

Studies on natural occurrence of entomopathogenic nematodes in Vietnam

Chau N. Nguyen* and Dieter Sturhan**

* Department of Nematology, Institute of Ecology and Bioresources, VAST,
18 Hoang Quoc Viet, Hanoi, Vietnam,

**Formerly: Institut für Nematologie und Wirbeltierkunde, BBA,
Toppheideweg 88, 48161 Münster, Germany.

Accepted for publication 19 November 2005

Summary. A total of 459 soil samples taken from the north to the south of Vietnam and from the coast to the high mountains were studied for the presence of *Steinernema* and *Heterorhabditis*. Using the baiting method with wax-moth larvae, 4.8% of all samples proved to be positive for entomopathogenic nematodes, and using direct extraction of the nematodes from soil, 28% of the samples were positive. Ten *Steinernema* species and four *Heterorhabditis* species could be distinguished, based mainly on morphological characters of the infective juveniles. Five of these species have been described from Vietnam (*Steinernema tami*, *S. loci*, *S. sangi*, *S. thanhi* and *Heterorhabditis baujardi*). With the exception of *H. indica*, all the other 'species' found could not be identified; several of the populations isolated appeared to be representatives of undescribed species. The highest diversity of entomopathogenic nematodes was found in forests, with eight *Steinernema* and three *Heterorhabditis* species.

Key words: Distribution, entomopathogenic nematodes, forests, occurrence, *Steinernema*, *Heterorhabditis*, Vietnam.

Entomopathogenic nematodes of the genera *Steinernema* and *Heterorhabditis* are promising biological control agents for many important insect pests and thus have attracted particular attention worldwide. More than 50 species in both genera are currently known, most of them described in recent years, among them several species from the East-Asian region. Numerous surveys on the occurrence and distribution of these nematodes have been conducted in many regions worldwide, including east and south-east Asian countries such as Japan (Yoshida *et al.*, 1998), Korea (Lee *et al.*, 1996), Malaysia (Mason *et al.*, 1996) and Indonesia (Griffin *et al.*, 2000).

Studies of entomopathogenic nematodes in Vietnam have started only recently. Nguyen and Nguyen (1997) recorded *Heterorhabditis* and *Steinernema* from Vietnam for the first time, and subsequently more data on natural occurrence of these nematodes were added (Nguyen *et al.*, 1999). Pathogenicity tests were made with indigenous *Steinernema* and *Heterorhabditis* isolates obtained with the *Galleria mellonella* baiting technique (Nguyen *et al.*, 1999). Subsequently, several isolates of entomopathogenic nematodes previously unknown to science have been described from

Vietnam: *Steinernema tami* Luc, Nguyen, Reid & Spiridonov, 2000, *S. sangi* Phan, Nguyen & Moens, 2001, *S. loci* Phan, Nguyen & Moens, 2001, *S. thanhi* Phan, Nguyen & Moens, 2001, *S. robustispiculum* Phan, Subbotin, Waeyenberge & Moens, 2005, *Heterorhabditis baujardi* Phan, Subbotin, Nguyen & Moens, 2003 and *H. indica* Poinar, Karanukar & David, 1992 (Phan *et al.*, 2003). Of the new species *S. tami*, *S. sangi* and *S. robustispiculum* were recorded from forest soil, *S. loci* from sea beach and *S. thanhi* from garden soil near the beach. The description of *H. baujardi* is based on three populations collected in forests, and *H. indica* has been recorded for six different forests.

The present paper summarises observations on the occurrence and distribution of entomopathogenic nematodes in Vietnam. Most of the studies were conducted in the years from 1999 to 2002 using the baiting method with larvae of the wax-moth, *Galleria mellonella*, as bait. In addition, a method of direct extraction of infective juveniles from soil samples was used, and identification of entomopathogenic nematodes found in the nematode suspensions was made. The morphometrics and some additional morphological

characteristics of the infective juveniles of the *Steinernema* and *Heterorhabditis* species found are presented. Several of the species identified have been described as new species; however, the majority of populations could not be identified as members of any of the species of *Steinernema* and *Heterorhabditis* currently known.

MATERIAL AND METHODS

The survey for entomopathogenic nematodes has been extended to all main regions of Vietnam, where a total of 459 soil samples were collected in several different and typical biotopes of the country. Among the samples, 65 were from fields, 66 from orchards, 27 from coast line and 301 from forests. The soil samples were taken from the upper 0-35 cm soil layer and each consisted of 3-4 cores. From trees, the cores originated from around the base of a single tree or of adjacent trees of the same species. In the laboratory each soil sample was gently mixed and separated into two subsamples of 250 ml volume each. One part of the soil was used for baiting with *Galleria mellonella* larvae.

A total of 104 samples of the second part of the soil samples were used for direct extraction of nematodes from soil using a sieving-decanting procedure combined with a still-filtering technique (Nguyen & Nguyen, 1993). Sieving was first through a screen with 0.5 mm openings to remove coarse litter and pebbles, followed by filtering through a 200 mesh (75 µm openings) sieve to retain the nematode sediment. After gentle washing, the sediment was transferred to special 75 mm wide and 20 mm high sieves with 75 µm openings. Each of these sieves was placed in a Petri dish (90 mm diameter) and left for 48 h at room temperature. The nematode suspensions obtained were killed by gentle heat, fixed with TAF and microscopically checked for the presence of infective-stage juveniles of *Steinernema* and *Heterorhabditis*. Nematodes from the isolates obtained by the baiting method were killed and fixed in the same way. Infective-stage juveniles of all samples were processed to anhydrous glycerine and mounted on permanent microscope slides for subsequent microscopic studies. Most voucher slides are deposited in the slide collection of the Department of Nematology, Hanoi, and some slides are in the German Nematode Collection, Münster.

RESULTS

A total of 22 of the 459 soil samples baited with *Galleria* revealed entomopathogenic nematodes (= 4.8%). The method of direct extraction from soil

yielded 29 of the 104 samples 'positive' for entomopathogenic nematodes (= 28%). Based on morphometrics and other morphological characteristics of the infective juveniles, ten *Steinernema* and four *Heterorhabditis* species could be distinguished (Table 1). Three of the *Steinernema* isolates obtained were meanwhile described as 'new' species (*S. loci*, *S. sangi*, *S. thanhi*) being isolated by the baiting method; of all other species or populations identified, no stages other than infective juveniles were available, and frequently only a few specimens or even a single specimen were isolated.

Most of the species were found in tropical primary and secondary forests (eight *Steinernema* and four *Heterorhabditis* species), one *Steinernema* species in a household orchard, another *Steinernema* species and a *Heterorhabditis* species in sandy soil close to the coast. One *Heterorhabditis* species was isolated by the baiting method from a temperate mountain region 2000 m above sea level but, unfortunately, this isolate could not be maintained in the laboratory because it failed to produce a second generation on *Galleria mellonella*. Table 1 summarises details about habitat, location and method of recovery.

At the coast line (Thach Ha, Ha Tinh prov.), *S. loci* and *Heterorhabditis* sp. 4 occurred together, in a sample from Muong Phang forest in Dien Bien province *Steinernema* sp.1 occurred together with *Steinernema* sp. 6, in another sample from the same forest *Steinernema* sp.1 plus *Steinernema* sp. 2, and in two samples from tropical forest in Cuc Phuong national park *Steinernema* sp. 2 occurred in a mixed population with *Steinernema* sp. 3, and *Steinernema* sp. 4 occurred together with *Heterorhabditis* sp. 1.

Morphometrics of the separated species of *Steinernema* are given in Table 2, and morphometrics of the *Heterorhabditis* species are given in Table 3. Additional morphological characteristics are summarized briefly below. For details about the identified *Steinernema* species see the original descriptions (Phan *et al.*, 2001a, b).

Steinernema sp. 1

A medium-sized *Steinernema* species with juvenile morphological characters of the *S. affine* group. In its morphometrics and other morphological characters it closely resembles *S. affine*; the excretory pore is in a slightly more anterior position and the hyaline tail portion slightly longer. Body of heat-relaxed specimens C-shaped, lip region continuous with body contour, conoid and mostly slightly flattened anteriorly.

Table 1. Entomopathogenic nematodes from Vietnam obtained in the present study, their prevalence in various biotopes, sites of occurrence and method of recovery.

No.	Species	Biotope and locality (number of positive samples)	Method of recovery
1	<i>Steinernema</i> sp. 1	Primary tropical forest, Ngoc Linh mountain, 1000 m above sea level, Quang Nam prov. (3)	Extraction
		Secondary tropical forest, Muong Phang, Dien Bien prov. (6)	Extraction
		Primary tropical forest, Thuong Xuan, Thanh Hoa prov. (2)	Extraction
		Primary tropical forest, Ba Vi national park, Ha Tay prov. (3)	Extraction
		Primary tropical forest, Cuc Phuong national park, Ninh Binh prov. (2)	Extraction
2	<i>Steinernema</i> sp. 2	Primary tropical forest, Cuc Phuong national park, Ninh Binh prov. (2)	Baiting and extraction
		Primary tropical and secondary forest, Muong Phang, Dien Bien prov. (1)	Extraction
		Forest, Cuc Phuong national park, Ninh Binh prov. (1)	Extraction
3	<i>Steinernema</i> sp. 3	Stone forest, Ha Long, Thach An, Cao Bang prov. (2)	Extraction
		Primary tropical forest, Cuc Phuong national park, Ninh Binh prov. (1)	Extraction
4	<i>Steinernema</i> sp. 4	Secondary bamboo forest, Dien Bien, Lai Chau prov. (1)	Extraction
		Primary tropical forest, Cuc Phuong national park, Ninh Binh prov. (1)	Extraction
5	<i>Steinernema</i> sp. 5	Secondary bamboo forest, Quan Ba, Ha Giang prov. (1)	Extraction
6	<i>Steinernema</i> sp. 6	Secondary bamboo forest, Muong Phang, Dien Bien prov. (1)	Extraction
7	<i>Steinernema</i> sp. 7	Primary tropical forest, Cuc Phuong national park, Ninh Binh prov. (1)	Extraction
8	<i>Steinernema loci</i>	Bush tree on coastline, sandy soil, 150 m from sea, Thach Ha, Ha Tinh prov. (1)	Baiting and extraction
		Stone forest, Dong Loan, Cao Bang prov. (1)	Extraction
9	<i>Steinernema thanhi</i>	Household orchard, Tinh Gia, Thanh Hoa prov. (1)	Baiting and extraction
10	<i>Steinernema sangi</i>	Primary tropical forest, Thuong Xuan, Thanh Hoa prov. (1)	Baiting and extraction
11	<i>Heterorhabditis</i> sp. 1	Secondary forest, Dien Bien, Lai Chau prov. (2)	Baiting and extraction
		Primary tropical forest, Cuc Phuong national park, Ninh Binh prov. (1)	Extraction
12	<i>Heterorhabditis</i> sp. 2	Soil around base of a wild tea, temperate mountain, 2000 m above sea level, Sapa, Lao Cai prov. (1)	Baiting and extraction
13	<i>Heterorhabditis</i> sp. 3	Primary tropical forest, Nam Cat Tien national park, Dong Nai prov. (1)	Extraction
14	<i>Heterorhabditis</i> sp. 4	Bush tree on coastline, sandy soil, 150 m from sea, Thach Ha, Ha Tinh prov. (1)	Baiting and extraction
		Trees at coast, sandy soil, Cu Lao Cham Islands, Quang Nam prov. (1)	Extraction

Table 2. Morphometrics of infective juveniles of *Steinernema* species from Vietnam.

Character	<i>Steinernema</i> sp. 1 n=20	<i>Steinernema</i> sp. 2 n=26	<i>Steinernema</i> sp. 3 n=20	<i>Steinernema</i> sp. 4 n=4	<i>Steinernema</i> sp. 5 n=1	<i>Steinernema</i> sp. 6 n=1	<i>Steinernema</i> sp. 7 n=8	<i>Steinernema</i> <i>loci</i> * n=50	<i>Steinernema</i> <i>thanhi</i> * n=50	<i>Steinernema</i> <i>sangi</i> * n=50	<i>Steinernema</i> <i>tami</i> * n=20
Body length (μm)	657 \pm 58 526-776	500 \pm 25 466-539	580 \pm 36 512-629	362 \pm 30 310-389	1038	1009	537 \pm 26 500-582	986 \pm 45 896-1072	851 \pm 57 720-960	753 \pm 18 704-784	530 \pm 40 400-600
Body width (μm)	24.8 \pm 2.7 21.6-30.8	21.4 \pm 0.9 21.6-30.8	21.5 \pm 1.6 19.6-25.0	21.5 \pm 1.6 19.6-25.0	29.9	28.5	23.7 \pm 1.3 21-25	36.5 \pm 2.7 30.4-44.8	31.4 \pm 2.5 27.3-2-39.2	34.5 \pm 2.2 29.6-39.6	23 \pm 2 19-29
Anterior end to excr. pore (μm)	51.2 \pm 4.2 41.6-57.3	31.2 \pm 1.3 28.5-34.3	33.2 \pm 1.5 30.4-35.3	33.2 \pm 1.5 30.4-35.6	74.5	70.0	54.6 \pm 1.9 52-57.5	80.1 \pm 3.3 71.2-86.4	74.8 \pm 3.8 67.6-84.4	50.5 \pm 1.8 46.0-53.6	36 \pm 2 34-41
Oesophagus length (μm)	112 \pm 6.4 101-124.5	101 \pm 4.2 93-110	112 \pm 6.8 97-126	85.5 \pm 1.4 83-87	144	139	115 \pm 5 107-125	141 \pm 5.8 126-155	129 \pm 6.4 115-146	127 \pm 3.8 120-138	117 \pm 4 110-123
Tail length (μm)	64 \pm 7.7 54-83	45 \pm 1.9 42-49	52 \pm 2.2 47-55	36 \pm 2.4 33-39	71	88	63 \pm 1.5 61-66	75 \pm 4.0 66-83	63 \pm 4.4 52-72	81.3 \pm 3 76-89	50 \pm 4 42-57
Hyaline tail length (μm)	34.3 \pm 5.3 27.4-50.9	19.1 \pm 1.3 16.2-21.5	15.4 \pm 1.5 12.0-17.6	15.2 \pm 1.2 13.2-16.2	38	50	34.4 \pm 1.9 31-37	Not available	Not available	Not available	Not available
a	26.6 \pm 2.3 20.7-30.0	23.5 \pm 1.1 20.7-26.9	26.9 \pm 1.6 23.6-30.5	21.1 \pm 0.3 20.6-21.5	34.7	34.5	22.7 \pm 1.2 20.6-24.4	27.1 \pm 1.4 23.9-29.7	27.1 \pm 1.9 23.3-33.2	21.8 \pm 1.2 19.3-24.3	23 \pm 2 19-28
b	5.8 \pm 0.3 5.0-6.5	4.8 \pm 0.2 4.5-5.4	5.2 \pm 0.3 4.8-5.9	4.2 \pm 0.3 3.7-4.5	7.2	7.2	4.7 \pm 0.1 4.4-4.8	7.0 \pm 3.6 6.3-7.9	6.6 \pm 0.32 5.8-7.6	5.9 \pm 1-0.7 5.5-6.3	5 \pm 0.3 3.7-5.1
c	10.4 \pm 0.5 9.3-11.6	11.0 \pm 0.5 9.3-11.8	11.2 \pm 0.5 10.0-12.2	10.0 \pm 0.5 9.3-10.8	14.7	11.4	8.6 \pm 0.5 7.9-9.3	13.1 \pm 0.85 11.4-15.4	13.5 \pm 0.7 12.0-15.5	9.2 \pm 0.3 8.6-10.1	11 \pm 0.5 9-11
c'	4.4 \pm 0.5 3.8-6.0	4.0 \pm 0.2 3.7-4.4	3.7 \pm 0.4 3.2-4.6	4.1 \pm 0.2 3.9-4.4	3.6	5.6	4.5 \pm 0.3 4.1-5.2	57 \pm 3 52-63	58 \pm 4 52-67	40 \pm 1.7 36-44	Not available
EP (%) ¹	45.5 \pm 2.4 40.4-49.5	30.8 \pm 1.1 28.1-32.6	29.5 \pm 1.8 24.4-32.4	32.8 \pm 0.4 32.4-33.5	51.7	50.3	47 \pm 2.3 43-51	56.8 \pm 2.6 51.63-62.5	58.1 \pm 3.2 51.9-66.6	39.7 \pm 1.6 36.2-44.0	31 \pm 0 28-34
H (%) ²	53.7 \pm 3.6 46.2-61.1	42.2 \pm 2.8 36.7-51.2	30.0 \pm 3.0 22.6-33.9	42.1 \pm 3.0 39.6-47.2	53.5	56.8	55 \pm 2.2 50.5-58	46 \pm 3 40-52	40 \pm 4 33-46	49 \pm 2.6 44-52	40 \pm 10 30-50

¹ Anterior end to excretory pore in % of oesophagus length.

² Length of hyaline tail portion in % of tail length.

* Data from original descriptions.

Table 3. Morphometrics of infective juveniles of *Heterorhabditis* species from Vietnam obtained in the present study.

Character	<i>Heterorhabditis</i> sp. 1 n=22	<i>Heterorhabditis</i> sp. 2 n=24	<i>Heterorhabditis</i> sp. 3 n=6	<i>Heterorhabditis</i> sp. 4 n=20
Body length (µm)	538 ± 30 509-605	510 ± 31 446-576	525 ± 14.5 507-540	482 ± 67.5 332-614
Body width (µm)	18.3 ± 1.1 15.7-20.6	20.4 ± 1.1 18.4-22	23.7 ± 1.8 21.2-25.8	25.6 ± 3.6 19.5-35.5
Anterior end to excr. pore (µm)	98.2 ± 3.9 95-109.6	76.6 ± 5.8 67.6-88.3	93.4 ± 3.9 89.2-97.5	89 ± 12 69.5-110
Oesophagus length (µm)	113 ± 3.5 110.3-121.5	110.3 ± 3 103-117	118.9 ± 2.4 115-122.4	113.5 ± 14 90.5-137
Tail length (µm)	96.5 ± 9.3 88.2-127.4	88.4 ± 6.8 74.5-102	36 ± 4.2 30.4-41.4	62 ± 12 44.5-87
a	29.3 ± 1.1 28.2-32.4	24.9 ± 1.8 21.6-30	22 ± 0.7 21-23	19.4 ± 2.4 13.5-22.5
b	4.7 ± 0.2 4.6-5.6	4.6 ± 0.2 4.0-5.1	4.6 ± 0.1 4.5-4.8	4.3 ± 0.4 3.3-5.4
c	5.5 ± 0.3 4.7-5.7	5.8 ± 0.3 5.2-6.5	14.8 ± 2 13-17.7	8.1 ± 1.4 6-11.7
c'	8.3 ± 0.6 7.4-9.8	6.3 ± 0.5 5.1-7.4	5.2 ± 0.3 4.7-5.7	Not available
EP (%)	87 ± 2.5 81.5-91.5	69 ± 4.7 61.7-78.6	77 ± 2.2 74.4-81	78 ± 4 71-86

Lateral fields with indistinct marginal lines, prominent submarginal lines and three indistinct inner lines. Bacterial vesicle large, generally more than two body widths long, with large bacteria. Tail usually with a slight depression at the dorsal side, tail terminus pointed, non-refractile.

Steinernema sp. 2

A small *Steinernema* species with excretory pore in an anterior position. Body slightly curved to almost straight. Lip region rounded, not offset from body contour. Lateral fields with marginal dorsal line rather indistinct, submarginal dorsal line prominent and extending as an elevated ridge to almost tail end; ventral marginal ridge of lateral field consisting of two close lines, rather distinct; inner four lines indistinct. Tail concave at ventral side, curved ventrad, terminus slender, finely pointed.

Steinernema sp. 3

A rather short *Steinernema* species with the excretory pore in an anterior position and with a short hyaline tail portion. Body of heat-relaxed specimens curved to almost straight. Most of the infective juveniles still within second-stage cuticle (which is rarely observed in *Steinernema* juveniles extracted from soil). Anterior end rather wide, continuous with body contour and rounded anteriorly. Lateral field with submarginal lines distinct and usually also the dorsal marginal line, other lines indistinct. Tail straight, evenly conoid, terminus pointed or finely rounded. In measurements and other morphological characters this species is close to *S. carpocapsae* and *S. websteri*.

Steinernema sp. 4

An extremely small species, shorter than any of the *Steinernema* species described so far, with excretory pore in anterior portion of body. Body slightly curved to the ventral side, lip region rounded, continuous, tail conoid and slightly concave at ventral side. Tail terminus sharply pointed. Bacterial vesicle small. Lateral field with dorsal marginal and submarginal lines and ventral marginal lines distinct, inner lines indistinct.

Steinernema sp. 5

Only a single, poorly preserved infective juvenile found, belonging to the *S. glaseri* group: Body >1000 µm long, lip region broad, excretory pore posterior to middle of oesophagus, lateral field wide, with nine evenly spaced and developed lines, tail straight, narrowing in hyaline posterior part, c-ratio rather high.

Steinernema sp. 6

Also only one specimen found, with rather long body, but probably not a member of the 'glaseri' group. Body almost straight. Lip region rounded. Tail straight and more or less uniformly tapering to a pointed terminus; hyaline tail portion more than 50% of tail length. Excretory pore located behind middle of oesophagus. Lateral field with marginal and/or submarginal lines rather distinct and inner lines indistinct.

Steinernema sp. 7

Infective juveniles rather short, with curved body, excretory pore in mid-oesophagus region, straight conical tail with dorsal depression, large bacterial vesicle containing long bacteria, lateral fields with only the submarginal lines distinct.

Main character distinguishing this species from all described *Steinernema* species is the asymmetrical and oblique lip region, which is recessed towards the dorsal side.

***Heterorhabditis* spp.**

Four populations, which may be representatives of separate species, could be distinguished using morphometrics of the infective-stage juveniles. In most of the morphological characters, *Heterorhabditis* sp. 1 and *Heterorhabditis* sp. 2 resemble *H. indica* and *H. baujardi*. Both species are rather widely distributed in Vietnam; they are morphologically differentiated only by spicule shape in male, but they are nearly the same in infective-stage juveniles characters (Phan *et al.*, 2003a). Two populations of *Heterorhabditis* sp. 1 extracted from soil samples of Muong Phang forest and *Heterorhabditis* sp. 2 extracted from a soil sample of Sapa mountain forest are distinguished only by the distance of the anterior end to excretory pore and index 'c'. *Heterorhabditis* sp. 3 is mainly characterised in having the shortest tail among the four 'species' differentiated. In *Heterorhabditis* sp. 4, the average body length and index 'a' are less than in any of the currently known *Heterorhabditis* species (Tab.2).

DISCUSSION

The survey revealed that entomopathogenic nematodes are widely distributed in Vietnam, occurring from the north to the south of the country, and from the coastal area to the high mountain region. The trap method with *G. mellonella* larvae as a bait appears to indicate a low prevalence of *Steinernema* and *Heterorhabditis* (4.8% positive samples) but direct extraction of infective juveniles from soil indicated a much greater incidence of entomopathogenic nematodes (28% positive samples). The greater efficiency may be due to the fact that the wax-moth *Galleria* is not a suitable host for certain of the *Steinernema* and *Heterorhabditis* species and that entomopathogenic nematodes occurring at low population densities are unable to cause an infection of the host.

The species diversity is high in Vietnam. Besides *S. loci*, *S. sangi*, *S. thanhi* and *S. robustispiculum* previously reported from Vietnam (Phan *et al.*, 2001a, b, 2003b), seven additional *Steinernema* 'forms' or 'species' could be distinguished by morphological characteristics of the infective-stage juveniles, one of these possibly being *S. tami*, a species described from the primary forest in Cat Tien National Park (Luc *et al.*, 2000). Four *Heterorhabditis* 'species' were distinguished, two of

which have infective juvenile morphological characters that are close to those of *H. indica* and *H. baujardi*, and at least one of the remaining two 'species' obviously represents an undescribed species. *Heterorhabditis indica* has been reported from ten sites in Vietnam and the description of *H. baujardi* is based on isolates from three localities in Vietnam (Phan *et al.*, 2003a).

Surveys for entomopathogenic nematodes in other Asian countries, which were all conducted using the *Galleria* baiting method, differed in part from the results obtained for Vietnam. In Japan, 10% of the soil samples were positive; eight *Steinernema* and two *Heterorhabditis* species were found (Yoshida *et al.*, 1998). In 200 samples from various biotopes in Korea, four revealed *Steinernema* and one *Heterorhabditis* (Lee *et al.*, 1996). In Malaysia, entomopathogenic nematodes were recovered from 10% of 425 samples assayed; two genetic types of *Steinernema*, *Heterorhabditis indica* and a new *Heterorhabditis* species were found (Mason *et al.*, 1996). In Indonesia, 20.3% of the sites sampled were positive for entomopathogenic nematodes (*H. indica*, two types of *Steinernema*), but all records were only from coastal sites (Griffin *et al.*, 2000). Also in Sri Lanka, entomopathogenic nematodes were only found in the coastal area (33 of 129 samples positive), where three *Steinernema* and two *Heterorhabditis* genetic types were distinguished, while none of 189 inland sites, including natural forests, provided a positive assay (Amarsinghe *et al.*, 1994). In Pakistan, entomopathogenic nematodes were detected from 12.4% of the sampling sites, with three *Steinernema* and 59 *Heterorhabditis* isolates obtained (Shahina *et al.*, 1998).

The number of species occurring in Vietnam appears to be higher than in most of these countries, as well as in most other countries worldwide, where surveys have been conducted. Five identified *Steinernema* species and two *Heterorhabditis* species had previously been reported for Vietnam. The present study has shown that many additional species are present in the country, among them most probably also species new to science. The species diversity is particularly high in natural forests but entomopathogenic nematodes appear to be almost absent in agricultural ecosystems.

Extension of the present survey in Vietnam is highly recommended, with improvement of the baiting method by using different trap hosts, the more frequent use of direct extraction of infective juveniles from soil and the inclusion of molecular methods for identification and discrimination of

isolates, populations or even a few selected specimens obtained by direct extraction from soil.

ACKNOWLEDGEMENTS

The senior author would like to thank the Deutscher Akademischer Austauschdienst, Bonn (DAAD) for a grant, which enabled him to undertake a research visit to Münster, Germany, in 1999. A visit to Vietnam by the second author in 2005 was financially supported by the Deutsche Forschungsgemeinschaft (DFG), Bonn-Bad Godesberg. The financial support for the surveys in Vietnam granted by the Vietnam National Program for Basic Research in Natural Sciences is also acknowledged.

REFERENCES

- Amarasinghe, L. D., Hominick, W. M., Briscoe B. R. & Reid, A. P. 1994. Occurrence and distribution of entomopathogenic nematodes in Sri Lanka. *Journal of Helminthology* 68: 277-286.
- Griffin, C. T., Chaerani, R., Fallon, D., Reid, A. P. & Downes, M. J. 2000. Occurrence and distribution of the entomopathogenic nematodes *Steinernema* spp. and *Heterorhabditis indica* in Indonesia. *Journal of Helminthology* 74: 143-150.
- Jian, H., Reid, A. P. & Hunt, D. J. 1997. *Steinernema ceratophorum* n. sp. (Nematoda: Steinernematidae), a new entomopathogenic nematode from north-east China. *Systematic Parasitology* 37: 115-125.
- Lee, S. M., Lee, D. W. & Choo, H. Y. 1996. Isolation of entomopathogenic nematodes and entomopathogenic fungi in the southern parts of Korea. *FRI Journal of Forest Science (Seoul)* 53: 110-116 (in Korean, with English summary).
- Liu, J. 1994. A new species of the genus *Heterorhabditis* from China. *Acta Zootaxonomica Sinica* 19: 268-272 (in Chinese).
- Luc, P. V., Nguyen, K. B., Reid, A. P. & Spiridonov, S. E. 2000. *Steinernema tami* sp.n. (Rhabditida: Steinernematidae) from Cat Tien Forest, Vietnam. *Russian Journal of Nematology* 8: 33-43.
- Mason, J. M., Razak, A. R. & Wright, D. J. 1996. The recovery of entomopathogenic nematodes from selected areas within Peninsular Malaysia. *Journal of Helminthology* 70: 303-307.
- Nguyen, N. C. & Nguyen, V. T. 1993. Modified method for extraction of nematodes from soils and plant tissues. *Achievements of Science and Technology Applied into Practice. NCST* 1: 41-45.
- Nguyen, N. C. & Nguyen, V. T. 1997. First records of natural occurrence of entomopathogenic nematodes in Vietnam. *Journal of Biology* 14: 24-29 (in Vietnamese, with English summary).
- Nguyen, N. C., Vu, T. M., Lai, P. H. & Ngo, X. T. 1999. Pathogenicity of two indigenous nematodes against insect pests in Vietnam. *Journal of Biology* 21: 109-118 (in Vietnamese, with English summary).
- Nguyen, N. C., Nguyen, V. T., Lai, P. H. & Phan, K. L. 1999. Preliminary surveys on natural occurrence of entomopathogenic nematodes in Vietnam. *Journal of Biology* 21: 94-100 (in Vietnamese, with English summary).
- Phan, K. L., Nguyen, N. C. & Moens, M. 2001a. *Steinernema sangi* sp.n. (Rhabditida: Steinernematidae) from Vietnam. *Russian Journal of Nematology* 9: 1-7.
- Phan, K. L., Nguyen, N. C. & Moens, M. 2001b. *Steinernema loci* sp.n. and *Steinernema thanhi* sp.n. (Rhabditida: Steinernematidae) from Vietnam. *Nematology* 3: 503-514.
- Phan, K. L., Subbotin, S. A., Nguyen, N. C. & Moens, M. 2003a. *Heterorhabditis baujardi* sp.n. (Rhabditida: Heterorhabditidae) from Vietnam and morphometric data for *H. indica* populations. *Nematology* 5: 367-382.
- Phan, K. L., Nguyen, N. C. & Moens, M. 2003b. Natural distribution of entomopathogenic nematodes (Rhabditida: *Steinernema* and *Heterorhabditis*) in Vietnam. In: *Problems of Basic Research in Life Sciences, Proceedings of the 2nd National Conference in Life Sciences, Hue, July 25-26, 2003*. Science and Technics Publishing House, Hanoi: 670-673 (in Vietnamese, with English summary).
- Phan, L. K., Subbotin, S. A., Waeyenberge, L., Moens, M. 2005. A new entomopathogenic nematode, *Steinernema robustispiculum* n.sp. (Rhabditida: Steinernematidae), from Chumomray National Park in Vietnam. *Systematic Parasitology* 60: 23-32.
- Shahina, F., Anis, M., Zainab, S. & Maqbool, M. A. 1998. Entomopathogenic nematodes from soil samples collected from Sindh, *Pakistan. Pakistan Journal of Nematology* 16: 41-50.
- Stock, S. P., Choo, H. Y. & Kaya, H. K. 1997. An entomopathogenic nematode, *Steinernema monticulum* sp. n. (Rhabditida: Steinernematidae) from Korea with a key to other species. *Nematologica* 43: 15-29.
- Stock, S. P., Samsook, V. & Reid, A. P. 1998. *Steinernema siamkaya* n. sp. (Rhabditida: Steinernematidae), an entomopathogenic nematode from Thailand. *Systematic Parasitology* 41: 105-113.
- Yoshida, M., Reid, A. P., Briscoe, B. R. & Hominick, W. M. 1998. Survey of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) in Japan. *Fundamental and Applied Nematology* 21: 185-198.
- Xu, Z., Wang, G. & Li, X. 1991. A new species of the genus *Steinernema* (Rhabditida: Steinernematidae). *Zoological Research* 12: 17-20 (in Chinese, with English summary).

C. N. Nguyen, D. Sturhan. Исследования по естественному распространению энтомопатогенных нематод во Вьетнаме.

Резюме. Было исследовано 459 проб почвы собранных от Севера до Юга Вьетнама, и от побережья до высокогорья. Пробы были обследованы на присутствие нематод родов *Steinernema* и *Heterorhabditis*. С использованием метода приманок с гусеницами большой вошинной моли энтомопатогенные нематоды были выявлены в 4.8% всех проб почвы. Метод прямого выделения личинок энтомопатогенных нематод выявил их в 28% всех проб. Основываясь на особенностях морфологии личинок, десять видов *Steinernema* и четыре вида *Heterorhabditis* могут быть дифференцированы. Пять из этих видов были описаны из Вьетнама (*Steinernema tami*, *S. loci*, *S. sangi*, *S. thanhi* and *Heterorhabditis baujardi*). За исключением *H. indica*, все другие выявленные формы не могут быть определены до вида. Несколько из выделенных популяций явно представляют новые виды. Наивысшее разнообразие фауны энтомопатогенных нематод было обнаружено в тропических лесах (восемь видов *Steinernema* и три вида *Heterorhabditis*).
