PRELIMINARY SURVEY OF ENTOMOPATHOGENIC NEMATODES IN UPPER NORTHERN THAILAND

Apichat Vitta[1,2,3], Chamaiporn Fukruksa[1], Thatcha Yimthin[1], Kitsakorn Deelue[1], Chutima Sarai[1], Raxsina Polseela[1,2,3] and Aunchalee Thanwisai[1,2,3]

[1]Department of Microbiology and Parasitology, [2]Center of Excellence in Medical Biotechnology (CEMB), Faculty of Medical Science, Naresuan University; [3]Center of Excellent for Biodiversity, Faculty of Sciences, Naresuan University, Phitsanulok, Thailand

Abstract. Entomopathogenic nematodes (EPNs) of the genera *Steinernema* and *Heterorhabditis* are used as biocontrol agents for insect pests. Survey of indigenous EPNs provides not only the diversity aspects but also the contribution in pest management in local areas. The objective of this study was to survey EPNs in upper northern Thailand. Nine hundred seventy soil samples were obtained from 194 sites in upper northern region of Thailand; of these 60 (6.2%) had EPNs in 2 genera: *Steinernema* (32 isolates) and *Heterorhabditis* (28 isolates). Most EPNs were isolated from loam with a soil temperature of 24-38°C, a pH of 1.5-7.0 and a soil moisture content of 0.5-6.8%. Molecular identification based on sequencing of a partial region of an internal transcribed spacer was performed for *Heterorhabditis* and the 28S rDNA for *Steinernema*. A BLASTN search of known sequence EPNs revealed 24 isolates of *S. websteri* and one isolate of *S. scarabaei* were identified; closely related to *S. websteri* (accession no. JF503100) and *S. scarabaei* (accession no. AY172023). The *Heterorhabditis* species identified were: *H. indica* (11 isolates), *H. gerrardi* (2 isolates) and *Heterorhabditis* sp (8 isolates). Phylogenetic analysis revealed 11 isolates of *Heterorhabditis* were related to *H. indica*; 2 isolates were related to *Heterorhabditis gerrardi* and 8 isolates were closely related to *Heterorhabditis* sp SGmg3. The study results show the genetic diversity of EPNs and describe a new observation of *S. scarabaei* and *H. gerrardi* in Thailand. This finding is new and provides important information for further study on using native EPNs in biological control.

Keywords: *Steinernema, Heterorhabditis*, entomopathogenic nematodes, phylogeny

INTRODUCTION

Entomopathogenic nematodes or EPNs of the genera *Steinernema* and *Heterorhabditis* are found in diverse geographical regions except Antarctica (Hominick, 2002). *Steinernema* is symbiotically associated with *Xenorhabdus* bacteria and *Heterorhabditis* with *Photorhabdus* (Boemare, 2002). EPNs have been used as biological control agents for insect pests since they have symbiotic bacteria that can kill parasitised insects within 48 hours (Smart, 1995; Denno *et al*, 2008). EPNs have been

Correspondence: Apichat Vitta, Department of Microbiology and Parasitology, Faculty of Medical Science, Naresuan University, Phitsanulok 65000, Thailand.
Tel: +66 (0) 55 964653; Fax: +66 (0) 55 964770
E-mail: apichatv@nu.ac.th

found to be effective agaisnt several types of insects, including termites, mustard saw flies, cabbage leaf webbers, fig moths, beetles, rice stem borers, and white grubs (Divya and Sankar, 2009).

Approximately 100 species of *Steinernema* and at least 26 species of *Heterorhabditis* have been documented worldwide (Poinar, 1990; Cimen *et al*, 2016; Malan *et al*, 2016; Shahina *et al*, 2016). However, there is little information about EPNs in Thailand. Several surveys of EPNs have been conducted in several provinces of Thailand and 7 species of EPN have been identified: *Steinernema siamkayi*, *S. minutum*, *S. websteri*, *S. khoisanae*, *Heterorhabditis indica*, *H. bacteriophora* and *H. baujardi* (Stock *et al*, 1998; Maneesakorn *et al*, 2010, 2011; Thanwisai *et al*, 2012; Vitta *et al*, 2015). This is a low number compared to the 126 species of EPNs identified worldwide. The most common species, found widespread in Thailand, are *S. websteri* and *H. indica* (Thanwisai *et al*, 2012). The climate and ecology of Thailand are conducive to the existence of EPNs. The objective of this study was to survey EPNs in 8 provinces of upper northern Thailand to provide information for potential use as a biological control agents for insect pests. Molecular identification was used and phylogenetic analysis was performed to identify the isolates and compare them with others world-wide.

MATERIALS AND METHODS

Sampling location and soil collection

Eight provinces of northern Thailand in which the study was conducted were: Chiang Mai, Chiang Rai, Nan, Phayao, Phrae, Lampang, Lamphun, and Mae Hong Son. Soil samples were collected randomly in each province from roadside verges, areas with fruit crops, field crops, forest areas, rice fields, and the banks of rivers and ponds. A total of 194 sites were sampled collecting 970 soil samples between March and September 2013. Soil sample collection was performed as described by Thanwisai *et al* (2012). Approximately 500 g of each soil sample was collected using a hand shovel and put into a plastic bag. The *in situ* soil temperature of each sample was measured using a soil survey instrument (Model: KC-300, Yancheng Kecheng Optoelectronic Technology, Jiangsu, China). The soil pH and moisture content of each sample was measured using a soil pH and moisture tester (Model: DM-15, Takemura Electronic Works, Chiba, Japan). The collection site was noted for each sample along with the latitude, longitude, and altitude using the Garmin Nüvi 1250 GPS navigator (Garmin, Taipei, Taiwan). The soil texture was also recorded. Soil samples were kept and transported at ambient temperature to the Department of Microbiology and Parasitology, Faculty of Medical Science, Naresuan University.

Isolation of entomopathogenic nematodes

EPNs were isolated from the soil samples using the *Galleria mellonella* baiting technique (Bedding and Akhurst, 1975). White traps were used for isolating emerging infective juvenile EPNs from *G. mellonella* cadavers (White, 1927). To maximize recovery of EPNs, all soil samples were re-baited using fresh *G. mellonella* larvae. To confirm the entomopathogenicity and amplify the number of EPNs, larval nematodes were collected and re-exposed to *G. mellonella* larvae. This follows the method of Thanwisai *et al* (2012). Larval nematodes were kept at 13°C in distilled water prior to molecular identification.

Identification of entomopathogenic nematodes

Polymerase chain reaction (PCR) and

sequencing of a partial region of the 28S rDNA gene and an internal transcribed spacer (ITS) were performed according to Hominick *et al* (1997) and Stock *et al* (2001). Genomic DNA samples of larval nematodes were extracted using a protocol described previously by Thanwisai *et al* (2012). Thermocycling was performed in the Applied Biosystems Thermal cycler (Carlsbad, CA) as follow for the 28S rDNA gene: 95°C for 5 minutes; followed by 35 cycles of 94°C for 1 minute, 55°C for 30 seconds and 72°C for 45 seconds; and a final extension at 72°C for 7 minutes, for the ITS region: 95°C for 5 minutes; followed by the 35 cycles of 94°C for 1 minute, 50°C for 30 seconds, 72°C for 1 minute; and the final extension at 72°C for 7 minutes. The PCR products were visualized on an ethidium bromide stained agarose gel and purified using a Gel/PCR DNA Fragments Extraction Kit (Geneaid Biotech, New Taipei City, Taiwan). Sequencing was performed by Macrogen (Seoul, Korea). To identify EPN species, the isolated nucleotide was compared with a nucleotide database using BLASTN search (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi).

Phylogenetic analysis of entomopathogenic nematodes

Phylogenetic analysis of the EPN isolates was performed using MEGA software version 5.05 (Tamura *et al*, 2011). Multiple sequences of known EPN species were downloaded from the NCBI database, and aligned with the sequences identified in this study using the ClustalW program (Thompson *et al*, 1994). Neighbor joining trees were reconstructed using the Kimura-2-parameter model with MEGA software, version 5.05. Bootstrap analysis was performed with 1,000 replicates. *Steinernema poinari* and *Heterorhabditis megidis* were used as the out-group.

RESULTS

Isolation of entomopathogenic nematodes

Of the total of 970 soil samples collected from 194 sites, 60 samples (6.2%) yielded EPNs. The preliminary genus identification was performed based on the morphology and color of the insect cadavers. The two genera identified were *Steinernema* (32 isolates, 3.3% of the total soil samples) and *Heterorhabditis* (28 isolates, 2.9% of the total soil samples) (Table 1). Most EPN isolates (85%) were found in loam with a pH range of 1.5-7.0 and temperature range of 27-38ºC. The soil textures of the 60 samples that yielded EPNs isolates were: loamy (85.0%), sandy (8.3%) and sandy and loamy (6.7%). The soil textures that did not yield EPNs were loamy (78.1%), sandy (4.9%), sandy and loamy (13.6%) and clay (3.4%). The soil pH levels of the EPN-positive and EPN-negative samples did not differ significantly ($p = 0.052$). The soil temperatures of the EPN-positive and EPN-negative samples did not differ significantly ($p = 0.968$). The soil moisture of the EPN-positive and EPN-negative samples did not differ significantly ($p = 0.561$).

Identification and phylogeny of *Heterorhabditis*

A partial region of the ITS sequence in the *Heterorhabditis* isolates was determined by PCR and sequenced. PCR products varied by strain and had a range of 800-850 bp. Twenty-one ITS sequences for *Heterorhabditis* were obtained. After a BLASTN search of the trimmed sequences of 512-527 bp in length against a known sequence database, 21 *Heterorhabditis* (accession nos. KU759911-KU759931) isolates were identified as *H. indica* (11 isolates), *Heterorhabditis* sp SGmg3 (8 isolates), and *Heterorhabditis gerrardi* (2 isolates). They

Table 1
Soil samples positive for entomopathogenic nematodes from various pasts in Thailand.

Province in Thailand	No. of soil sites	No. of soil samples	No. of soil samples positive for EPNs (%)		
			Heterorhabditis No. (%)	*Steinernema* No. (%)	Total No. (%)
Chiang Mai	21	105	3 (2.8)	2 (1.9)	5 (4.7)
Chiang Rai	20	100	0 (0.0)	3 (3.0)	3 (3.0)
Nan	22	110	4 (3.6)	2 (1.8)	6 (5.4)
Phayao	21	105	10 (9.5)	4 (3.8)	14 (13.3)
Phrae	23	115	0 (0.0)	5 (4.3)	5 (4.3)
Lampang	25	125	6 (4.8)	5 (4.0)	11 (8.8)
Lamphun	26	130	2 (1.5)	3 (2.3)	5 (3.8)
Mae Hong Son	36	180	3 (1.6)	8 (4.4)	11 (6.1)
Total	194	970	28 (2.9)	32 (3.3)	60 (6.2)

showed 99-100% similarity. Fig 1 shows the Neighbor joining tree constructed using 512-527 bp of the partial ITS from the 21 *Heterorhabditis* sequences along with the sequences downloaded from GenBank. The Thai *Heterorhabditis* isolates in the present study could be divided into 3 groups. Group 1 included 11 study isolates and an ITS sequence for *H. indica* (accession no. AY321483) derived from the NCBI database. Group 2 included 8 study isolates closely related to *Heterorhabditis* sp SGmg3 (accession no. FJ751864). Group 3 contained the remaining 2 Thai isolates (eCM17.3_TH and eLPO13.3_TH) which were most closely related to *Heterorhabditis gerrardi* (accession no. FJ152545).

Identification and phylogeny of *Steinernema*

The PCR products of the partial sequence for the 28S rDNA were approximately 870 bp in size when compared with the standard DNA ladder. Twenty-five sequences for *Steinernema* were obtained. A BLAST search against a known sequence database revealed these 25 isolates of *Steinernema* (accession nos. KU564093-KU564117) were *Steinernema websteri* (24 isolates) with 98-99% certainty and *Steinernema scarabaei* (1 isolate) with 100% identity. Interestingly, *S. scarabaei* has never been reported in Thailand. It was isolated from loam with a pH of 6.6, at 27°C, and 2% soil moisture content, which was sampled from a forested area in Pai District, Mae Hong Son Province at N19° 23′ 10.8″ E098° 25′ 11.7″ at 519 meters above sea level. A neighbor joining tree reconstructed using the 621-632 bp of the partial 28S rDNA from the 25 *Steinernema* sequences and with the sequences downloaded from GenBank are shown in Fig 2. A total of 24 sequences matched sequences from *S. websteri* (accession no. JF503100), *S. anatoliense* (GU569043), and *S. carpocapsae* (FJ860020.1) and only one Thai isolate (eMH9.2_TH) matched *S. scarabaei* (accession no. AY172023).

DISCUSSION

In the current study, we determined the presence of entomopathogenic nematodes (EPNs) among soil samples collected from 8 provinces in upper northern Thai-

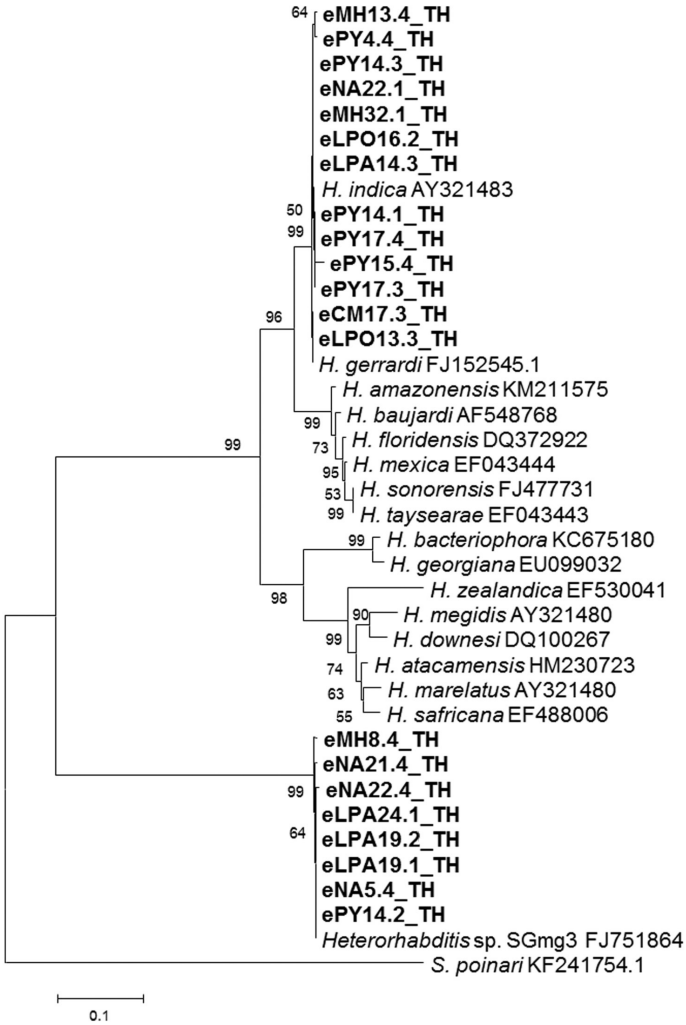

Fig 1–Neighbor joining tree based on a 512-527 bp region of ITS for 21 *Heterorhabditis* isolates from upper northern Thailand and *Heterorhabditis* sequences downloaded from GenBank. The numbers of bootstrap percentages with clades supported above the 50% level.

land. Sixty out of 970 soil samples (6.2%) were positive for EPNs belonging two genera: *Heterorhabditis* and *Steinernema*. Forty-five EPN isolates were identified using sequence analysis of a partial sequence of 28S rDNA and ITS. In the remaining 15 EPN isolates, the genomic DNA could not be determined due to contamination with protozoa or fungi. EPNs have been reported from North and South America, Australia, Europe, Asia and Africa. In our study, we found *Steinernema* isolates in greater numbers than *Heterorhabditis* isolates. This is consistent with studies from Europe where more isolates of *Steinernema* were found (Hominick *et al*, 1995). This may be due to *Steinernema* having a greater ability to adapt to its environment than *Heterorhabditis* (Nyasani *et al*, 2008).

In Thailand, Tangchitsomkid and Sontirat (1998) reported first finding of genera *Steinernema* and *Heterorhabditis* in soil samples from 8 other provinces in Thailand (Kanchanaburi, Phichit, Kalasin, Maha Sarakham, Khon Kaen, Nong Khai, Roi ET, Sa Kaeo). *Steinernema saimkayi* was isolated in Phetchabun Province and was identified as a novel species (Stock *et al*, 1998). *S. minutum*, a second novel species discovered in Thailand, was found in soil samples collected from Chumphon Province in southern Thailand (Maneesakorn *et al*, 2010). *H. indica*, symbiotically associated with *Photorhabdus luminescens*, has been found in Khon Kaen and Krabi Provinces in Thailand (Maneesakorn *et al*, 2011). Thanwisai *et al* (2012) found *S. websteri* and *S. khoisanae* in several provinces of Thailand; *S. websteri* was found in Khon

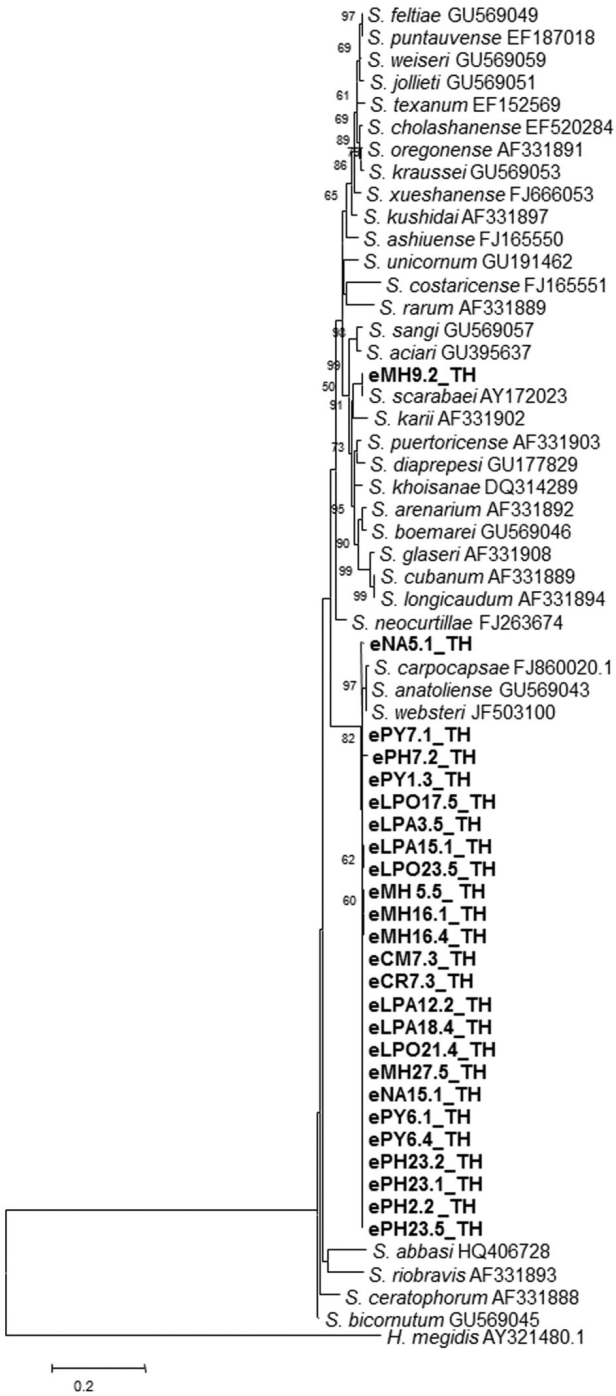

Fig 2–Neighbor joining tree based on a 621-632 bp region of the 28S rDNA for 25 *Steinernema* isolates from upper northern Thailand and *Steinernema* sequences downloaded from GenBank. The numbers of bootstrap percentages with clades supported above the 50% level.

Kaen, Phetchabun, Suphan Buri, Nakhon Nayok, and Kanchanaburi Provinces. *H. indica, H. bacteriophora, H. baujardi, Heterorhabditis* sp SGmg3 and *Heterorhabditis* sp SGgi have been reported in Thailand (Thanwisai *et al*, 2012). Our findings are consistent with previous studies (Thanwisai *et al*, 2012; Vitta *et al*, 2015) that showed the most common species of EPNs in upper northern Thailand are *H. indica* and *S. websteri* which have been found in Chiang Mai, Chiang Rai, Nan, Phayao, Phrae, Lampang, Lamphun, and Mae Hong Son Provinces, the eight evaluated in our study. This suggests the distribution of *H. indica* and *S. websteri,* is nation-wide in Thailand. However, unlike previous study (Thanwisai *et al*, 2012), we did not find *S. khoisanae*, *H. bacteriophora* and *H. baujardi,* which could be due to a low density of these 3 EPNs in Thailand. We also found, a single isolate of *S. scarabaei* collected from a soil sample from Mae Hong Son Province; this is the first recorded identification of this EPN in Asia.

S. scarabaei was first isolated from scarabs in New Jersey, USA (Stock and Koppenhöfer, 2003); its symbiotic bacterium is *Xenorhabdus koppenhoeferi* (Tailliez *et al*, 2006). This EPN showed potential efficacy against the larvae of the Asiatic garden

beetle (Koppenhöfer and Fuzy, 2003). In the present study, *S. scarabaei* was isolated from loam with a soil temperature of 27°C and a pH of 6.6. These soil parameters are similar to those where the first isolate in New Jersey was identified (Stock and Koppenhöfer, 2003). *S. scarabaei* has been shown to have good infectivity in loamy sand and its density is high in all soil types (Koppenhöfer and Fuzy, 2003). The natural insect host of *S. scarabaei* in Thailand is unknown. The scarab beetle may be the host for *S. scarabaei* in Thailand, since it is commonly found here (Sukapanpotharam, 1979; Bouchard *et al*, 2009).

Most of the soil samples in our study were negative for EPNs. The EPNs isolated in our study were from loam with a soil temperature of 24-38°C, a pH of 1.5-7.0, and a soil moisture content of 0.5-6.8%. In our study, *Steinernema* and *Heterorhabditis* were more commonly found in loam than in other soil types, similar to the findings of Thanwisai *et al* (2012). Soil temperature, pH and moisture are important biotic factors for EPN survival, movement and infectivity (Molyneux, 1986; Kung *et al*, 1990, 1991; Rohde *et al*, 2010; Yadav and Lalramliana, 2012). *S. websteri* and *H. indica*, the most common EPNs in Thailand, can live in a wide range of soils.

A phylogenetic tree based on the 28S rDNA region was determined to identify the relationship between *Steinernema* Thai isolates and other strains isolated from several geographical areas. The twenty-four isolates of *Steinernema* were closely related to *S. websteri*, *S. carpocapsae*, *S. anatoliense* and *S. scarabaei*. Phylogenetic analysis of the 21 *Heterorhabditis* isolates found 11 of these were *H. indica*, which has been reported from several provinces of Thailand and 8 isolates were of unknown species. Of the 8 unknown species, all isolates were closely related to *Heterorhabditis* sp SGmg3, which has been reported from Khon Kaen, Kanchanaburi, and Petchabun Provinces in Thailand (Thanwisai *et al*, 2012). The remaining two isolates were related to *Heterorhabditis gerrardi*, which has not been isolated previously from Thailand but it was first reported from Australia (Plichta *et al*, 2009).

In conclusion, we have extended the body of knowledge regarding EPNs in Thailand. EPNs were isolated from several soil types with wide ranges in temperature, moisture and pH. Low concentrations of *Steinernema* (3.3%) and *Heterorhabditis* (2.9%) were found in the soil samples collected. *S. websteri* and *H. indica* were the most common EPNs found in our study. *Steinernema scarabaei* has never previously been reported from Asia. *Heterorhabditis gerrardi* has never previously been reported from Thailand. Phylogenetic tree analysis based on the ITS for *Heterorhabditis* and the 28S rDNA gene for *Steinernema* provided molecular information about the genetics related to the taxonomy of EPNs. This finding is new and provides important information for further study on applying native EPNs in biological control that contribute to minimize the use of chemical pesticide. Further studies of local EPNs are needed by nematologists, entomologists and parasitologists. The symbiotic bacteria, *Xenorhabdus* and *Photorhabdus* and their roles also require further study.

ACKNOWLEDGEMENTS

We would like to thank Naresuan University for the financial support (Grant Number R2556B060). We would also like to thank Miss Nisarat Chonklin, Miss Sudarat Inyoung, Miss Khanittha Phumdoung, Miss Nongluck Lertjarukkulthaworn, Mr Repee Thummepak and

Mr Waranun Yotpunya for the soil collections. Many thanks to Mr Roy Morien of the Naresuan University Language Centre (NULC) for his editing assistance and advice regarding the English in this document.

REFERENCES

Bedding RA, Akhurst RJ. A simple technique for the detection of insect parasitic rhabditid nematode in soil. *Nematologica* 1975; 21: 109-10.

Boemare NE. Biology, taxonomy and systematics of *Photorhabdus* and *Xenorhabdus*. In: Gaugler R, ed. Entomopathogenic nematology. Wallingford: CABI Publishing, 2002: 35-56.

Bouchard P, Grebennikov VV, Smith ABT, Douglas H. Biodiversity of Coleoptera. In: Foottit RG, Adler PH, eds. Insect biodiversity. London: Blackwell Publishing, 2009: 265-302.

Cimen H, Půža V, NermuŤ J, Hatting J, Ramakuwela T, Hazir S. *Steinernema biddulphi* n. sp., a new entomopathogenic nematode (Nematoda: Steinernematidae) from South Africa. *J Nematol* 2016 48: 148-58.

Denno RF, Gruner DS, Kaplan I. Potential for entomopathogenic nematodes in biological control: a meta-analytical synthesis and insights from trophic cascade theory. *J Nematol* 2008; 40: 61-72.

Divya K, Sankar M. Entomopathogenic nematodes in pest management. *Indian J Sci Technol* 2009; 2: 53-60.

Hominick WM. Biogeography. In: Gaugler R, ed. Entomopathogenic nematology. Wallingford: CABI Publishing, 2002: 115-43.

Hominick WM, Briscoe BR, del Pino FG, *et al*. Biosystematics of entomopathogenic nematodes: current status, protocols and definitions. *J Helminthol* 1997; 71: 271-98.

Hominick WM. Reid AP, Briscoe BR. Prevalence and habitat specificity of Steinernematid and Heterorhabditid nematodes isolated during soil surveys of the UK and the Netherlands. *J Helmintol* 1995; 69: 27-32.

Koppenhöfer AM, Fuzy EM. Biological and chemical control of the Asiatic garden beetle, *Maladera castanea* (Coleoptera: Scarabaeidae). *J Econ Entomol* 2003; 96: 1076-82.

Kung SP, Gaugler R, Kaya HK. Influence of soil pH and oxygen on persistence of *Steinernema* spp. *J Nematol* 1990; 22: 440-5.

Kung SP, Gaugler R, Kaya HK. Effects of soil temperature, moisture, and relative humidity on entomopathogenic nematode persistence. *J Invertebr Pathol* 1991; 57: 242-9.

Malan AP, Knoetze R, Tiedt LR. *Steinernema jeffreyense* n. sp. (Rhabditida: Steinernematidae), a new entomopathogenic nematode from South Africa. *J Helminthol* 2016; 90: 262-78.

Maneesakorn P, An R, Daneshvar H, *et al*. Phylogenetic and cophylogenetic relationships of entomopathogenic nematodes (*Heterorhabditis*: Rhabditida) and their symbiotic bacteria (*Photorhabdus*: Enterobacteriaceae). *Mol Phylogenet Evol* 2011; 59: 271-80.

Maneesakorn P, Grewal PS, Chandrapatya A. *Steinernema minutum* sp. nov. (Rhabditida: Steinernema): a new entomopathogenic from Thailand. *Int J Nematol* 2010; 20: 27-42.

Molyneux AS. *Heterorhabditis* spp. and *Steinernema* (= Neoaplectana) spp.: temperature, and aspects of behavior and infectivity. *Exp Parasitol* 1986; 62: 169-80.

Nyasani JO, Kimenju JW, Olubayo FM, Shibairo SI, Mutua GK. Occurrence of entomopathogenic nematodes and their potential in the management of diamondback moth in Kale. *Asian J Plant Sci* 2008; 7: 314-8.

Plichta KL, Joyce SA, Clarke D, Waterfield N, Stock SP. *Heterorhabditis gerrardi* n. sp. (Nematoda: Heterorhabditidae): the hidden host of *Photorhabdus asymbiotica* (Enterobacteriaceae: gamma-Proteobacteria). *J Helmintol* 2009; 83: 309-20.

Poinar GO Jr. Taxonomy and biology of Steinermematidae and Heterorhabditidae. In: Gaugler R and Kaya HK, eds. Entomopathogenic nematodes in biological control. Boca Raton: CRC Press, 1990: 23-61.

Rohde C, Moino A Jr, Silva MA, Carvalho FD, Ferreira CS. Influence of soil temperature and moisture on the infectivity of entomopathogenic nematodes (Rhabditida: Heterorhabditidae, Steinernematidae) against larvae of *Ceratitis capitata* (Wiedeman) (Diptera: Tephritidae). *Neotrop Entomol* 2010; 39: 608-11.

Shahina F, Tabassum KA, Salma J, Mehreen G, Knoetze R. *Heterorhabditis pakistanense* n.sp. (Nematoda: Heterorhabditidae) a new entomopathogenic nematode from Pakistan. *J Helminthol* 2016 April 7: 1-4 (Epub ahead of print).

Smart GC. Entomopathogenic nematodes for the biological control of insects. *J Nematol* 1995; 27(4S): 529-34.

Stock SP, Campbell JF, Nadler SA. Phylogeny of *Steinernema* Travassos 1927 (Cephalobina: Steinernematidae) inferred from ribosomal DNA sequences and morphological characters. *J Parasitol* 2001; 87: 877-89.

Stock SP, Koppenhöfer AM. *Steinernema scarabaei* n. sp. (Rhabditida: Steinernematidae), a natural pathogen of scarab beetle larvae (Coleoptera: Scarabaeidae) from New Jersey, USA. *Nematology* 2003; 5: 191-204.

Stock SP, Somsook V, Reid AP. *Steinernema siamkayai* n. sp. (Rhabditida: Steinernematidae), an entomopathogenic nematode from Thailand. *Syst Parasitol* 1998; 41: 105-13.

Sukapanpotharam V. Scarab beetle communities in deciduous dipterocarp and dry evergreen forests in northern Thailand. *Nat Hist Bull Siam Soc* 1979; 28: 55-100.

Tailliez P, Pagès S, Ginibre N, Boemare N. New insight into diversity in the genus *Xenorhabdus*, including the description of ten new species. *Int J Syst Evol Microbiol* 2006; 56: 2805-18.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* 2011; 28: 2731-9.

Tangchitsomkid N, Sontirat S. Occurrence of entomopathogenic nematode in Thailand. *Kasetsart J (Nat Sci)* 1998; 32: 347-54.

Thanwisai A, Tandhavanant S, Saiprom N, *et al*. Diversity of *Xenorhabdus* and *Photorhabdus* spp. and their symbiotic entomopathogenic nematodes from Thailand. *PLOS One* 2012; 7(9): e43835.

Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* 1994; 22: 4673-80.

Vitta A, Yimthin T, Fukruksa C, *et al*. Distribution of entomopathogenic nematodes in lower northern Thailand. *Southeast Asian J Trop Med Public Health* 2015; 46: 564-73.

White GF. A method for obtaining infective nematode larvae for cultures. *Science* 1927; 66: 302-3.

Yadav AK, Lalramliana. Soil moisture effects on the activity of three entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) isolated from Meghalaya, India. *J Parasit Dis* 2012; 36: 94-8.