*Biodiversity and Conservation* **12:** 375–386, 2003.


# Diversity and distribution of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) in Turkey

SELÇUK HAZIR[1], NEVIN KESKIN[1], S. PATRICIA STOCK[2,*], HARRY K. KAYA[3] and SERVET ÖZCAN[4]

*[1]Department of Biology, Faculty of Science, University of Hacettepe, Beytepe, 06532 Ankara, Turkey; [2]Department of Plant Pathology, University of Arizona, 1140E South Campus Dr, Tucson, AZ 85721-0036, USA; [3]Department of Nematology, University of California, One Shields Ave., Davis, CA 95616-8668, USA; [4]Department of Biology, Faculty of Art and Science, University of Erciyes, 38039 Kayseri, Turkey; [*]Author for correspondence (e-mail: spstock@ag.arizona.edu; fax: +1-520-621-9290)*

Received 21 September 2001; accepted in revised form 14 February 2002

**Key words:** *Heterorhabditis bacteriophora*, Soil nematodes, *Steinernema affine*, *Steinernema feltiae*, Survey

**Abstract.** The diversity and distribution of entomopathogenic nematodes in the families Steinernematidae and Heterorhabditidae were assessed throughout an extensive soil survey in Turkey during 1999 and 2000. Entomopathogenic nematodes were recovered from six out of seven regions sampled, with 22 positive sites (2%) out of 1080 sites sampled. A single nematode isolate was recovered at each of the positive sites, of which 15 were steinernematid isolates and seven were heterorhabditid isolates representing a total of four species. Based on morphometric and molecular data, the nematode species were identified as *Heterorhabditis bacteriophora*, *Steinernema feltiae*, *S. affine*, and *Steinernema* n. sp. The most common species was *S. feltiae*, which was isolated from 10 sites in six regions, followed by *H. bacteriophora* from seven sites in five regions, *S. affine* from four sites in two regions, and *Steinernema* n. sp. from one site. *Heterorhabditis bacteriophora* and *S. feltiae* have been found in many parts of the world, whereas *S. affine*, so far, has only been recovered in Europe until our survey. *Steinernema affine* was isolated from the European (Marmara) as well as the Asiatic region (Middle Anatolia) of Turkey. A new undescribed *Steinernema* sp. was isolated from the most eastern region (East Anatolia) of Turkey. Soils of the positive sites were classified as sandy, sandy loam, or loam (68.2%) and sandy–clay–loam or clay loam (31.8%) and the pH ranged from 5.6 to 7.9. The habitats from which the entomopathogenic nematodes were isolated were broadly classified as disturbed (59.1%), which included agricultural fields and poplar planted for lumber and wind breaks, and undisturbed (40.9%), which included pine forest, grassland, marsh and reed sites. *Steinernema feltiae*, *S. affine*, and *H. bacteriophora* were recovered from both disturbed and undisturbed habitats. The new *Steinernema* sp. was recovered from grassland. Our survey showed that these nematodes occur widely throughout Turkey, but at a frequency below that reported for other parts of the world.

## Introduction

Entomopathogenic nematodes of the genera *Steinernema* Travassos and *Heterorhabditis* Poinar are obligate pathogens that infect a wide range of insects in the laboratory, but in the field they infect only soil insects and a few other soil invertebrates (Kaya and Gaugler 1993). These nematodes are mutualistically

associated with bacteria in the genus *Photorhabdus* Boemare, Akhurst and Mourant for *Heterorhabditis* or *Xenorhabdus* Thomas and Poinar for *Steinernema* (Boemare et al. 1997; Burnell and Stock 2000). The life cycle of all known entomopathogenic nematode/bacterium complexes is similar. The only stage that survives outside of the host is the non-feeding, third-stage infective juvenile (IJ). The IJ carries cells of the bacterial symbiont in its intestine. When the IJ finds a susceptible host, it invades and penetrates into the host's hemocoel through natural openings (i.e., anus, mouth, or spiracles). The IJ then releases the symbiotic bacterium that kills the host within 48 h by septicemia. The bacterium produces antibiotics that prevent other microorganisms from colonizing the cadaver. In addition to serving as a food source for the nematode, the bacterium digests the host tissues, thereby providing suitable nutrients for nematode growth and development. *Steinernema* species are amphimictic, and a male and female IJ must enter the insect host for successful reproduction to occur, whereas *Heterorhabditis* species are hermaphroditic and only one IJ in the host is sufficient for successful reproduction. In the second generation of both nematode genera, reproduction is amphimictic (Poinar 1990). However, a hermaphroditic steinernematid species was recently isolated from Indonesia by Griffin et al. (2001).

Entomopathogenic nematodes can provide effective biological control of some important soil insect pests and pests that occur in cryptic habitats (Georgis and Manweiler 1994; Koppenhöfer 2000). Accordingly, there is an intense interest to isolate these nematodes from different regions of the world that are climatically adapted and have the potential for biological control of pests in that area. In addition, many countries are concerned about the introduction of exotic entomopathogenic nematodes, because they may have a negative impact on non-target organisms (Bathon 1996). Hence, surveys have been conducted in many parts of the world demonstrating their wide spread occurrence and providing an indication of which species are indigenous for a given area. Hominick et al. (1996) summarized the results of these surveys conducted prior to 1996. Since then a number of other surveys have been conducted and new species and strains of entomopathogenic nematodes have been isolated. Surveys in Europe include those by Steiner (1996), Midituri et al. (1997), Constant et al. (1998), Shishiniova et al. (1998), Mráček et al. (1999), Sturhan and Lišková (1999), Mráček and Bečvář (2000), and Rosa et al. (2000). In other parts of the world, surveys were conducted in Asia by Mason et al. (1996), Josephrajkumar and Sivakumar (1997), Tangchitsomkid and Sontirat (1998), Yoshida et al. (1998), Vyas et al. (1999), Griffin et al. (2000), Iraki et al. (2000), Luc et al. (2000) and Liao et al. (2001), in North America by Stock et al. (1999), and in South America by Rosales and Suarez (1998).

In Turkey, Özer et al. (1995) conducted a preliminary survey and found five positive isolates out of 106 soil samples. Out of the five isolates, only one was identified to species as *S. carpocapsae* (Weiser) from the Black Sea region, but subsequently, this isolate was re-identified as *S. feltiae* (Filipjev) by A.P. Reid (Hominick et al. 1996). The other four *Steinernema* isolates were only identified to the generic level. There is still a paucity of information on the diversity of entomopathogenic nematode species in Turkey and a need to find biological control

agents for soil pests. Accordingly, we conducted an intensive survey throughout Turkey for entomopathogenic nematodes and identified the isolates using a combination of classical and molecular taxonomic approaches.

## Materials and methods

### *Geography and collection of soil samples*

Turkey is located at a latitude of 36° north and a longitude of 26° east, is 1660 km long and 550 km wide, and occupies an area of 814 578 $km^2$, of which 790 200 $km^2$ are located in Asia and 24 378 $km^2$ in Europe (http://www.turkey.org/start.html). It is divided into seven regions, i.e. Black Sea, Marmara, Aegean, Mediterranean, Middle Anatolia, East Anatolia, and Southeast Anatolia. Its climate varies from Mediterranean to temperate.

Soil samples were collected from the seven regions in 1999 and 2000. The sampling sites were within 150 m along passable roads and were selected based on accessibility and habitat (see Table 1) with at least 10 km distance between sites. Within a given site, a sample of ca. 1 kg made up of a composite from three subsamples was taken. Each subsample was obtained using a hand trowel from the upper 15–20 cm within an area of 10 $m^2$. Samples were placed in a polyethylene bag to minimize dehydration and transported in a cooler to the laboratory. At each site, the soil temperature was taken and notation about the habitat (vegetation) was recorded. The hand trowel was sterilized with 70% ethanol before leaving the sampling site.

### *Isolation of nematodes*

The soil samples were stored at 15 °C in the laboratory and processed within 1 week of collection. The 1 kg soil sample was thoroughly mixed, ca. 240 cc of subsample was placed into a 250-cc plastic container, five last instar larvae of the wax moth *Galleria mellonella* (L.) were placed on the soil, and the container was covered with a lid and inverted (Bedding and Akhurst 1975; Kaya and Stock 1997). The containers were held at room temperature (22–25 °C) for a period of 15 days. Dead larvae were collected at 3-day intervals for 15 days after set-up and transferred to White traps to collect the emerging IJs (Kaya and Stock 1997). The IJs were pooled from each sample and were used to infect fresh *G. mellonella* larvae to verify their pathogenicity and allow for progeny production for identification (Kaya and Stock 1997).

Soil that was not used for baiting for nematodes was maintained at room temperature. Soil samples that were positive for entomopathogenic nematodes were analyzed by the Soil and Fertilizer Research Institute of Turkey for soil type, organic matter, and pH (Table 1).

*Table 1. Heterorhabditis* and *Steinernema* species isolated from various regions of Turkey showing the soil type, soil temperature at time of collection, pH and habitat between April 1999 and October 2000.

| Genus/species | Region | Locality | Soil type | Soil temp. (°C) | Organic matter (%) | pH | Habitat | Sampling time |
|---|---|---|---|---|---|---|---|---|
| *H. bacteriophora* | Mediterranean | Adana | Sandy loam | 18 | 4.2 | 7.2 | Pine forest | Apr 1999 |
| *H. bacteriophora* | Mediterranean | Icel | Sandy loam | 16 | 3.7 | 6.8 | Grassland | Apr 1999 |
| *H. bacteriophora* | Marmara | Kirklareli | Sandy–clay–loam | 20 | 1.9 | 7.4 | Sunflower field | Aug 1999 |
| *H. bacteriophora* | Marmara | Kirklareli | Sandy loam | 21 | 1.5 | 6.3 | Fallow field | Aug 1999 |
| *H. bacteriophora* | Middle Anatolia | Aksaray | Sandy loam | 23 | 2 | 7.5 | Clover field | Jun 2000 |
| *H. bacteriophora* | Southeast Anatolia | Mardin | Sandy–clay–loam | 17 | 3.1 | 7.7 | Grassland | May 2000 |
| *H. bacteriophora* | Black Sea | Tokat | Sandy loam | 18 | 3.2 | 7.6 | Poplar | Jul 2000 |
| *S. affine* | Marmara | Istanbul | Clay loam | 18 | 3.8 | 5.6 | Grassland | Aug 1999 |
| *S. affine* | Marmara | Kirklareli | Loam | 19 | 4.7 | 7.3 | Fallow field | Aug 1999 |
| *S. affine* | Marmara | Tekirdag | Sandy–clay–loam | 14 | 2.9 | 6.4 | Fallow field | Sep 1999 |
| *S. affine* | Middle Anatolia | Kayseri | Sandy loam | 14 | 2.8 | 7.7 | Poplar | Aug 1999 |
| *S. feltiae* | Middle Anatolia | Burdur | Sandy loam | 11 | 5.1 | 7.6 | Marsh (*Juncus*)[a] | Jun 1999 |
| *S. feltiae* | Middle Anatolia | Kirsehir | Sandy–clay–loam | 20 | 3.5 | 7.4 | Poplar hedgerow | Jun 2000 |
| *S. feltiae* | Middle Anatolia | Kirsehir | Sand | 20 | 0.8 | 7.7 | Poplar | Jun 2000 |
| *S. feltiae* | Middle Anatolia | Sivas | Sandy loam | 13 | 2.5 | 7.9 | Poplar | Oct 2000 |
| *S. feltiae* | East Anatolia | Van | Loam | 14 | 5 | 7.8 | Reed[b] | Sep 2000 |
| *S. feltiae* | East Anatolia | Van | Loam | 16 | 4.1 | 7.5 | Poplar-clover | Sep 2000 |
| *S. feltiae* | Black Sea | Sinop | Sandy loam | 10 | 4.1 | 6.1 | Roadside edge[c] | Nov 1999 |
| *S. feltiae* | Marmara | Canakkale | Clay loam | 18 | 2.4 | 7.7 | Reed[b] | Sep 1999 |
| *S. feltiae* | Middle Anatolia | Eskisehir | Loam | 21 | 5 | 7.4 | Grassland | Sep 1999 |
| *S. feltiae* | Black Sea | Rize | Sandy loam | 15 | 5.5 | 5.9 | Grassland | Jul 2000 |
| *Steinernema* n. sp.[d] | East Anatolia | Kars | Sandy–clay–loam | 15 | 5.3 | 7.3 | Grassland | Jun 2000 |

[a]Marsh containing *Juncus* sp.; [b]Reed, *Phragmites* sp.; [c]Roadside edge with grass; [d]Putative new species.

*Taxonomic studies*

*Morphological characterization*

For morphological studies, nematodes were examined live or heat-killed in 60 °C Ringer's solution. The heat-killed nematodes were placed in triethanolamine–formalin (TAF) fixative (Kaya and Stock 1997) and processed to anhydrous glycerine for mounting (Seinhorst 1959). Observations were made from live and mounted specimens using a Nikon Eclipse E600 microscope equipped with differential interference contrast optics. Specimen measurements were made using Scion Image software v. 1.62c (Frederick, Maryland, USA) that was calibrated with a stage micrometer. For morphometric characterization of the isolates, 25 first-generation males and 25 IJs were randomly selected from different *G. mellonella* cadavers.

The following morphometric characters were analyzed: total body length, greatest body width, distance from anterior end to excretory pore, distance from anterior end to nerve ring, distance from anterior end to base of esophagus, tail length, width at anus/cloaca, and values of ratio a, b, c, d (expressed as D%) and e (expressed as E%). Additionally, the length of the spicules, length of the gubernaculum, and number and arrangement of genital papillae were considered.

According to their morphological characteristics, all isolates were placed into similar species-groups using taxonomic criteria suggested by Stock and Kaya (1996) and Hominick et al. (1997). Additionally, morphological features of males and IJs of representative isolates of each species-group were examined using scanning electron microscopy. For this purpose specimens were processed following protocols described by Kaya and Stock (1997).

*Molecular characterization*

In addition to morphological studies, all isolates were characterized molecularly by analysis of the LSU (large subunit) for steinernematids and ITS (internal transcribed spacer) for heterorhabditids of rDNA sequences according to Stock et al. (2001). The resulting sequences were compared to a library of 21 *Steinernema* species (Stock et al. 2001).

To further confirm the identity of the *Steinernema* isolates recovered, cross-breeding tests were conducted according to Kaya and Stock (1997). At least one representative isolate of each species-group was cross-bred with known isolates of the species-group they were assigned to. The following species were used to confirm their identity: *S. feltiae* (SN strain), *S. carpocapsae* (All strain) and *S. affine* (UK strain). In all cases, controls consisted of crosses between the same species/isolate.

## Results

Entomopathogenic nematodes were recovered from 22 of the 1080 soil samples (2%) collected in 1999 and 2000 (Figure 1). Out of the 22 positive samples, 15 steinernematid (68.2%) and seven heterorhabditid (31.8%) isolates were recovered from six of the seven regions.

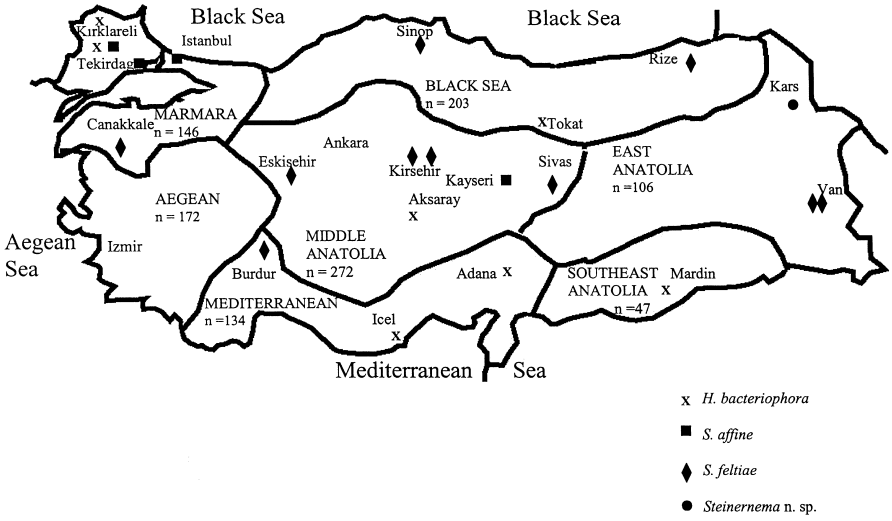


*Figure 1.* Map of Turkey showing the positive sites where entomopathogenic nematodes were recovered. The names in capital letters represent the seven regions in Turkey and the *n* number below the name of each region shows the total number of soil samples taken within the region. The total number of soil samples that were taken for all regions was 1080.

Morphological and molecular identification demonstrated that two of the isolates recovered were *Steinernema feltiae* and *S. affine* (data not shown). These identifications were confirmed by cross-breeding experiments. Four isolates from Middle Anatolia, two isolates each from Black Sea and East Anatolia, and one isolate each from Mediterranean and Marmara (45.5% of the total positive samples) cross-bred with the known *S. feltiae* (SN strain). Three isolates from Marmara and one isolate from Middle Anatolia (18.2%) cross-bred with the known *S. affine* strain. An undescribed *Steinernema* species (4.5%) from East Anatolia, similar to *S. carpocapsae* in size but with some morphological differences and no successful cross-breeding with known *S. carpocapsae*, *S. affine*, or *S. feltiae* strains, was molecularly distinct from other steinernematid species and is considered to be a new species. Morphological and molecular identification of all heterorhabditid isolates showed that they were *H. bacteriophora* (Poinar) and were found in the regions Middle Anatolia, Mediterranean, Southeast Anatolia, and Black Sea.

The habitats from which entomopathogenic nematodes were isolated can be classified broadly as disturbed (59.1%, $n = 13$) and undisturbed (40.9%; $n = 9$). The disturbed habitats include the poplar trees that have been planted as wind breaks and/or lumber sources, agricultural fields (i.e., sunflower, clover, orchard, fallow, and poplar/clover) and the roadside edge (Table 1). Three species of nematodes, *S. feltiae*, *S. affine*, and *H. bacteriophora*, were isolated from the diverse agricultural fields and represented 31.8% of the positive isolates. Similarly, the same three nematode species were isolated from the poplar habitat (including the poplar/clover) and represented 27.4% of the positive isolates. The same three nematode

species and *Steinernema* n. sp. were recovered from undisturbed habitats (i.e., pine forest, grassland, marsh, reed).

Generally, the entomopathogenic nematodes were isolated from the 'lighter' sandy loam and loam soils (Table 1). Soils of the positive sites were classified as sandy, sandy loam, or loam (68.2%) and sandy–clay–loam or clay loam (31.8%). There were no obvious trends associated with soil temperature at the time of sampling, pH or organic matter (Table 1).

## Discussion

Our study has documented the occurrence and natural distribution of entomopathogenic nematodes in Turkey. These nematodes were recovered from six out of the seven regions in Turkey with the Aegean region having no positive entomopathogenic nematode sites. However, in conjunction with another study, two steinernematid isolates were recovered from the Aegean region in 2001, demonstrating that these nematodes are widespread throughout Turkey (S. Hazir, unpublished data).

Rosa et al. (2000) have summarized the rate of recovery of entomopathogenic nematodes from various soil surveys conducted throughout the world. Our recovery rate of 2% is one of the lowest, but is in line with the preliminary survey conducted by Özer et al. (1995) in Turkey who reported a recovery rate of 4.7%. Other surveys with 5% or less recovery include Northern Ireland by Blackshaw (1988) at 3.8%, the Azorean archipelago, Portugal by Rosa et al. (2000) at 3.9%, Korea by Choo et al. (1995) at 4.6%, and Italy by Ehlers et al. (1991) at 5%. The highest recovery rate for entomopathogenic nematodes was in England by Hominick and Briscoe (1990) at 48.6%. Most surveys show a recovery rate from soils between 6 and 35% (Rosa et al. 2000). In more recent surveys not cited by Rosa et al. (2000), the recovery rate of entomopathogenic nematodes was generally between 8 and 30% (Midituri et al. 1997; Griffin et al. 1999, 2000; Stock et al. 1999).

In Turkey, the most common entomopathogenic nematode species isolated was *S. feltiae*, followed by *H. bacteriophora*. Both of these entomopathogenic nematode species are widely distributed throughout the world (Hominick et al. 1996). *Steinernema feltiae*, for example, has been found in tropical Hawaii (USA) as well as in the temperate regions of Europe (Hominick et al. 1996). Even though it has been isolated from the tropics, it is a species that is adapted to cold temperatures (Wright 1992; Hazir et al. 2001). As indicated by Hominick et al. (1996), the global distribution of *S. feltiae* suggests that it is either an efficient disperser and/or an ancient species that was present before continental drift occurred. Once it disperses, it is an effective colonizer, probably adapting to local insect hosts in the habitat. Although not as widely distributed as *S. feltiae*, *H. bacteriophora* has been found from the tropics (Constant et al. 1998) to temperate regions of the world (Hominick et al. 1996). In Turkey, with the exception of an *H. bacteriophora* isolate from Middle Anatolia, six of the seven isolates were found in the warmer climatic areas. *H. bacteriophora*, based on developmental temperatures, appears to be a species

more adapted to tropical and subtropical areas (Grewal et al. 1994), but it has been recovered from Hungary and Germany, two countries that are considered to be temperate (Mráček and Jenser 1988; Glare et al. 1993; Griffin et al. 1999). A comparison of *H. bacteriophora* geographic isolates, as has been done for *S. feltiae* (Hazir et al. 2001), may provide insights into its ecological niche.

In contrast to *S. feltiae* and *H. bacteriophora*, *S. affine*, up to our survey, has been found only in Europe. Its most eastern European distribution is in the Slovak Republic as reported by Sturhan and Lišková (1999). In Turkey, three isolates have been recovered from the European region (Marmara), whereas one isolate was recovered from the Asian region (Middle Anatolia). As far as we are aware, this would be the first report of this species outside of Europe. However, as with many entomopathogenic nematode species that initially appear to be restricted to a given geographical area, as more surveys are conducted and nematode species recovered and identified, many of them have a wider distribution than previously thought.

*Steinernema affine* has been found in a number of different habitats, including along riverbanks (Sturhan and Lišková 1999) and in grassland (Steiner 1996; Midituri et al. 1997; Sturhan and Lišková 1999), apple orchard (Mráček and Bečvář 2000), woodland (Midituri et al. 1997), roadside verge (Midituri et al. 1997), and cultivated areas (Sturhan and Lišková 1999). Originally described by Bovien (1937), *S. affine* was isolated from bibionid fly larvae that inhabited barley fields and grassland. The larvae of this fly feed on roots and decaying organic matter. Although we did not know the natural insect host for this species in Turkey, our findings match much of the habitats in which *S. affine* was found in other parts of Europe.

The isolation of a putative new species of *Steinernema* was an interesting finding. It represented one of our most eastern Turkish isolates. Although the average length of the infective juveniles and first generation males of *S. carpocapsae* (547 and 1450–1890 $\mu$m, respectively; see Poinar 1979) and *Steinernema* n. sp. (544 and 1532 $\mu$m, respectively) were similar, cross-breeding between the two 'isolates' did not occur (S. Hazir and S.P. Stock, unpublished data). Additionally, molecular data strongly indicated that this isolate is a new undescribed species.

An important indicator determining whether entomopathogenic nematodes occur in the environment is soil type. Generally, entomopathogenic nematodes have been isolated from soils with high sand content and low clay content. In our survey, the majority of samples that tested positive for entomopathogenic nematodes were sand to sandy loam soils (Table 1). These results are similar to those reported by a number of other researchers (e.g., Blackshaw 1988; Hominick and Briscoe 1990; Griffin et al. 1991; Hara et al. 1991; Liu and Berry 1995; Midituri et al. 1997; Stock et al. 1999). For pH, we isolated entomopathogenic nematodes from slightly acidic (pH 5.6) to slightly alkaline (pH 7.9) soils. This agrees with other studies where the pH of entomopathogenic nematode positive soil samples varied from 4.6 to 8 (e.g., Mráček and Bečvář 1988; Hara et al. 1991; Griffin et al. 1994).

Our survey has shown that entomopathogenic nematodes occur throughout Turkey, with the most common species being *S. feltiae*, followed by *H. bacteriophora*, *S. affine*, and a putative *Steinernema* n. sp. We believe that the isolation

of *S. affine* is the first report for Asia. These four nematode species open the prospect for using them in biological control programs against insect pests in Turkey. We found that the prevalence of these nematodes in Turkish soils is low compared with other surveys that have been conducted in other parts of the world. The low recovery rate of these nematodes in this survey could be attributed to the selection of sampling sites and/or use of *G. mellonella* as the bait insect. The distribution of entomopathogenic nematodes can be highly aggregated; hence, our small soil sample size would have reduced the chances for isolating the nematodes (Hominick et al. 1996). The site we selected may reflect variation in the availability of suitable host species (Mráček and Webster 1993), and it is difficult to find steinernematids or heterorhabditids in the soil in the absence of susceptible hosts. To overcome this, a greater volume of soil and more sites may have increased our prevalence rate. The other reason may be that we were unable to detect entomopathogenic nematode species that were more host specific. In using *Galleria*, we isolated only those species that have a relatively broad host range or only infect lepidopteran larvae. Inclusion of a bait insect species other than lepidopteran larvae would have been too expensive and limit the extensiveness of our survey. In the future, the incorporation of coleopteran and dipteran bait insects may increase our knowledge on the diversity and distribution of these important natural control agents of insects.

## Acknowledgements

This study was supported by the Scientific and Technical Research Council of Turkey (TUBITAK). We thank S. Nadler for use of microscope and video image software for conducting the morphometric observations and for facilitating equipment for molecular studies, and the Genetics Resources Conservation Program at UC Davis for their continued support for maintenance of the entomopathogenic nematode collection.

## References

Bathon H. 1996. Impact of entomopathogenic nematodes on non-target hosts. Biocontrol Science and Technology 6: 421–434.

Bedding R.A. and Akhurst R.J. 1975. A simple technique for the detection of insect parasitic rhabditid nematodes in soil. Nematologica 21: 109–110.

Blackshaw R.P. 1988. A survey of insect parasitic nematodes in Northern Ireland. Nematologica 21: 109–110.

Boemare N.E., Givaudan A., Brehelin M. and Laumond C. 1997. Symbiosis and pathogenicity of nematode–bacterium complexes. Symbiosis 22: 21–45.

Bovien P. 1937. Some types of association between nematodes and insects. Videnskabelige Meddelelser fra Dansk Naturhistorisk Forening 101: 1–114.

Burnell A.M. and Stock S.P. 2000. *Heterorhabditis*, *Steinernema* and their bacterial symbionts – lethal pathogens of insects. Nematology 2: 31–42.

Choo H.Y., Kaya H.K. and Stock S.P. 1995. Isolation of entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) in Korea. Japanese Journal of Nematology 25: 44–51.

Constant P., Marchay L., Fischer-Le Saux M., Briand-Panoma S. and Mauléon H. 1998. Natural occurrence of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) in Guadeloupe islands. Fundamental and Applied Nematology 21: 667–672.

Ehlers R.U., Deseö K.V. and Stackebrandt E. 1991. Identification of *Steinernema* spp. (Nematoda) and their symbiotic bacteria *Xenorhabdus* spp. from Italian and German soils. Nematologica 37: 360–366.

Georgis R. and Manweiler S.A. 1994. Entomopathogenic nematodes: a developing biological control technology. Agricultural Zoology Reviews 6: 63–94.

Glare T.R., Jackson T.A. and Zimmermann G. 1993. Occurrence of *Bacillus Popilliae* and 2 nematode pathogens in populations of *Amphimallon solstitialis* (Col, Scarabaeidae) near Darmstadt, Germany. Entomophaga 38: 441–450.

Grewal P.S., Selvan S. and Gaugler R. 1994. Thermal adaptation of entomopathogenic nematodes: niche breadth for infection, establishment, and reproduction. Journal of Thermal Biology 19: 245–253.

Griffin C.T., Chaerani R., Fallon D., Reid A.P. and Downes M.J. 2000. Occurrence and distribution of the entomopathogenic nematodes *Steinernema* spp. and *Heterorhabditis indica* in Indonesia. Journal of Helminthology 74: 143–150.

Griffin C.T., Dix I., Joyce S.A., Burnell A.M. and Downes M.J. 1999. Isolation and characterization of *Heterorhabditis* spp. (Nematoda: Heterorhabditidae) from Hungary, Estonia, and Denmark. Nematology 1: 321–332.

Griffin C.T., Joyce S.A., Dix I., Burnell A.M. and Downes M.J. 1994. Characterization of *Heterorhabditis* (Nematoda: Heterorhabditidae) from Ireland and Britain by molecular and cross-breeding techniques, and the occurrence of the genus in these islands. Fundamental and Applied Nematology 17: 245–253.

Griffin C.T., Moore J.F. and Downes M.J. 1991. Occurrence of insect parasitic nematodes (Steinernematidae, Heterorhabditidae) in the Republic of Ireland. Nematologica 37: 92–100.

Griffin C.T., O'Callaghan K.M. and Dix I. 2001. A self-fertile species of *Steinernema* from Indonesia: further evidence of convergent evolution amongst entomopathogenic nematodes? Parasitology 122: 181–186.

Hara A.H., Gaugler R., Kaya H.K. and LeBeck L.M. 1991. Natural populations of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) from the Hawaiian islands. Environmental Entomology 17: 211–216.

Hazir S., Stock S.P., Kaya H.K., Koppenhöfer A.M. and Keskin N. 2001. Developmental temperature effects on five geographic isolates of the entomopathogenic nematode *Steinernema feltiae* (Nematoda: Steinernematidae). Journal of Invertebrate Pathology 77: 243–250.

Hominick W.M. and Briscoe B.R. 1990. Occurrence of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) in British soils. Parasitology 100: 295–302.

Hominick W.M., Briscoe B.R., del-Pino F.G., Heng J., Hunt D.J., Kozodoy E. et al. 1997. Biosystematics of entomopathogenic nematodes: current status, protocols and definitions. Journal of Helminthology 71: 271–298.

Hominick W.M., Reid A.P., Bohan D.A. and Briscoe B.R. 1996. Entomopathogenic nematodes – biodiversity, geographical distribution and the convention on biological diversity. Biocontrol Science and Technology 6: 317–331.

Iraki N., Salah N., Sansour M.A., Segal D., Glazer I., Johnigk S.A. et al. 2000. Isolation and characterization of two entomopathogenic nematode strains, *Heterorhabditis indica* (Nematoda, Rhabditida), from the West Bank, Palestinian Territories. Journal of Applied Entomology 124: 375–380.

Josephrajkumar A. and Sivakumar C.V. 1997. A survey for entomopathogenic nematodes in Kanyakumari district, Tamil Nadu, India. Indian Journal of Entomology 59: 45–50.

Kaya H.K. and Gaugler R. 1993. Entomopathogenic nematodes. Annual Review of Entomology 38: 181–206.

Kaya H.K. and Stock S.P. 1997. Techniques in insect nematology. In: Lacey L.A. (ed.), Manual of Techniques in Insect Pathology. Academic Press, New York, pp. 281–324.

Koppenhöfer A.M. 2000. Nematodes. In: Lacey L.A. and Kaya H.K. (eds), Field Manual of Techniques in Invertebrate Pathology. Kluwer, Dordrecht, the Netherlands, pp. 283–301.

Liao C.Y., Tang L.C., Pai C.F., Hsiao W.F., Briscoe B.R. and Hou R.F. 2001. A new isolate of the entomopathogenic nematode, *Steinernema abbasi* (Nematoda: Steinernematidae), from Taiwan. Journal of Invertebrate Pathology 77: 78–80.

Liu J. and Berry R.E. 1995. Natural distribution of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) in Oregon soils. Environmental Entomology 24: 159–163.

Luc P.V., Nguyen K.B., Reid A.P. and 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. and Wright D.J. 1996. The recovery of entomopathogenic nematodes from selected areas within Peninsular Malaysia. Journal of Helminthology 70: 303–307.

Midituri J.S., Waeyenberge L. and Moens M. 1997. Natural distribution of entomopathogenic nematodes (Heterorhabditidae and Steinernematidae) in Belgian soils. Russian Journal of Nematology 5: 55–65.

Mráček Z. and Bečvář S. 2000. Insect aggregations and entomopathogenic nematode occurrence. Nematology 2: 297–301.

Mráček Z., Bečvář S. and Kindlann P. 1999. Survey of entomopathogenic nematodes from the families Steinernematidae and Heterorhabditidae (Nematoda: Rhabditida) in the Czech Republic. Folia Parasitologica 46: 145–148.

Mráček Z. and Jenser G. 1988. First report of entomogenous nematodes of the families Steinernematidae and Heterorhabditidae from Hungary. Acta Phytopathologica et Entomologica Hungarica 23: 153–156.

Mráček Z. and Webster J.M. 1993. Survey of Steinernematidae and Heterorhabditidae (Rhabditida: Nematoda) in western Canada. Journal of Nematology 25: 710–717.

Özer N., Keskin N. and Kirbaş Z. 1995. Occurrence of entomopathogenic nematodes (Steinernematidae: Heterorhabditidae) in Turkey. Nematologica 41: 639–640.

Poinar G.O. Jr. 1979. Nematodes for Biological Control of Insects. CRC Press, Boca Raton, Florida.

Poinar G.O. Jr. 1990. Taxonomy and Biology of *Steinernema* and *Heterorhabditis*. In: Gaugler R. and Kaya H.K. (eds), Entomopathogenic Nematodes for Biological Control. CRC Press, Boca Raton, Florida, pp. 23–61.

Rosa J.S., Bonifassi E., Amaral J., Lacey L.A., Simoes N. and Laumond C. 2000. Natural occurrence of entomopathogenic nematodes (Rhabditida: *Steinernema*, *Heterorhabditis*) in the Azores. Journal of Nematology 32: 215–222.

Rosales A.L.C. and Suarez H.Z. 1998. Nemátodos entomopatógenos como posibles agentes de control del gorgojo negro del platano *Cosmopolites sordidus* (Germar 1824) (Coleoptera: Curculionidae). Boletin de Entomologia Venezolana 13: 123–140.

Seinhorst J.W. 1959. A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. Nematologica 4: 117–128.

Shishiniova M., Budurova L. and Gradinarov D. 1998. Contribution to the fauna of the entomopathogenic nematodes/rhabditida: Steinernematidae, Heterorhabditidae from Bulgaria II. Biotechnology and Biotechnological Equipment 12: 104–108.

Steiner W. 1996. Distribution of entomopathogenic nematodes in the Swiss Alps. Suisse Journal de Zoologie 103: 439–452.

Stock S.P. and Kaya H.K. 1996. A multivariate analysis of morphometric characters of *Heterorhabditis* species (Nemata, Heterorhabditidae) and the role of morphometrics in the taxonomy of the species of the genus. Journal of Parasitology 82: 806–813.

Stock S.P., Campbell J.F. and Nadler S.A. 2001. Phylogeny of *Steinernema* Travassos, 1927 (Cephalobina: Steinernematidae) inferred from ribosomal DNA sequences and morphological characters. Journal of Parasitology 87: 877–889.

Stock S.P., Pryor B.M. and Kaya H.K. 1999. Distribution of entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) in natural habitats in California, USA. Biodiversity and Conservation 8: 535–549.

Sturhan D. and Lišková M. 1999. Occurrence and distribution of entomopathogenic nematodes in the Slovak Republic. Nematology 1: 273–277.

Tangchitsomkid N. and Sontirat S. 1998. Occurrence of entomopathogenic nematodes in Thailand. Kasetsart Journal of Natural Sciences 32: 347–354.

Vyas R.V., Pharindera Y., Choudhary R., Patel N.S. and Yadav P. 1999. Natural incidence of entomopathogenic nematodes in central north Gujarat. In: Dhawan S.C. (ed.), Proceedings of National Symposium on Rational Approaches in Nematode Management for Sustainable Agriculture, Anand, India, 23–25 November, 1998. Nematological Society of India, New Delhi, India, pp. 86–88.

Wright P.J. 1992. Cool temperature reproduction of steinernematid and heterorhabditid nematodes. Journal of Invertebrate Pathology 60: 143–152.

Yoshida M., Reid A.P., Briscoe B.R. and Hominick W.M. 1998. Survey of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) in Japan. Fundamental and Applied Nematology 21: 185–198.