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Distribution of entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) in natural habitats in California, USA

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Abstract. A total of 270 soil samples from 30 different habitats in 10 geographic regions of California were evaluated for the presence of rhabditid entomopathogenic nematodes. Nematodes were isolated from 26.3% of the samples. The recovered isolates were identified as *Steinernema carpocapsae*, *S. feltiae*, *S. kraussei*, *S. longicaudum*, *S. oregonense*, *Heterorhabditis marelatus* and *H. bacteriophora*. Among the steinernematids, *S. kraussei* and *S. feltiae* were the most commonly encountered species, generally occurring in acidic soils high in organic matter. Among the heterorhabditids, *H. bacteriophora* was isolated along the southern coast, whereas *H. marelatus* was recovered along the northern coast of California. Steinernematids were recovered from coniferous forests, oak woodlands and grasslands whereas heterorhabditids were isolated from coastal marshes.

Key words: California, insect-pathogenic nematodes, natural habitats, survey

Introduction

Entomopathogenic nematodes (EPN) in the families Steinernematidae and Heterorhabditidae are obligate parasites of soil-inhabiting insects and have great potential as biological control agents of many insect pests. They are found in a variety of soil habitats, and the various species and isolates exhibit considerable variation in terms of host range, reproduction, infectivity and conditions for survival (i.e. temperature, soil moisture, etc.) (Bedding et al. 1983; Bedding 1990). To further advance the use of EPN as biological control agents, locally-adapted species or isolates from native non-disturbed habitats need to be identified and their unique characteristics documented.

Surveys for EPN have been conducted in temperate, subtropical and tropical regions (Hominick et al. 1996). In the US, surveys have been conducted in Puerto Rico (Roman and Beavers 1983), Florida (Beavers et al. 1983), North Carolina (Akhurst and Brooks 1984), Hawaii (Hara et al. 1991), New Jersey (Gaugler et al. 1992), Tennessee (Rueda et al. 1993) and Oregon (Liu and Berry

1995). In California, several steinernematid and heterorhabditid nematodes have been recovered from agricultural areas and golf courses (Poinar 1990; Stock et al. 1996), however, no systematic survey has been conducted to document the occurrence of these nematodes from natural or non-disturbed habitats. In contrast to human modified areas, natural habitats are more likely uncontaminated by introduced nematodes and offer a better chance for finding native species. California is unique in the diversity of habitats found, and may contribute to an equally diverse distribution of EPN. The objective of this study was to survey EPN diversity in a variety of undisturbed (non-agricultural) habitats of California and identify the recovered isolates using classical and molecular taxonomy.

Methods

Collection and isolation of nematodes

Soil samples were collected from 10 landscape regions of California (Durrenberger 1959) between March and June 1996. Within each region, 1–4 of the predominant habitats (vegetation types) were selected for sampling (Table 1). Three sites were selected within each habitat and 3 samples were collected at each site. Each soil sample (approximately 1 kg) was a composite of 5 random subsamples taken at a depth of 10–20 cm in an area of 20 m^2. Samples were taken at least 100 m apart at each site. Samples were placed in polyethylene bags to prevent water loss and were kept in coolers (ca. 15 °C) during transit to the laboratory.

EPN were recovered from soil samples by the insect baiting technique (Bedding and Akhurst 1975). Five *Galleria mellonella* (L.) larvae were placed in 250 ml plastic containers (5 containers/sample) with moistened soil obtained from each sample. Containers were covered with a lid, turned upside down and kept at room temperature (20 ± 3 °C). After 7–8 days, all insects were recovered, and parasitized cadavers were individually placed in White traps to allow the emergence of the infective-stage juveniles. Emerging nematodes were pooled for each sample and used to infect fresh *G. mellonella* larvae to produce nematodes for identification and establishment of cultures. Soil samples with negative nematode recovery were baited a second time to confirm results of the first test.

For each sampling site, a portion of the soil from each of the 3 samples (ca. 100 g) was combined and analyzed for the following soil characteristics: pH, organic matter, sand, silt and clay content. Soil analysis was performed by the Division of Agricultural and Natural Resources (DANR) Analytical Laboratory at the University of California, Davis. Positive samples (EPN present) were correlated with landscape region, habitat and soil characteristics (Table 2).

Table 1. Landscape regions, natural habitats and sampling time.

Landscape regions	Habitats	Sampling time
R1. Klamath-Siskiyou	Mixed coniferous forest	5/96
	Grassland	6/96
	Chaparral	6/96
R2. Northeast volcanic	Mixed coniferous forest	5/96
	Grassland	5/96
	Chaparral	6/96
R3. Sierra Nevada foothills and low coastal mountains	Mixed coniferous forest	5/96
	Grassland	6/96
	Chaparral	6/96
	Oak woodland	4/96
R4. Great Valley	Oak woodland	5/96
	Grassland	5/96
	Wetland	6/96
R5. Sierra Nevada	Mixed coniferous forest (> 5000 ft.)	6/96
	Grassland (meadow) (> 5000 ft.)	6/96
	Lodgepole pine forest (> 9000 ft.)	6/96
R6. Southwest mountains and valleys	Mixed coniferous forest	3/96
	Oak woodland	3/96
	Chaparral	3/96
	Grassland	3/96
R7. Desert and desert mountains	Pinion-juniper forest	3/96
	Joshua tree forest	3/96
	Desert scrub	3/96
	Grassland	3/96
R8. Northern coastal strip	Grassland	5/96
	Coastal chaparral	5/96
	Coastal marsh	5/96
R9. Southern coastal strip	Grassland	3/96
	Coastal chaparral	3/96
	Coastal marsh	3/96
R10. Redwoods	Redwood forest	5/96

Taxonomic studies

Nematode isolates recovered were identified by morphological criteria using standard light microscopy and scanning electron microscopy (SEM) according to Kaya and Stock (1997). For biometric studies, approximately 30 adult males of first and second generation and 30 infective juveniles from selected representatives of each isolate were fixed in triethanolamine formalin (TAF) and processed to glycerin (Kaya and Stock 1997). Morphometrical analysis was done with the aid of Jandel Java software™ with a high resolution video

Table 2. EPN-positive sites within California landscape regions.

Region	Habitat	pH	% OM	Soil type	No. positive samples /Total no. samples	Nematode species
R1: Klamath-Siskiyou	MCF	6.3	7.13	Sandy loam	3/3	*S. carpocapsae*
R2: NE Volcanic	GL	6.3	4.31	Sandy loam	3/3	*S. feltiae*
R3: Sierra foothills and low coastal mountains	OW	5.5	6.46	Sandy loam	2/3	*S. kraussei*
	OW	6.3	5.01	Loam	3/3	*S. kraussei*
	OW	6.2	2.81	Sandy loam	1/3	*S. kraussei*
R4: Great Valley	OW	7.0	2.83	Silt loam	3/3	*S. carpocapsae*
	OW	5.4	2.41	Sandy loam	3/3	*S. carpocapsae*
	OW	6.2	5.96	Sandy loam	3/3	*S. carpocapsae*
R5: Sierra Nevada	MCF	6.8	6.46	Sandy loam	3/3	*S. longicaudum*
	MCF	6.7	5.24	Sandy loam	3/3	*S. longicaudum*
	LPF	6.1	4.67	Sandy loam	3/3	*S. kraussei*
	LPF	6.2	5.49	Sandy loam	3/3	*S. kraussei*
	M	5.5	5.30	Sandy loam	3/3	*S. kraussei*
R6: SW Mountains and valleys	MCF	5.6	5.13	Sandy loam	3/3	*S. oregonense*
	MCF	5.0	5.39	Sandy loam	3/3	*S. oregonense*
	MCF	6.1	4.81	Sandy loam	3/3	*S. oregonense*
	OW	5.5	5.35	Sandy loam	3/3	*S. kraussei*
	OW	5.2	2.44	Sandy loam	3/3	*S. kraussei*
	OW	6.3	2.81	Sandy loam	3/3	*S. kraussei*
R8: North Coast	CM	7.2	2.77	Loam	3/3	*H. marelatus*
	CM	6.4	3.96	Sandy loam	3/3	*H. marelatus*
	CM	6.3	2.10	Loamy sand	3/3	*H. marelatus*
R9: South Coast	CM	7.1	1.27	Sand	3/3	*H. bacteriophora*
	CM	6.3	1.10	Loamy sand	3/3	*H. bacteriophora*
	GL	5.3	5.56	Loam	2/3	*S. feltiae*

Abbrevations: MCF – mixed coniferous forest; GL – grassland; OW–oak woodland; LPF – lodgepole pine forest; M – meadow; CM – coastal marsh.

camera. In general, and for all the nematode stages (first and second generation adults and infective juveniles), the following characters were analyzed: total length, greatest 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%). In particular, within the males, testis reflexion, length and width of the spicules, length and width of gubernaculum and number and arrangement of genital papillae were also considered. For first and second generation females, morphology of the vulva, V% and shape of tail were also examined. First generation males and infective juveniles of selected representatives from each group were examined by SEM for confirmation of observations done with light microscopy. According to their morphology, all isolates were placed into different species groups using taxonomic criteria suggested by Stock and Kaya (1996) and Stock et al. (1997).

To further confirm the identity of the *Steinernema* isolates recovered, cross-breeding tests were conducted according to Kaya and Stock (1997). Representative isolates of each species group were cross-bred with each other and with known isolates of the species group they were assigned and were more closely related (Table 3). With the exemption of *S. longicaudum* (no 'type isolate' was available), the following species were considered to confirm identity of the species: *S. feltiae* (SN strain), *S. carpocapsae* (ALL strain), *S. kraussei* (Westphalia and Nash strains), *S. oregonense* (OS10 strain). Similarly, each of the *Heterorhabditis* isolates recovered were cross-bred with each other and with reference isolates OH10 (*H. marelatus*) and Chino Hill (*H. bacteriophora*) using the lipid agar method of Dix et al. (1992). In both cases, controls consisted of crosses between the same isolate/species.

In addition to morphological and cross-breeding studies, two randomly selected isolates of each identified *Steinernema* species were sent for molecular characterization to A. P. Reid (International Institute of Parasitology, St. Albans, UK) (Figures 2–3) (Table 3). These isolates were subjected to RFLP analysis (Reid and Hominick 1992) and the resulting band patterns were compared to an RFLP library representing of over 35 *Steinernema* species.

Results

EPN were recovered from 71 (26.3%) of the 270 soil samples collected from 12 distinct habitats in 8 out of the 10 surveyed regions (Figure 1). No nematodes were recovered from soil samples collected from the desert and redwood regions. With the exception of two sites in Region 3 (Sierra Nevada foothills and

Table 3. Hybridization tests of selected EPN isolates from California.

Isolates/species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
S. carpocapsae isolates																																
1. R1-mcf21[a]	+																															
2. R4-ow12[a]	+	+				−			−																							
3. R4-ow21	+	+	+			−				−																						
4. R4-ow33	+	+	+	+				−																								
5. S. c. [1] (All)	+	+	+	+	+	−				−						−					−	−								−	−	
S. feltiae isolates																																
6. R2-gl32[a]	−					+																										
7. R9-gl11[a]	−				−	+	+		−														−									
8. S. f.[2] (SN)	−	−				+	+	+		−		−		−	−					−									−		−	
S. kraussei isolates																																
9. R3-ow11						−				+																						
10. R3-ow24						−				+	+																					
11. R3-ow31[a]										+	+	+							−													
12. R5-lpf11			−			−				+	+	+																				
13. R5-lpf23[a]	−	−							+		+	+																				
14. R5-gl12		−	−			−			+		+	+	+					+														
15. R6-ow12						−			+		+										−	−	−									
16. R6-ow23	−	−				−				+	+				+		+				−	−										
17. R6-ow31	−	−				−				+	+	+				+	+				−	−										
18. S. k[3] (Westphalia)			−	−	−	−			+	+	+	+	+	+	+	+	+	+	+	−		−	−			−			−	−		
19. S. k[3] (Nash)			−	−	−	−			+	+	+	+	+	+	+	+	+	+		−		−	−			−			−	−		
S. longicaudum isolates																																
20. R5-mcf12[a]																				+												
21. R5-mcf23 (B2)[a]																				+	+											
S. oregonense isolates																																
22. R6-mcf13[a]	−	−		−		−			−												−	+										
23. R6-mcf22			−							−			−							−		+	+									

Table 3. (Continued)

Isolates/species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
24. R6-mcf32[a]		−								−				−						−		+	+	+								
25. S. o.[4] (OS10)			−	−		−			−		−						−					+	+	+	+							
H. bacteriophora isolates																																
26. R9-cm12																										+			−	−	−	−
27. R9-cm22																										+	+		−	−	−	−
28. H. b.[5] (Chino Hills)																										+	+	+	−	−	−	
H. marelatus isolates																																
29. R8-cm11																										−		−	+			
30. R8-cm22																										−	−	−	+	+		
31. R8-cm31																										−	−		+	+	+	
32. H. m.[6] (OH10)																										−	−	−	+	+	+	+

Abbreviations: [1] *S. carpocapsae*; [2] *S. feltiae*; [3] *S. kraussei*; [4] *S. oregonense*; [5] *H. bacteriophora*; [6] *H. marelatus*. +: progeny; −: no progeny; in blank: no crosses.
[a] Submitted for molecular characterization (RFLP analysis).

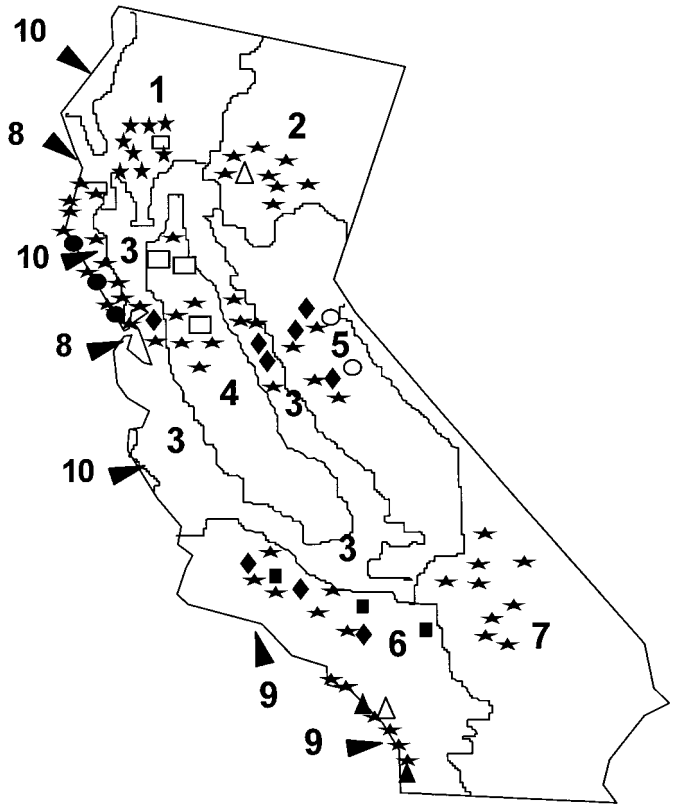

Figure 1. Geographic regions of California showing sampling sites. 1. Klamath-Siskiyou; 2. Northeast Volcanic; 3. Sierra foothills and low coastal mountains; 4. Great Valley; Sierra Nevada; 6. Southwest Mountains and Valleys; 7. Desert and desert mountains; 8. North coastal strip; 9. South coastal strip; 10. Redwoods. Positive sampling sites: (□) *S. carpocapsae*; (△) *S. feltiae*; (◆) *S. kraussei*; (■) *S. oregonense*; (○) *S. longicaudum*; (▲) *H. bacteriophora*; (●) *H. marelatus*; (★) negative sampling sites.

low coastal mountains) and one site in Region 9 (southern coastal strip), all positive sampling sites yielded 3 out of 3 EPN-positive samples (Table 2). Of the isolates recovered, 80% were steinernematids and 20% were heterorhabditids. Nematodes were identified as: *Steinernema feltiae* (Filipjev 1934), *S. kraussei* Steiner 1923 (Figure 2), *S. carpocapsae* (Weiser 1934), *S. longicaudum* Shen and Wang 1992 (Figure 3), *S. oregonense* Liu and Berry 1996a, *Heterorhabditis bacteriophora* Poinar 1975 and *H. marelatus* Liu and Berry 1996b.

Steinernematids were more ubiquitous than the heterorhabditids, having been isolated from 7 out of 10 regions. *S. kraussei* was the most abundant (32.8% of the total positive samples) and widely distributed species. It was found in 3 different landscape provinces – the Sierra Nevada foothills and

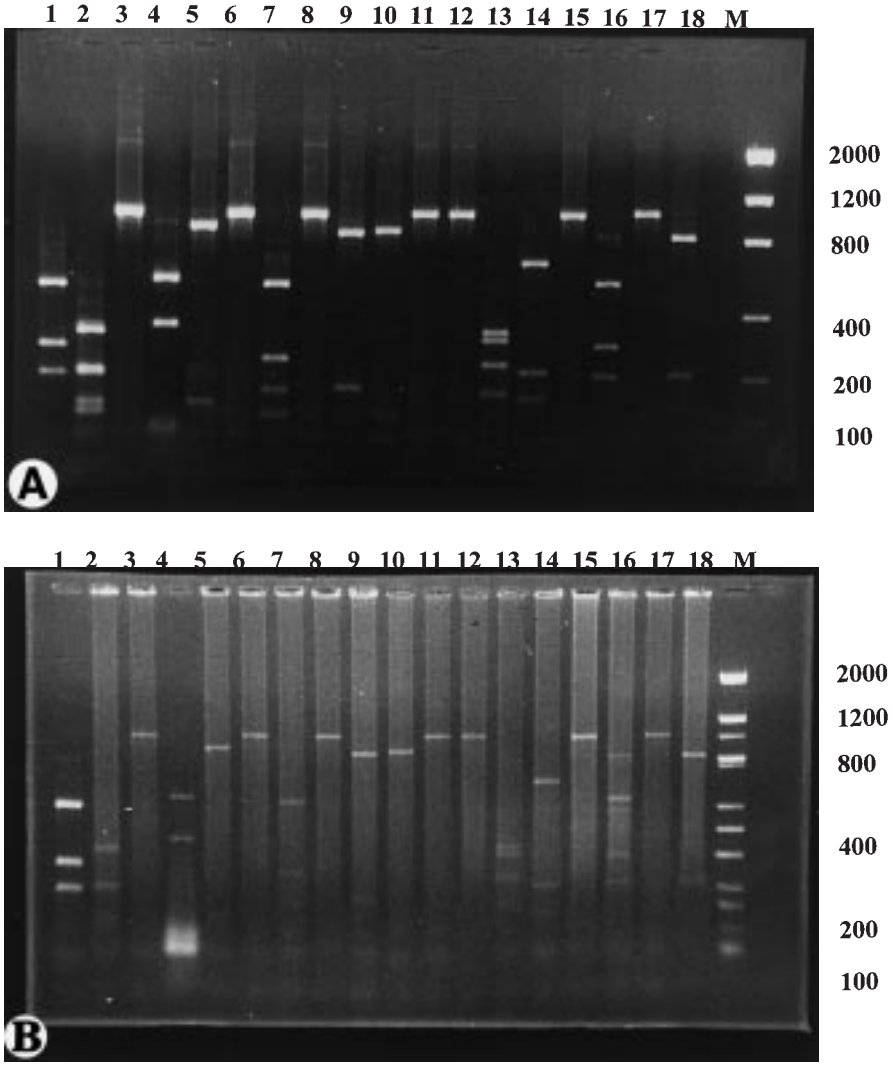

Figure 2. PCR amplified products from the internal transcribed spacer (ITS) of *S. kraussei* digested with 17 different enzymes. Fragments were separated with ethidium bromide stained 1.5% (W/V) agarose gel. A. Westphalia isolate; B. R3-OW24 isolate (California). Lane 1 is a digest of *S. feltiae* (UK, site 76) with *Alu* I. Lanes 2–18 are individual digests of *S. kraussei* with the following restriction enzymes: 2. *Alu* I; 3. *Bst* 0 I; 4. *Dde* I; 5. *ECOR* I; 6. *Hae* III; 7. *Hha* I; 8. *Hind* III; H inf I; 10. *Hpa* I; 11. *Kpm* I; 12. *Pst* I; 13. *Pvu* I; 14. *Rsa* I; 15. *Sal* I; 16. *Sau* A I; 17. *Sau* 96; 18. *Xba* I; 19. molecular weight marker and the band sizes are shown in base pairs.

coastal mountains, Sierra Nevada and SW mountains and valleys. *S. feltiae* was recovered in the northeast volcanic and southern coast regions. *S. carpocapsae* was isolated in the Klamath-Siskiyou and Great Valley. *S. oregonense*

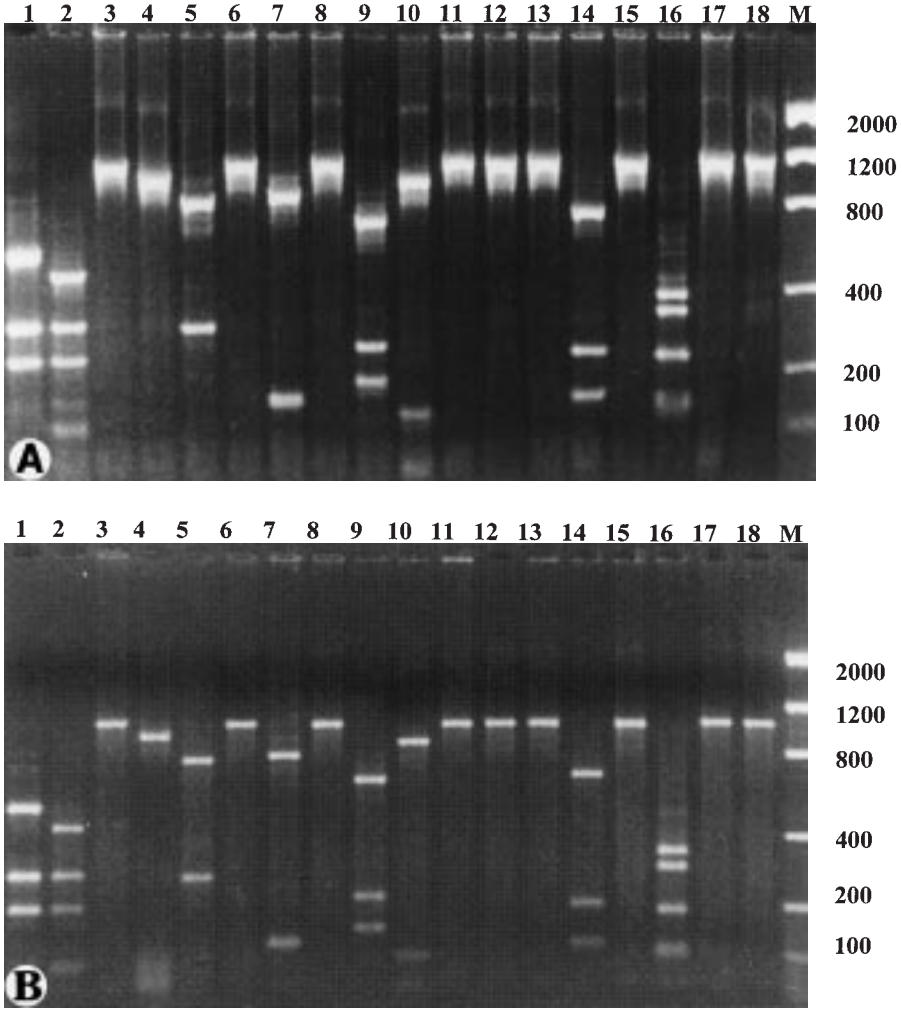

Figure 3. PCR amplified products from the internal transcribed spacer (ITS) of *S. longicaudum* digested with 17 different enzymes. Fragments were separated with ethidium bromide stained 1.5% (W/V) agarose gel. A. Chinese isolate; B. R5-mcf23 (B2) isolate (California). Lane 1 is a digest of *S. feltiae* (UK, site 76) with *Alu* I. Lanes 2–18 are individual digests of *S. longicaudum* with the following restriction enzymes: 2. *Alu* I; 3. *Bst* 0 I; 4. *Dde* I; 5. *ECOR* I; 6. *Hae* III; 7. *Hha* I; 8. *Hind* III; H inf I; 10. *Hpa* I; 11. *Kpm* I; 12. *Pst* I; 13. *Pvu* I; 14. *Rsa* I; 15. *Sal* I; 16. *Sau* A I; 17. *Sau* 96; 18. *Xba* I; 19. molecular weight marker and the band sizes are shown in base pairs.

and *S. longicaudum* were isolated from the SW mountains and valleys and the Sierra Nevada, respectively (Table 1).

The *Heterorhabditis* species were restricted to the Northern and Southern coastal regions, but with a distinct geographic delimitation between the two species (Table 1). *H. marelatus* was exclusively isolated along the northern

coastal strip, and *H. bacteriophora* was only recovered along the south coastal strip.

Habitats

The majority of the positive samples (48 of 71) were recovered from woodlands (coniferous forest (33.8%) and oak forests (33.8%)). Coastal marshes and grasslands yielded the remaining positive samples (23.9% and 8.5%, respectively). No nematodes were recovered from soil samples taken from chaparral habitats, redwood forest, or any of the desert habitats (scrub, chaparral and Joshua tree forest).

In terms of species diversity, the coniferous forests were the richest habitats, yielding 4 out of the 5 species of *Steinernema* recovered. Next were the coastal marshes, where two *Heterorhabditis* species (*H. marelatus*, *H. bacteriophora*) and one steinernematid (*S. feltiae*) were recovered. Oak woodlands were characterized by the presence of *S. kraussei* and *S. carpocapsae*, and the grasslands were represented by *S. kraussei* and *S. feltiae*.

In terms of EPN recovery, several habitats had significantly higher recovery frequencies than others. Most notable were the oak woodlands in which 100% of the sites sampled were positive in all regions where they were sampled. Also showing 100% recovery rate were the mixed coniferous forests of the southwest mountains and the north coastal marshes. South coastal marshes, Sierra Nevada mixed coniferous forests, and Sierra Nevada lodge-pole pine forests had recovery frequencies of 67%.

Soil type

Soil types classified from sand to clay with a pH that ranged from acidic (4.2) to slightly alkaline (7.2). In general, steinernematids were found in sandy loam soils that ranged from acidic (pH 5.0) to neutral (Table 2). The organic matter content of the nematode-positive soil samples varied from 2.4% to 7.1%. As for the *Heterorhabditis* species, *H. bacteriophora* was recovered from sand to loamy sand soils, with a pH ranging from slightly acidic to slightly alkaline (6.3–7.1). The organic matter content of these soils was the lowest recorded from all the positive soil samples (Table 2). The soil types where *H. marelatus* was found ranged from loam to sandy loam. The pH of the soils was similar to those for *H. bacteriophora* (6.3–7.2). The organic matter content for these soils was higher than that for *H. bacteriophora* (2.1–3.9% vs. 1.1–1.2%).

Discussion

The present study has shown for the first time the occurrence of EPN in non-disturbed habitats in California. Moreover, an accurate identification (to the species level) of all the isolates recovered was accomplished.

In this survey, 26.3% of the samples taken were nematode-positive with a predominance of steinernematids (80%) over heterorhabditids (20%). Most of the *Steinernema* species were recovered from coniferous and oak forests. Even though no nematodes were recovered from soil samples taken from chaparral habitats, redwood forest, or any of the desert habitats, a more intensive survey of these habitats would be necessary to fully confirm these results.

Among the steinernematids, *S. kraussei* was the most commonly encountered species in California. It was isolated above 7000 ft from coniferous forests and a grassland in the Sierra Nevada Mountains; at 3000 ft in oak woodlands in the Valley SW Mountains and Valleys and near sea level in oak woodlands in the Sierra Foothills and Coastal Mountains. This is in agreement with European studies in which this species has been found at both high (coniferous forests) and low elevations (meadows) (Mracek et al. 1992; Steiner 1996). Although this is the first report of *S. kraussei* in California, recent studies have confirmed the presence of this species in US (New York) and Canada (Alberta and British Columbia) (Stock et al. unpublished).

An unexpected finding from this survey was the recovery of *S. longicaudum* from coniferous forests (lodgepole pine) in the Sierra Nevada mountains. This species was originally isolated from an orchard in China (Shen 1992), and has subsequently been isolated from Australia (Hominick et al. 1996). This is the first report of this species in North America and expands its known geographic range.

S. oregonense was originally isolated from a grassland in Oregon (Liu and Berry 1996). In our survey, this species was found in mixed coniferous forests in the Southwest Mountains and Valleys, expanding the habitat range and natural distribution of this nematode to Southern California.

S. carpocapsae was recovered from oak woodlands in the Great Valley and mixed coniferous forests in the Klamath-Siskiyou region. However, this species has also been reported from an apple orchard (Poinar 1990) and golf courses in the foothills of the Sierra Nevada (Kaya and Koppenhofer pers. comm.), which suggests this species may be widely distributed in California.

S. feltiae was recorded from a grassland and a coastal marsh in Southern California, and from grasslands in the Northeast volcanic region. This is in agreement with results from surveys conducted in other parts of the world, where grasslands seem to be the habitat of preference for this species (Boag et al. 1992; Hominick et al. 1995; Miduturi et al. 1996; Steiner 1996).

In this survey, *Heterorhabditis* species, were isolated in coastal marshes from both North and South coastal strips. This is in agreement with other studies

where heterorhabditids have been found to be more common in sandy coastal soils (Griffin et al. 1991; Hara et al. 1991; Liu and Berry 1995). However, previous studies have reported the presence of *H. bacteriophora* from orchards and golf courses in California (Stock et al. 1996) indicating this species is not restricted to the coastal strips.

Like other surveys (Hara et al. 1991, Griffin et al. 1994), our study showed that sandy loam soils seem to be the preferred soil types for EPN. This is supported by the fact that mobility and survival of EPN are favored in soils with a high sand content, whereas soils with high clay content restrict nematode movements (Molyneux and Bedding 1984; Kung et al. 1990). In terms of pH tolerance, EPN were found in soils with a pH ranging from acidic (pH 5) to slightly alkaline (pH 7.2). This agrees with other studies where the pH of nematode-positive soil samples varied from 4.6 to 8 (Hara et al. 1991; Griffin et al. 1994).

Our results extend the geographic range of several species. The presence in California of *S. longicaudum* indicates this species has a more extended geographic distribution. According to Hominick et al. (1996) this species has been isolated from two distant locations China and Australia. In view of this, the Californian isolate of *S. longicaudum*, as well as the Chinese and Australian, may represent a species with a Pacific Rim distribution or relic populations of a previously widespread species. The recovery of *S. oregonense* in California extends the known geographic range of this species further south than previous reports (Liu and Berry 1996a, 1996b; Stock et al. 1996). The finding of *S. carpocapsae*, *S. feltiae* and *H. bacteriophora* in California supports the notion sustained by Hominick et al. (1996) that these three species are ubiquitous.

An important finding of this survey is that at all the EPN-positive sites, only one single nematode species was recovered. Furthermore, at over 90% of the EPN-positive sites, all three samples collected contained nematodes. This information should be considered in future surveys in California so that emphasis can be done on sampling more sites rather than multiple samples per site.

Finally, the richness of species encountered in this survey, reflects the diversity of habitats found in California and suggests the adaptability of EPN to a wide range of habitats. This is a very encouraging aspect since the nematodes isolated during this survey may contribute to the expanded use of native isolates of entomopathogenic nematode in future biological control programs in California and other areas with similar climates.

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Westphalia isolate of. *S. kraussei* and OS10 isolate of *S. oregonense*, respectively.

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References

Akhurst RJ and Brooks WM (1984) The distribution of entomophilic nematodes (Heterorhabditidae and Steinernematidae) in North Carolina. Journal of Invertebrate Pathology 44: 140–145

Beavers JB, Mc Coy CW and Kaplan DT (1983) Natural enemies of subterranean. *Diaprepes abbreviatus* (Coleoptera: Curculionidae) larvae in Florida. Environmental Entomology 12: 840–843

Bedding RA (1990) Logistics and strategies for introducing entomopathogenic nematode technology in developing countries. In: Gaugler R and Kaya HK (eds) Entomopathogenic Nematodes for Biological Control, pp. 233–248, CRC, Boca Raton, FL

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

Bedding RA, Molyneux AS and Akhurst RJ (1983) *Heterorhabditis* spp., *Neoaplectana* spp. and *Steinernema kraussei*, interspecific and intra specific differences in infectivity to insects. Experimental Parasitology 55: 248–257

Boag B, Neilson R and Gordon SC (1992) Distribution and prevalence of the entomopathogenic nematode *Steinernema feltiae* in Scotland. Annals of Applied Biology 121: 355–360

Dix I, Burnell AM, Griffin CT and Joyce SA (1992) The identification of biological species in the genus *Heterorhabditis* (Nematoda: Heterorhabditidae) by cross-breeding second generation amphimictic adults. Parasitology 104: 509–518

Durrenberger RW (1959) The Geography of California in Essays and Readings. Brewster Publications, CA

Gaugler R, Campbell JF, Selvan S and Lewis EE (1992) Large-scale inoculative releases of the entomopathogenic nematode *Steinernema glaseri*: assessment 50 years later. Biological Control 2: 181–187

Griffin CT, Moore JF and Downes MJ (1991) Occurrence of insect parasitic nematodes (Steinernematidae, Heterorhabditidae) in the Republic of Ireland. Nematologica 37: 92–100

Griffin CT, Joyce SA, Dix I, Burnell AM and Downes MJ (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

Hara AH, Gaugler R, Kaya HK and Lebeck LM (1991) Natural populations of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) from the Hawaiian islands. Environmental Entomology 20: 211–216

Hominick WM, Reid AP and Briscoe BR (1995) Prevalence and habitat specificity of steinernematid and heterorhabditid nematodes isolated during soil surveys in the UK and the Netherlands. Journal of Helminthology 69: 27–32

Hominick WM, Reid AP, Bohan DA and Briscoe BR (1996) Entomopathogenic nematodes-biodiversity, geographical distribution and the convention on biological diversity. Biocontrol Science and Technology 6: 317–331

Kaya HK and Stock SP (1997) Techniques in insect Nematology. In: Lacey LA (ed) Manual of Techniques in Insect Pathology, pp 281–324. Academic Press, New York

Kung SPR, Gaugler and Kaya HK (1990) Influence of soil, pH and oxygen on persistence of *Steinernema* spp. Journal of Nematology 22: 440–445

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

Liu J and Berry RE (1996a) *Steinernema oregonensis* n. sp. (Rhabditida: Steinernematidae) from Oregon, USA. Fundamental and Applied Nematology 19: 375–380

Liu J and Berry RE (1996b) *Heterorhabditis marelatus* n. sp. (Rhabditida: Heterorhabditidae) from Oregon. Journal of Invertebrate Pathology 67: 48–54

Miduturi JS, Moens M, Hominick WM, Briscoe BR and Reid AP (1996) Naturally occurring entomopathogenic nematodes in the province of West-Flanders, Belgium. Journal of Helminthology 7: 319–327

Molyneux AS and Bedding RA (1984) Influence of soil texture and moisture on the infectivity of *Heterorhabditis* sp. D1 and *Steinernema glaseri* for larvae of the sheep blowfly, *Lucilia cuprina*. Nematologica 30: 358–365

Mracek Z, Weiser J, Bures M and Kahounova L (1992) *Steinernema kraussei* (Steiner, 1923) (Nematoda-. Steinernematidae) rediscovery of its type locality in Germany. Folia Parasitologica 39: 181–182

Poinar GO, Jr. (1990) Taxonomy and Biology of *Steinernema* and *Heterorhabditis*. In: Gaugler R and Kaya HK (eds) Entomopathogenic nematodes for biological control, pp 23–61. CRC, Boca Raton, FL

Reid AP and Hominick WM (1992) Restriction fragment length polymorphisms within the ribosomal DAN repeat unit of British entomopathogenic nematodes (Rhabditida: Steinernematidae). Parasitology 105: 317–323

Roman J and Beavers JB (1983) A survey of Puerto Rican soils for entomogenous nematodes which attack *Diaprepes abbreviatus* (L.) (Coleoptera: Curculionidae). Journal of Agriculture of the University of Puerto Rico 67: 311–316

Rueda LM, Osawaru SO, Georgei LL and Harrison RE (1993) Natural occurrence of entomogenous nematodes in Tennessee nursery soils. Journal of Nematology 25: 181–188

Shen CP (1992) Description of an Entomopathogenic Nematode, *Steinernema longicaudum* sp. nov. and its Application. Proceedings of the 19th International Congress of Entomology, Beijin, China, pp 220–231

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

Stock SP and Kaya HK (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 SP, Strong DR and Gardner SL (1996) Identification of *Heterorhabditis* (Nemata: Heterorhabditidae) with a new species isolated from the larvae of the ghost moth caterpillar *Hepialis californicus*, (Lepidoptera: Hepialiidae) from the Bodega Bay Marine Preserve. Fundamental and Applied Nematology 19: 585–592

Stock SP, Choo HY and Kaya HK (1997) An entomopathogenic nematode *Steinernema monticolum* sp. n. (Rhabditida: Steinernematidae) from Korea with a key to other species. Nematologica 43: 15–29