Natural occurrence of entomopathogenic nematodes in North China

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Summary. The diversity and distribution of entomopathogenic nematodes (EPN) in North China was studied during 2007 and 2008. A total of 2780 soil samples were taken from six provinces (Shanxi, Henan, Jilin, Liaoning, Heilongjiang and Inner Mongolia). Two hundred and five sites were positive for the occurrence of EPN; 103 isolates were steinernematids and 102 isolates were heterorhabditids. Morphological, morphometric and molecular data were used to identify the nematode species. Seven species of Steinernematidae were found: *Steinernema feltiae* (Filipjev, 1934) Wouts, Mráček, Gerdin & Bedding, 1982, *S. ceratophorum* Jian, Reid & Hunt,1997, *S. longicaudum* Shen & Wang, 1992, *S. litorale* Yoshida,*2004*, *S. hebeiense* Chen, Li, Yan, Spiridonov & Moens, 2006, *S. carpocapsae* (Weiser, 1955) Wouts, Mráček, Gerdin & Bedding, 1982, and *S. monticolum* Stock, Choo & Kaya, 1997. Three *Heterorhabditis* species were obtained: *i.e., Heterorhabditis bacteriophora* Poinar, 1976, *H. megidis* Poinar, Jackson & Klein, 1987, and *H. indica* Poinar, Karunakar & David, 1992. There were also three undescribed *Steinernema* species which were distinct from known steinernematids in morphological, morphometric and molecular characteristics. According to the morphological traits of their IJ and males, these three species should be classified in the *S. feltiae* group. The EPN were recovered from a wide range of habitats including both natural and agricultural habitats. Nematodes were isolated from plain areas (38 m elevation) to high altitude areas (1723 m). The survey indicated that the occurrence of different nematode species was strongly associated to the prevailing climatic conditions, altitude, vegetation and soil types.
Key words: biological control, distribution, diversity, Heterorhabditidae, Steinernematidae, survey.

Entomopathogenic nematodes (EPN) of the families Heterorhabditidae and Steinernematidae are widely distributed and have a wide range of insect hosts (Adams & Nguyen, 2002; Hominick, 2002; Shapiro-Ilan *et al.,* 2002). Species of the genera *Heterorhabditis* Poinar, 1976 and *Steinernema* Travassos, 1927 are obligate insect pathogens that are symbiotically associated with bacteria of the genera *Photorhabdus* (Thomas & Poinar, 1979) Boemare, Akhurst & Mourant, 1993 and *Xenorhabdus* Thomas & Poinar, 1979, respectively (Sicard *et al*., 2004). Entomopathogenic nematodes are considered interesting candidates for biological control agents of agricultural pests given their ability to search for hosts, safety to non-target organisms and the environment, high reproductive potential, capacity for mass production and compatibility with agricultural chemicals (Kaya & Gaugler, 1993).

As there are large differences in the virulence of nematode species and strains against different species of pests, and environmental conditions might affect survival, reproductive potential and virulence of the EPN strains, indigenous isolates might be more suitable against local insect pests (Millar & Barbercheck, 2001). Furthermore, the introduction of non-native EPN species may have negative effects on non-target organisms (Bathon, 1996). Therefore, detailed and reliable bio-geographic data and native strain collections of EPN are important for the successful control of endemic pests.

During the past decades, heavy use of chemical insecticides for controlling pests has led to environmental pollution in some regions of China (Sun *et al.,* 2008). Unfortunately, few effective and sustainable biological control methods were developed, especially for some important soil insect pests and pests that occur in cryptic habitats.

Therefore, the detection of EPN that may be considered for the biological control of endemic insect pests in China would be a valuable contribution to the development and implementation of integrated pest management strategies.

China with its diverse climates, natural vegetations and agricultural ecosystems is rich in EPN diversity. Earlier surveys for EPN conducted in different parts of China have yielded some previously described species including *Steinernema glaseri* (Steiner, 1929) Wouts, Mráček, Gerdin & Bedding, 1982, *S. carpocapsae* (Weiser, 1955) Wouts, Mráček, Gerdin & Bedding, 1982, *Heterorhabditis bacteriophora* Poinar, 1975 (Han, 1994) and *H. indica* Poinar, Karunakar & David, 1992 (Fang *et al.,* 2004). In addition to these species several new species were detected: *viz. S. caudatum* Xu, Wang & Li (Xu *et al*., 1991), *S. longicaudum* Shen & Wang (Shen & Wang, 1991), *H. brevicaudis* Liu (Liu, 1994), *S. ceratophorum* Jian, Reid & Hunt (Jian *et al*., 1997), *S. websteri* Cultler & Stock (Cutler & Stock, 2003), *S. guangdongense* Qiu, Fang, Zhou, Pang & Nguyen (Qiu *et al*. 2004), *S. beddingi* Qiu, Hu, Zhou, Pang & Nguyen (Qiu *et al*. 2005a), *S. aciari* Qiu, Yan, Zhou, Nguyen & Pang 2005 (Qiu *et al*., 2005b), *S. leizhouense* Nguyen, Qiu, Zhou & Pang (Nguyen *et al*., 2006), *S. hebeiense* Chen, Li, Yan, Spiridonov & Moens (Chen *et al*., 2006b), *S. sichuanense* Mráček, Nguyen, Tailliez, Boemare & Chen (Mráček *et al*., 2006), *S. cholashanense* Nguyen, Půža & Mráček (Nguyen *et al*., 2008) and *S. xueshanense* Mráček, Liu & Nguyen (Mráček *et al*., 2009). With the exception for *S. ceratophorum* in Liaoning, *S. longicaudum* in Shangdong and *S. hebeiense* in Hebei, all of these species were found in the southern part of China. No systematic survey has been conducted on a regional scale to assess presence and diversity of EPN occurring in the north of the country. Therefore, we carried out an extensive survey on EPN in six Northern provinces during 2007 and 2008, in order to study the distribution of EPN and to obtain native EPN isolates that might be used for the biological control of local insect pests.

MATERIAL AND METHODS

Collection and isolation of the entomopathogenic nematodes. Soil samples were collected in the provinces Shanxi, Henan, Jilin, Liaoning, Heilongjiang and the east part of Inner Mongolia in North China (Fig. 1). The northeast zone (Heilongjiang, Jilin and Liaoning provinces) is the largest natural forest zone of China and includes the Daxing'an mountain range, the Xiaoxing'an Mountains and the neighbouring Changbai Mountains. Heilongjiang is located between the temperate and the cold zones with an average temperature of -31 to -15°C in January and an average temperature of 18 to 23°C in July. The average annual rainfall in this province ranges between 400 and 600 mm and is concentrated mostly in summer; the annual frost-free period lasts from three to four months or longer (Ma & Shu, 2008). Jilin is bordered by Heilongjiang in the north, Liaoning to the south and Inner Mongolia to the west; it has a distinct temperate continental monsoon climate with a yearly average temperature of 3-6°C and an annual rainfall of 400-900 mm. Liaoning is located in the southern part of China's northeast plain. The annual average temperature in the largest part of Liaoning is 7-11°C. This province has an annual rainfall of 600-1100 mm. Inner Mongolia shares borders with Russia and Mongolia in the north. It has a cold, long winter with frequent blizzards and a warm, short summer. The average temperature is 15-25°C in July and -30-10°C in January. The difference of temperature between day and night is great. The region has an annual precipitation of 100-500 mm, 80-150 frost-free days, and 2,700 hours of sunshine. Shanxi province is located on a plateau, which in turn is made up of higher ground to the east (Taihang Mountains) and the west (Lüliang Mountains), and a series of valleys in the centre. It also has a continental monsoon climate and is rather arid. The mean annual precipitation in this province ranges from 400 to 650 mm, with 60% of it concentrated between June and August. The average annual temperature is 9.4°C. Henan province is flat in the east and mountainous in the west and extreme south. Its average annual temperature is 12.8-15.5°C and the annual precipitation averages 600-1000 mm (Ma & Shu, 2008).

Each of the principal natural habitats (forest, shrubs and natural grassland) and groups of crops (arable crops, vegetables and fruits) was considered when selecting sampling sites. Soil samples (*ca* 400 cm^3) were composed of 3-6 sub-samples randomly taken at least 5 m apart at a depth of 2-20 cm. Sub-samples were mixed, placed in polyethylene bags to prevent water loss, transported to the laboratory and stored under cool conditions (12-15°C) until EPN evaluation. Associated vegetation, date, sampling location and soil characteristics were recorded. Data of average annual air temperature and rainfall were obtained from the local weather stations. Soils positive for EPN were analysed for pH and texture.

EPN were extracted from soil by the *Galleria mellonella* L. (Lepidoptera: Pyralidae) baiting method (Bedding & Akhurst, 1975). Samples were

moistened (10%) if too dry. Six last instars of *G. mellonella* were buried in each soil sample; dead larvae were collected and replaced by living ones until dead insects were no longer observed. Infective juveniles (IJ) were collected from the insect cadavers using the method of White (1927) and the emerging nematodes were collected and stored at 10°C.

Identification of the isolated nematodes. Nematodes were re-cultured using last instar larvae of *G. mellonella*. Only IJ collected during the week after their first emergence from the insect cadavers were used for the identification. First and second generation adults were dissected from the cadavers 3-5 and 8-11 days after nematode infection, respectively. For morphological observations, nematodes were examined live or heat-killed (60°C in Ringer's solution) and fixed in 4% formalin. Fixed nematodes were processed to glycerin according to De Grisse (1969) and mounted on slides using glass fibres to support the cover slip and avoid flattening of the nematodes. Cover slips were sealed with wax. At least 20 individuals of females, males and infective juveniles were randomly selected and measured using an Olympus BX50 light microscope with differential interference contrast optics and digital image software (Cell^D Soft Imaging System, Olympus Company, Japan). The morphological and morphometric features were compared with those in the original descriptions of these species. The nematodes were identified using the criteria suggested by Stock and Kaya (1996) and Hominick *et al*. (1997).

Molecular characteristics of the isolated nematodes. The species identification based on morphology and morphometrics was confirmed by molecular analysis. Nematode DNA was extracted from single IJ (Phan *et al*., 2005). A specimen was cut in 8 µl of worm lysis buffer (500 mM KCl, 100 mM Tris-Cl pH 8.3, 15 mM $MgCl_2$, 10 mM DTT, 4.5% Tween 20, 0.1% gelatine). The nematode fragments were transferred in 4 µl of the buffer to an Eppendorf tube to which 5 µl of double distilled water and 1 µl of proteinase K (600 µg ml^{-1}) were added. After freezing (-70°C for 15min) the tubes were incubated at 65°C for 1 h and then at 95°C for 10 min. For the amplification and sequencing of ITS rDNA the primers TW81 (5'-GTT TCC GTA GGT GAA CCT GC-3') and AB28 (5'-ATA TGC TTA AGT TCA GCG GGT-3') (Joyce *et al*., 1994) were used. The resulting sequences were edited and analysed using software packages Chromas 2.0 and BioEdit 7.01. The obtained sequences were blasted in GeneBank for comparison. A phylogenetic tree was constructed using the ITS rDNA sequences, *Caenorhabditis elegans* Maupas, 1900 (GeneBank accession X03680) was used as outgroup.

RESULTS

Collection and identification of EPN. Out of a total of 2780 soil samples that were collected in the six provinces, 205 (7.4%) were positive for EPN. According to the colour of the infected *G. mellonella* cadavers and the general morphology of the IJ, 103 and 102 isolates were sorted out as steinernematids and heterorhabditids, respectively. However, cadavers of *G. mellonella* infected by one isolate of *Steinernema* turned light cyan (*S. longicaudum* HDT-69) and another one turned light red brown (*S.* sp. 1 LFS-32), both different from the typical colour of cream to brown. *Galleria mellonella* cadavers infected by four isolates of *Heterorhabditis* turned to fresh orange (*H. indica* ZZ-71, KF-58; *H. megidis* JJY-177, JJY-113) and four isolates coloured the cadaver light greenish blue (*H. megidis* LFS-90, LFS-94, LBX-76, JJY-25), rather than the typical red colour. Morphological examination and sequence analysis enabled the EPN isolates to be identified as *S. carpocapsae*, *S. ceratophorum*, *S. feltiae* (Filipjev,

Table 1. Number of soil samples positive for entomopathogenic nematodes (*Steinernema* and *Heterorhabditis* spp.) in different provinces in North China.

Species	Henan	Shanxi	Heilongjiang	Jilin	Liaoning	Inner Mongolia	Total
Heterorhabditis bacteriophora	8	6	7	13	7	4	45
H. megidis	–	–	11	20	14	–	45
H. indica	12	–	–	–	–-	–	12
Steinernema feltiae	1	12	6	21	8	–	48
S. longicaudum	–	-	9	4	1	–	14
S. ceratophorum	–	1	5	4	2	–	12
S. hebeiense		1	4	7	–	–	12
S. litorale	5	1	–	–	–	–	6
S. carpocapsae	–	–	1	–	–	–	1
S. monticolum	–	–	–	–	1	–	1
Steinernema spp.	–	–	–	–	9	–	9
Positive soil samples (%)	3.5	4	11.2	10.5	10.8	4.4	7.4

1934) Wouts, Mráček, Gerdin & Bedding, 1982, *S. hebeiense*, *S. litorale*, Yoshida, 2004, *S. longicaudum*, *S. monticolum,* Stock, Choo & Kaya, 1997, *H. bacteriophora*, *H. megidis* Poinar, Jackson & Klein, 1987, and *H. indica* (Table 1). All isolates of these species fit the morphological and morphometric traits of the species. The analysis of ITS rDNA sequences confirmed the identification (99-100% similarity with information on sequences available on GeneBank). Nine isolates, all from Liaoning province, did not fit with any of the currently described species and could be divided into three different taxons of *Steinernema* based on their morphological, morphometrical and molecular characteristics. According the morphological traits of their IJ and males, these three species are to be classified in the *S. feltiae* group. From these, both *Steinernema* sp. 1 and *Steinernema* sp. 2 originated from coniferous forests, whereas *Steinernema* sp. 3 was sampled from soil under shrubs (Table 4). The natural hosts of these three species could not be identified; their reproductive rate on *G. mellonella* was lower than that of the other isolates. We are currently describing these species.

Table 2. Frequency of positive sites for entomopathogenic nematodes (*Steinernema* and *Heterorhabditis* spp.) from different types of habitats.

Habitat	Positive sites (%)
Arable cropland	4.5
Deciduous trees	10.8
Coniferous trees	12.1
Fruit orchards	10.3
Shrubs	10.6
Grassland	8.6
Vegetables	1.1
Swamps (riversides)	0

The most common species were *H. bacteriophora* and *S. feltiae*. *Heterorhabditis bacteriophora* was isolated from 45 sites from all geographic regions sampled. The 67% positive samples for *H. bacteriophora* originated from places below the elevation of 200 m; two isolates (4%) were collected from sites situated above 1000 m. *Steinernema feltiae* was detected in 48 sites in five provinces (not in Inner Mongolia). The 68% positive samples of *S. feltiae* were recovered between 200 m to 800 m; 20% were collected below 200 m. All positive soil samples of *H. indica* were obtained from Henan province. Except for one isolate recovered from Shanxi province, all isolates of *S. litorale* were collected in Henan province. *Heterorhabditis megidis* and *S. longicaudum* were only detected in Heilongjiang, Jilin and Liaoning provinces, the annual average temperature of the positive sampling sites was between 2°C to 7°C. The 80% positive samples for *H. megidis* were taken from the elevation of 200-500 m, while all the *S. longicaudum* isolates were sampled under the 200 m. *Steinernema ceratophorum* was detected in 12 locations in Heilongjiang, Jilin, Liaoning and Shanxi. All the positive soil samples of *S. ceratophorum* were taken at an elevation between 140m and 230m, except the one from Wutai Mountain in Shanxi province. The only isolate of *S. monticolum* was recovered from a soil sample from shrubbery near Jinzhou in Liaoning province and the single isolate of *S. carpocapsae* was isolated from a soybean field in Heilongjiang province (Table 4).

Table 3. Frequency of positive sites for entomopathogenic nematodes (*Steinernema* and *Heterorhabditis* spp.) from different soil textures.

Soil type	*Steinernema* spp.	*Heterorhabditis* spp.	Total positive
Sand	2.0	0	2.0
Sandy loam	4.0	5.2	9.2
Loam	4.2	4.2	8.4
Clay	0	1.3	1.3

Some isolates showed morphological variations. IJs of isolate TN13 (*S. hebeiense*) from Shuozhou (Shanxi province) split up into a group with spiky tails and another group with smooth tails. Hybridisations between these two groups yielded offspring that included both tail types. However, the rDNA-ITs sequences showed no differences (100% sequence similarity).

Habitats and soil type. On the whole, EPN occurred in almost all studied habitats, including forest, grassland, field crops (wheat, corn, bean and cotton), vegetable crops and fruit orchards (Table 2). No EPN were found in marsh areas or at riversides. EPN were most regularly found in forests [coniferous trees (12.1%) and deciduous trees (10.8%)], followed by shrubs (10.6%) and grasslands (8.6%). *Heterorhabditis bacteriophora* was found in a wide range of habitats including both natural and agricultural sites. *Steinernema feltiae* or *H. megidis* were rarely isolated from arable crops; only one isolate of *S. feltiae* was sampled from a pear orchard and two isolates of *H. megidis* were collected from an apple orchard. EPN were more common in sandy loam and loamy soils (Table 3). The soil texture of 80% of the samples positive for *H. megidis* and 58% samples positive for *S. feltiae* was loam, while the texture of 55% of the samples positive for *H. bacteriophora* was sandy loam. The pH of the soils positive for EPN ranged from acidic (4.5) to slightly alkaline (8.6). *Heterorhabditis indica* and *S. longicaudum* were mainly recovered

Table 4. Characteristics of the detection sites of entomopathogenic nematode species in North China.

Species	Habitat	North Latitude	East Longitude	Soil Texture	Soil pH	Altitude (m)	Average annual rainfall (mm)	Average annual temperature (°C)
Heterorhabditis bacteriophora	W, D, C, Cr, F (*)	33.57-47.57	112.28-130.42	sandy loam, loam, clay	6.8-8.6	38-1723	400-850	3.0 -16
H. megidis	W, C,D, F, Sh,	41.04-46.36	121.49-130.79	sandy loam, loam	4.5-6.5	81-652	420-850	2.0 -7.0
H. indica	Cr, W, D, V	33.57-35.33	112.80-114.33	sandy loam	6.7-7.8	67-208	550-1000	14 -16.4
Steinernema feltiae	D,W,C, Sh, F	34.52-47.75	110.92-130.74	Sand, sandy loam, loam,	5.2-7.7	147-1397	320-890	2.0 -14
S. longicaudum	W, Sh, D	41.57-47.83	121.29-129.76	sandy loam	7.0-7.8	64-159	408-590	2.0 -8.5
S. ceratophorum	W, D, Cr, C	41.02-48.02	122.99-129.66	sandy loam, loam	5.8-7.5	140-970	320-850	2.6-8.4
S. hebeiense	W, Sh, D,C,	38.96-47.423	112.24-130.19	sandy loam, loam	4.6-6.9	150-1528	415-883	2.4-6.2
S. litorale	W, D, V, Cr	34.06-38.67	111.93-114.33	sandy loam, loam	7.2-7.7	55-743	400-634	6.4-14.7
S. carpocapsae	Cr	44.91	129.95	loam	4.6	289	520	2.8
S. monticolum	Sh	41.53	121.49	loam	6.1	81	528.3	7.9
S. sp. 1	W, C	41.02-42.03	123.74-124.36	sandy loam, loam	5.7-6.0	220-420	650-850	6.1-7.0
S. sp. 2	C	40.89-42.08	124.03-124.28	sandy loam, loam	5.3-5.9	205-300	650-850	6.2-7.0
S. sp. 3	C	41.02-41.88	123.74-124.47	sandy loam, loam	5.5-6.8	151-260	761-850	4.6-7.0

(*) W: weeds; Sh: shrubs; D: deciduous trees; C: coniferous trees; Cr: cropland; F: fruit trees; V: vegetables

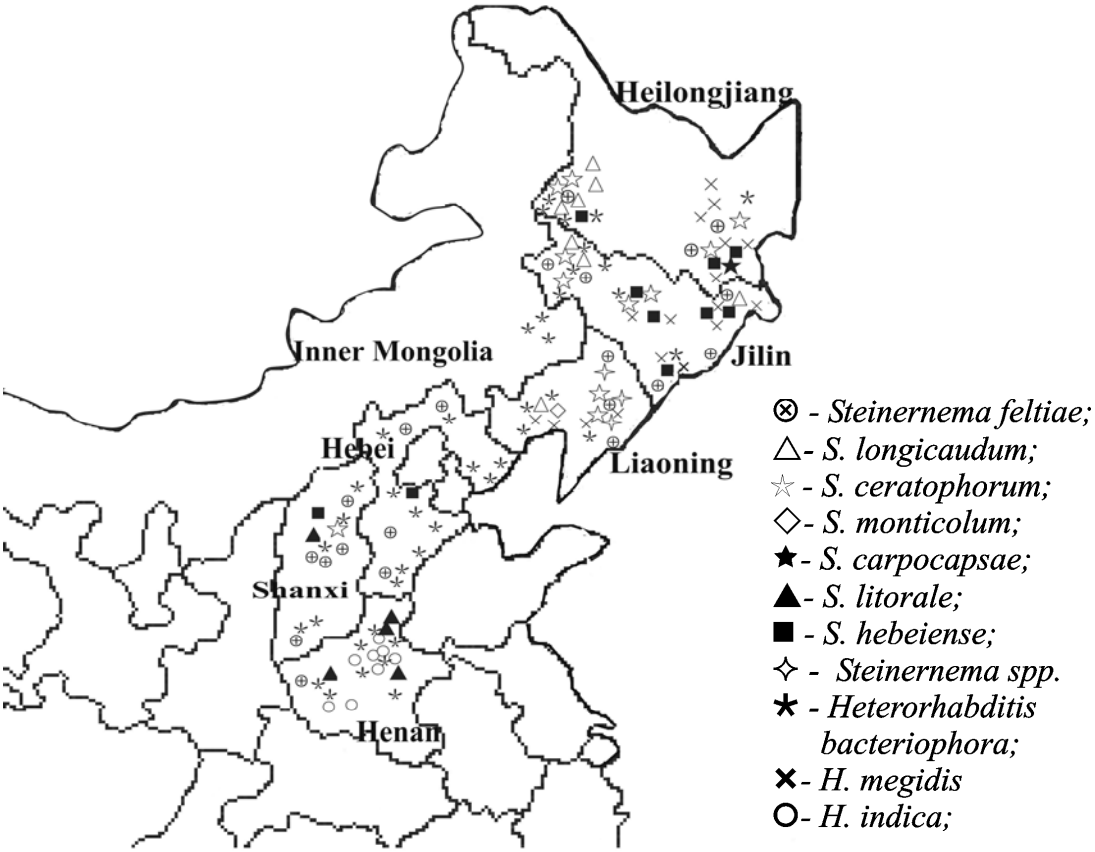

Fig. 1. Geographic location of sampling sites positive for entomopathogenic nematodes (*Steinernema* and *Heterorhabditis* spp.).

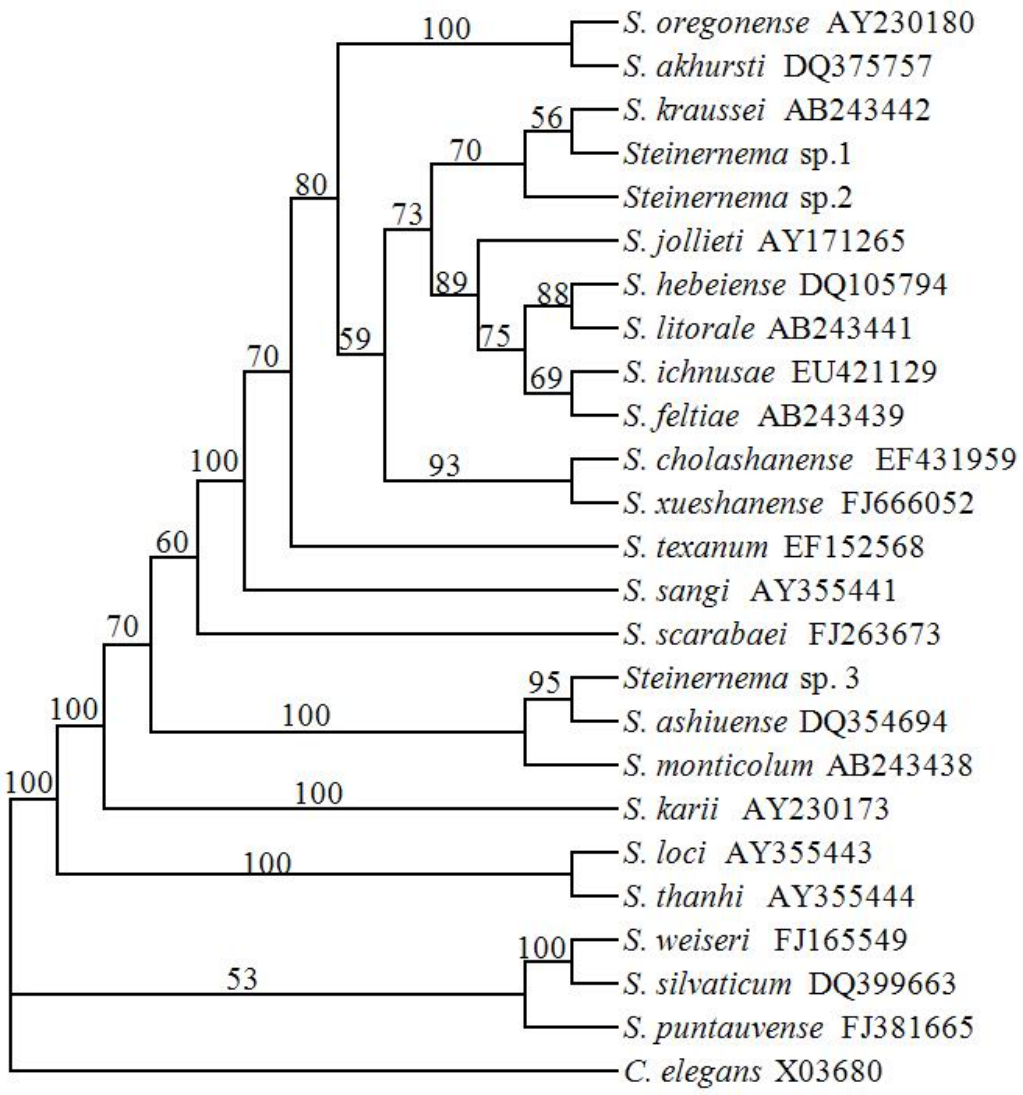

Fig. 2. Phylogenetic relationships based on the analysis of ITS regions of 24 species in the *Steinernema feltiae* group using *Caenorhabditis elegans* as an outgroup taxon. Numbers at the nodes represent bootstrap proportion for maximum parsimony.

from sandy loam soils with a slightly alkaline pH. Both *H. megidis* and *S. hebeiense* were recovered from loam soils with an acidic pH (Table 4).

Phylogenetic relationships of the new *Steinernema* spp. In this consensus tree of 24 species of the *S. feltiae* group (Fig. 2) (tree length=2098, CI=0.679, RI=0.635, RC=0.431, HI=0.321), *Steinernema* sp. 1 and *Steinernema* sp. 2 are closely related to *S. kraussei*; the similarity of *Steinernema* sp. 1 and *Steinernema* sp. 2 with *S. kraussei* was 95.6% and 94.5%, respectively. *Steinernema* sp. 3 and *S. ashiunense* Phan, Takemoto & Futai, 2006, form a monophyletic group with *S. monticolum* being their closest sister taxon, which is well supported by bootstrap proportion (95 and 100). The similarity of *Steinernema* sp. 3 with *S. ashiunense* was 94.6%.

DISCUSSION

Our finding of ten described species and three undescribed species demonstrates the high diversity of EPN in the surveyed area in North China. In conjunction with an earlier survey in Hebei (Chen *et al*., 2006a), our observations reveal *H. bacteriophora* to be the most commonly distributed. This species appears to be well adapted to both the humid warm-temperate climate and the continental monsoon climate prevailing in that part of the country. *Heterorhabditis bacteriophora* was present in almost all habitats sampled in our survey, including forest, arable cropland, shrubs, fruit orchard and vegetables. The distribution of *H. bacteriophora* was not influenced by altitude.

The distribution of *S. feltiae* is comparable to that of *H. bacteriophora*. *Steinernema feltiae* was

reported to prefer arable soils, grassland and woodland (Hominick, 2002). In our survey, the species was found broadly distributed in both grassland and woodland, but it was less abundant in field crops. We found *S. feltiae* more abundant above 300 m herewith confirming earlier findings by Rosa *et al*. (2000). *Heterorhabditis megidis* and *S. longicaudum* were only detected in Heilongjiang, Jinlin and Liaoning provinces. The first species was dominant in the three Northern provinces, which have a continental climate characterised by long, dry and cold severe winters, warm and rainy summers and a comparatively short and windy spring and autumn; the January mean temperature is below 0°C. *Heterorhabditis megidis* is widespread in the temperate regions of Europe (Hominick, 2002). It was also isolated in Greece (Menti *et al*., 1997), Israel (Glazer *et al*., 1993), Japan (Yoshida *et al*., 1998), Russia (Fischer-Le Saux *et al*., 1998) and Canada (Mráček & Webster 1993). *Steinernema longicaudum* was originally isolated from an orchard in Shandong province, China (a province bordering Hebei province). It had subsequently been recovered from Australia (Hominick *et al*., 1996), California, USA (Stock *et al*., 1999), and South Korea (Stock *et al*., 2001). Our results extend the known geographic range of *S. longicaudum* to more northern latitudes. Altitude had a clear influence on the distribution of both *H. megidis* and *S. longicaudum*.

Heterorhabditis indica was first isolated from soil in Coimbatore, Tamil Nadu, India (Poinar *et al*., 1992). It is a common species in tropical and subtropical zones (Hominick, 2002). We found this species in Henan province, which is situated in a warm temperate zone with the mean annual precipitation of 600-1000 mm. *Heterorhabditis indica* did not show a distinct habitat preference, it was detected in both natural and agricultural habitats. The influence of agricultural activities on *H. indica* seems to be less than for the other species sampled in our survey. *Steinernema litorale*, which until now had only been found in Japan, was detected in Henan and Shanxi provinces in our survey.

Steinernema litorale was originally detected in sandy coastal soils in the eastern part of Japan; its habitat includes grassland and pine forest land (Yoshida, 2004). All *S. litorale* isolates in our survey were recovered in inland areas, with associated vegetation including soybean, leek, weeds and poplar. *Steinernema ceratophorum* was originally recovered in arable fields in Liaoning and Jilin province (Jian *et al*., 1997); we found this species also in forest, grassland, and arable cropland in Heilongjiang and Shanxi province. This species is more abundant at low altitudes.

This wide-scale survey indicates that EPN occur more frequently in undisturbed soils than in other monitored habitats. EPN were more common in sandy loam and loamy soils than in clay or sand soils. Soil texture (Barbercheck, 1992), temperature, moisture, agronomic practices, soil antagonists (Patel & Wright 1996; Grewal *et al*., 1998), and host resources (Baur & Kaya, 2001) are major factors affecting nematode persistence in the soil environment. In this study, EPN were more abundant in the three northeast provinces (Heilongjiang, Jilin and Liaoning). The forest area and forest coverage rates are much higher in these provinces than in the other three provinces (Zhou *et al*., 2009).

Bacteria play a key role in the control of insects. They help the nematodes to overcome the humoral and cellular defences of insect hosts, to protect the insect cadaver from colonisation by other organisms, and as a substrate for growth and reproduction (Ciche *et al*., 2006). The parasitic success of EPN relies on their symbiotic bacteria. Usually the colour of *G. mellonella* cadavers that are infected by EPN will turn to cream to brown (*Steinernema* spp.) or red (*Heterorhabditis* spp.) within 24-48 h after nematode penetration. Some of our isolates caused an atypical discoloration on *G. mellonella*. The regular symbiotic bacteria of these three nematode species are *Xenorhabdus beddingii* (Akhurst) Akhurst & Boemare, *Photorhabdus luminescens* subsp. *akhurstii* Fischer-Le Saux, Viallard, Brunel, Normand & Boemare, and *P. luminescens* (Thomas and Poinar) Boemare, Akhurst & Mourant emend. Fischer-Le Saux, Viallard, Brunel, Normand & Boemare, respectively (Fischer-Le Saux *et al*., 1999). The symbiotic bacteria of the different isolates and their virulence will be the subject of further study.

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Ma Juan, Chen Shulong, Zou Yaxin, Li Xiuhua, Han Richou, De Clercq, P., Moens M. Энтомопатогенные нематоды северного Китая.

Резюме. Разнообразие и географическое распределение почвенных энтомопатогенных нематод в Сев. Китае исследовали в 2007-2008 г. Всего было собрано 2780 образцов почвы из шести провинций (Шанси, Хэнань, Цзилинь, Ляонин, Хэйлунцзян и Внутренняя Монголия. Энтомопатогенные нематоды были обнаружены в 205 образцах – было выделено 103 культуры штейернематид и 102 гетерорабдитид. Морфологические, морфометрические и молекулярные данные были использованы для определения выявленных нематод. Было обнаружено семь видов Steinernematidae: *Steinernema feltiae* (Filipjev) Wouts, Mráček, Gerdin & Bedding, *S. ceratophorum* Jian, Reid & Hunt, *S. longicaudum* Shen & Wang, *S. litorale* Yoshida, *S. hebeiense* Chen, Li, Yan, Spiridonov & Moens, *S. carpocapsae* (Weiser, 1955) Wouts, Mráček, Gerdin & Bedding и *S. monticolum* Stock, Choo & Kaya. Также было выявлено три вида *Heterorhabditis*: *Heterorhabditis bacteriophora* Poinar, *H. megidis* Poinar, Jackson & Klein, and *H. indica* Poinar, Karunakar & David. Обнаружено также три неописанных вида *Steinernema,* которые существенно отличаются от известных видов по морфологическим, морфометрическим и молекулярным признакам. В соответствии с их особенностями все эти три новых вида близки к *S. feltiae*. Энтомопатогенные нематоды были выявлены в самых разных биотопах, включая как природные, так и сельскохозяйственные ценозы. Нематод были обнаружены как на равнинах (высота 38 м над уровнем моря), так и в горных местностях (высота 1723 м). Обследование показало, что встречаемость нематод была тесно связана с климатическими условиями, высотой местности, типом растительности и почвами.