhabiting the woods.

The *H. megidis* species complex occurs widely in temperate zones in North America (Poinar *et al.*, 1987; Mráček & Webster, 1993) and Europe (Reid, 1994; Hominick *et al.*, 1995). There appears to be three closely related RFLPs types –Ohio, NWE, and Irish (Joyce *et al.*, 1994*a*; Reid, 1994; Hominick, *et al.*, 1996)– and the Japanese isolates were determined to be of the Ohio type. Many similarities between eastern Asian biota and eastern North American biota are also known (Pielou, 1979). Accordingly, it may be that Japanese *H. megidis* is a native species and not an introduced species such as the Australian *S. feltiae* (Akhurst & Bedding, 1986), because of its occurrence inland (Fig. 4) and coexistence with steinernematids (Table 2) in well-developed forest. However, as the distribution of the heterorhabditids in the coastal region of Ireland and Britain was suspected to be affected by transport by sea water (Griffin *et al.*, 1994*a*), more surveys in eastern Asia are necessary to show the distribution pattern of *H. megidis* and the origin of the Japanese isolates.

This survey indicates that the Japanese environment has a rich entomopathogenic nematode fauna, much of it specific to Japan, and that each nematode has a characteristic distribution pattern. Especially, nine of ten species were recorded from mountains. This may result from the geographic complexity and biological diversity of the mountain environment that provides numerous and various insects as potential hosts. As most of the land in Japan is mountainous, it might be speculated that most of the Japanese entomopathogenic nematodes were more adapted and/or specialized to a mountain environment. It is expected that other species or strains of steinernematids and heterorhabditids will be isolated from other environments, as many potential habitats remain to be explored.

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