First record on the distribution of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) in Southern Benin

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Summary. For the first time, surveys of entomopathogenic nematodes (EPN) were conducted in five departments in the Guinean zone of Southern Benin. Out of 84 prospected sites and 280 collected soil samples from agricultural and natural vegetation, 26 (31.0%) and 32 (11.4%) were positive for EPN, respectively. Identification of the EPN was based on analyses of sequences of the ITS rDNA region and morphological/morphometric investigations. Two species were found, *Heterorhabditis sonorensis* and *H. indica.* This is the first record of *H. sonorensis* since its description from the Sonora desert in Mexico. *Heterorhabditis sonorensis* was the most common species, showing a preference for semi-closed habitats such as citrus orchards, other fruit production fields and woodland with soils having sand and organic matter content ranging between 53.6-89.5% and 0.1-4.7%, respectively, and a pH from acidic (4) to neutral (7.1). Entomopathogenic nematodes were not recovered from crop fields (maize, cassava, groundnut and bean) and soil samples with less than 50% sand content. *Heterorhabditis indica* was associated with citrus orchards and fruit fields on sand to sandy clay soils, with pH slightly acidic (pH = 5.4-6.4), but not with woodland. Discriminant analysis identified five major environmental variables, longitude, organic matter content and texture (silt, sand and clay content) to be the most important abiotic factors determining the occurrence of EPN in soil from Southern Benin. Using these parameters, redundancy analysis revealed that *H. sonorensis* and *H. indica* prefer soils with high sand or organic matter content located in the more eastern longitude. No significant difference was observed in EPN species preferences taken individually, in terms of studied ecological parameters.

Key words: Benin, distribution, Guinean zone, *Heterorhabditis*, survey.

Entomopathogenic nematodes (EPN) are known since 1923 with the description of *Aplectana kraussei* Steiner, 1923, now *Steinernema kraussei* Travassos, 1927 (Nguyen & Hunt, 2007). Although nearly 40 nematode families have been isolated from soil inhabiting insects throughout the world, only two families, Steinernematidae Travassos, 1927 and Heterorhabditidae Poinar, 1976, are of major interest in agriculture because of their potential in regulating insect populations, particularly insect pests with a soil-dwelling phase (Kaya & Gaugler, 1993). They are associated with symbiotic bacteria belonging to the genus *Xenorhabdus* (*Steinernema* spp.) and *Photorhabdus* (*Heterorhabditis* spp.) (Boemare *et al.*, 1993). Entomopathogenic nematodes along with their bacterial symbionts are able to kill their insect hosts in a short period of time, usually within 24 to 48 h.

Numerous surveys have provided evidence of the omnipresence of these nematodes. Entomopathogenic nematodes have been isolated from many types of natural and managed habitats in a wide variety of soils throughout the world (Hominick, 2002); Antarctica is the only continent

from which they have not been recorded (Griffin *et al*., 1991). The African continent remains largely a fertile field for EPN exploration. At the end of the 20th century, only two *Steinernema* species, *S. feltiae* (Filipjev, 1934) Wouts, Mráček, Gerdin & Bedding, 1992 (Egypt) and *S. karii* Waturu, Hunt & Reid, 1997 (Kenya), and three *Heterorhabditis* species, *H. bacteriophora* Poinar, 1976 (Kenya and South Africa), *H. indica* Poinar, Karunakar & David, 1992 (Egypt and Kenya) and *H. taysearae* Shamseldean, El-Sooud, Abd-Elgawad & Saleh, 1996 (Egypt) had been recorded for this continent (Peters, 1996; Shamseldean *et al*., 1996; Waturu, 1998). More recent surveys in Africa revealed several new species: *S. yirgalemense* Nguyen, Tesfamariam, Gozel, Gaugler & Adams, 2005, *S. ethiopiense* Tamiru, Waeyenberge, Hailu, Ehlers, Půža & Mráček, 2012 (both from Ethiopia), *S. khoisanae* Nguyen, Malan & Gozel, 2006, *H. safricana* Malan, Nguyen, De Waal & Tiedt, 2008 (both from South Africa), *S. citrae* Malan, Knoetze & Moore, 2011 (South Africa), and *S. cameroonense* Kanga, Trinh, Waeyenberge, Spiridonov, Hauser & Moens, 2012 and *S. nyetense* Kanga, Trinh, Waeyenberge, Spiridonov, Hauser & Moens, 2012 (both from Cameroon) (Nguyen *et al.,* 2006; Nguyen & Hunt, 2007; Malan *et al*., 2008; Malan *et al*., 2011; Kanga *et al*., 2012; Tamiru *et al*., 2012). New strains of *H. indica* and *H. bacteriophora* were reported from Kenya and Egypt (Stack *et al*., 2000; Hominick, 2002), new isolates of *S. yirgalemense, S. karii* and *S. weiseri* Mráček, Sturhan & Reid, 2003 were found in the Central Rift Valley Region of Kenya (Nyasani *et al*., 2008) and a population of *S. yirgalemense* was detected in South Africa (Malan *et al*., 2011). In Ethiopia, the dominant species detected was *S. yirgalemense* (6.3%) next to two isolates of *H. bacteriophora* (0.7%) (Mekete *et al*., 2005). Kanga *et al*. (2012) reported from Cameroon new strains of *H. baujardi* Phan, Subbotin, Nguyen & Moens, 2003, a species originally described from Vietnam and later also recorded from Brazil.

Thus far, no study has been published on the EPN distribution in Benin. However, within the framework of the VLIR-UOS Own Initiative 2010 project on *Ecologically Sustainable Citrus Production in Benin*, the potential of indigenous EPN as an environmental friendly alternative control agent to the commonly used synthetic chemicals is being investigated. In this respect, surveys for EPN were conducted in 2010 and 2011 throughout the Southern part of Benin.

The current contribution presents the first records of EPN from Benin. It reports the occurrence of two *Heterorhabditis* species in Southern Benin and their distribution in agricultural and natural habitats.

MATERIALS AND METHODS

Site description and sampling characterisation. The Republic of Benin is situated in West Africa between the latitudes 6º10′N and 12º25′N and longitudes 0º45′E and 3º55′E. Three climate zones can broadly be distinguished (Akoègninou, 2004): *i*) the Northern zone located between 9°45′-12°25′N; *ii*) the transition zone located between 7°30′-9°45′N; and iii) the Southern zone located between 6°25′-7°30′N. Soil samples for our study were collected from the Southern zone where the climate is Guinean with two rainy seasons alternating with a long dry season (December-February). Two surveys of EPN were carried out during the rainy season in September 2010 and August 2011. In the prospected area, the mean annual rainfall varies from 1048.3 to 1604 mm; the mean annual temperature ranges from 26 to 28°C and the annual relative humidity from 75.5 to 81.7% (Table 1).

The first survey was mainly orientated towards citrus orchards (orange, lemon and grapefruit) with a high incidence of insect populations. During the second survey, samples were also taken in fields next to citrus orchards and included other fruit fields (mango, cashew, avocado, guava, banana, oil palm), annual and perennial crop fields (maize, cassava, groundnut, bean), and woodland (teak, acacia, eucalyptus). A total of 280 soil samples were collected from 84 sites distributed over 28 communities. At each site, a total of 3-4 samples, at least 10 m apart, were taken. Each soil sample (approximately 1.5 kg) consisted of a composite of 3-5 cores randomly taken in a 9-m² area; always close a tree or plant, at a depth of 0-15 cm and using a hand trowel. Samples were placed in polyethylene bags to prevent water loss and kept in coolers (*ca* 15°C) during transit to the laboratory.

At each site, data on sampling location, habitat (vegetation), longitude, latitude and altitude were recorded. For each sampling site, a subsample (*ca* 300 g) was analysed for the following physical and chemical characteristics: pH, organic matter, sand, silt, and clay content. Soil samples were processed at the Soil Analysis Laboratory of the Faculty of Agronomy Sciences, University of Abomey Calavi, Benin.

Nematode isolation. Entomopathogenic nematodes were recovered from soil samples using the insect baiting method (Bedding & Akhurst, 1975). Within a week after the sampling, a

subsample of *ca* 350 cm^3 was transferred to a 360-cm^3 plastic container to which five larvae of *Galleria mellonella* were added as bait. The containers were inverted and kept in the dark at ambient temperature of 27±2°C. Five days after incubation, the dead larvae were removed, rinsed successively with alcohol (70%) and water. Dead larvae that exhibited signs of infection with EPN, *i.e*., flaccid, soft, odourless larvae with either light to dark brown or reddish colour, were washed and placed in modified White traps (White, 1927). The larvae on the White trap were checked for emergence of nematodes after 1 week and thereafter daily. All nematodes emerging from dead larvae of the same sample were bulked and considered as one isolate. In the case of negative results, the isolation was repeated once to confirm results of the first observation.

To verify the pathogenicity of the collected nematodes and to establish new cultures, emerging nematodes were used to infect fresh *G. mellonella* larvae (Kaya & Stock, 1997). Then, dead larvae were collected and separately placed on a White trap. The emerged nematodes were collected alive in distilled water over a 2-week period and stored at 13°C. Isolate stocks were maintained on *G. mellonella* larvae being re-inoculated on a 2-month basis.

Nematode identification. Both molecular and morphological/morphometric approaches were used for the nematode identification, starting with the molecular analysis (Joyce *et al*., 1994; Stock, 2009).

Molecular characterisation and phylogenetic analysis. Molecular characterisation of the isolates was performed by analysis of the ITS rDNA sequences. Genomic DNA was extracted from a single nematode and the ITS regions and the 5.8S rRNA gene of the ribosomal DNA (rDNA) was amplified by PCR according to procedures described by Joyce *et al*. (1994). The obtained 707-bp sequences (Fig. 1) were compared with all DNA-sequences of *Heterorhabditis* species available in Genbank using the Basic Local Alignment Search Tool (BLAST) of the National Centre for Biotechnology Information (www.ncbi.com).

An alignment of the ITS1-5.8S-ITS2 sequences was generated using Clustal W (Thompson *et al.*, 1997) and used for phylogenetic analysis. A phylogenetic tree was produced using the Neighbour Joining method (Saitou & Nei, 1987). The analysis involved 48 nucleotide sequences (Fig. 1) and was conducted in MEGA5 (Tamura *et al*., 2011). In agreement with Nguyen *et al.* (2010) *Caenorhabditis elegans* (EU131007) was applied as outgroup.

Morphological characterisation. 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 & Stock, 1997) and processed to anhydrous glycerin for mounting (Seinhorst, 1959). Observations were made from live and mounted specimens using an Olympus BX51 microscope equipped with differential interference contrast optics and digital image software ($Cell^D$ Soft Imaging System, Olympus Company, Japan). For morphological characterisation of the isolates, 20 second-generation males and 30 infective juveniles (IJ) were randomly selected from different *G. mellonella* cadavers (Nguyen & Smart Jr., 1995). According to their morphological traits, isolates were placed into similar species-groups using taxonomic criteria suggested by Stock & Kaya (1996) and Hominick *et al.* (1997).

Data analysis. Diversity patterns among sampling sites were assessed by means of Principal Component Analysis (PCA) performed with environmental variables (latitude, longitude, altitude, habitat, pH, soil texture, silt, sand, clay, and organic matter content of the soil). To scrutinise the ecological trends of EPN isolates, the occurrence of EPN related to the environmental variables was first assessed with their recovery frequency (number positive samples/total number samples) (Liu & Berry 1995), expressed as percentage. Afterwards, Discriminant Analysis (DA) was used to determine which of the environmental variables were the best predictors of the presence of EPN in soil samples. These latter variables were used in Redundancy Analysis (RDA) to investigate differences in ecological preferences of the *Heterorhabditis* species found (Van den Wollenberg, 1977). The species matrix data was characterised by EPN species presence/absence and the analysis was conducted on centred response variables because species frequencies do not require standardisation. The selected environmental variables were used as explanatory variables. Statistical analyses were performed using STATISTICA 7 for PCA and DA, and XLSTAT 2012 for RDA agricultural and natural vegetation, 26 (31.0%) and 32 (11.4%) were EPN-positive, respectively. Molecular examination revealed that they all belonged to the genus *Heterorhabditis*. Twenty-nine isolates showed 100% sequence similarity with *H. sonorensis* Stock, Rivera-Orduño & Flores-Lara, 2009; 99% with *H. taysearae*; 98% with *H. mexicana* Nguyen, Shapiro-Ilan, Stuart, McCoy, James & Adams, 2004 and *H. floridensis* Nguyen, Gozel, Koppenhöfer & Adams, 2006; and 97% with *H. amazonensis* Andaló, Nguyen

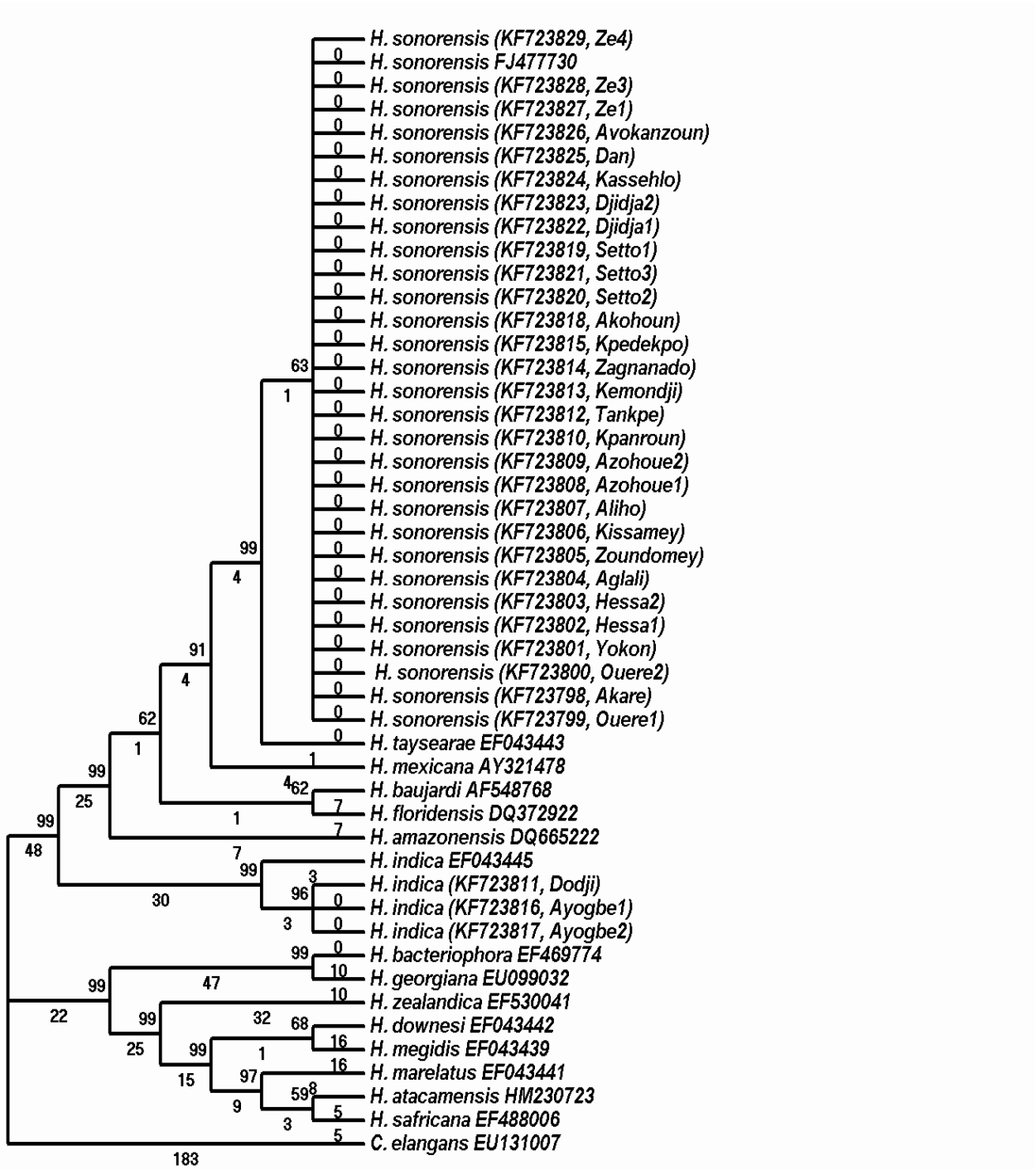

Fig. 1. Phylogenetic relationships of 15 *Heterorhabditis* species for which ITS sequences are available in Genbank, 32 *Heterorhabditis* isolates from Benin, and one outgroup species (*Caenorhabditis elegans*) based on analysis of ITS regions by Neighbour-Joining method. Tree length = 660.72. Numbers above the nodes indicate bootstrap value and numbers below branches represent base differences per sequence. Numbers after species name correspond to GenBank accession numbers, sample code.

Table 1. Sampled departments in Benin: geographic location and climate characteristics.

Department	Latitude	Longitude	Altitude* (m)	Annual precipitation* (mm)	Mean annual temperature* (°C)	Relative humidity* (%)*
Atlantique	02° 15′ 00″	06° 40′ 00″	27	1604	28.4	81
Couffo	01° 48′ 06″	06° 57′ 43″	121	1326.5	28.2	75.8
Mono	01° 43′ 00″	06° 38′ 00″	12	1048.3	27.8	81.5
Oueme	02° 36′ 00″	06° 30′ 00″	31	1553.4	28.4	80.8
Plateau	02° 41′ 00″	06° 58′ 00″	75	1305.2	27.6	81.7
Zou	01° 59′ 00″	07° 11′ 00″	121	1114.07	28.2	75.5

*Source: Agence pour la Sécurité de la Navigation Aérienne en Afrique et à Madagascar (ASECNA).

& Moino, 2006 and *H. baujardi*. The remainder of the selected isolates shared sequence similarity of 99% with *H. indica.*

RESULTS

Identification of isolates. Out of 84 prospected sites and 280 collected soil samples from agricultural and natural vegetation, 26 (30.95%) and 32 (11.43%) were EPN-positive, respectively. Molecular examination revealed that they all belonged to the genus *Heterorhabditis.* Twenty-nine isolates showed 100% sequence similarity with *H. sonorensis* Stock, Rivera-Orduño & Flores-Lara, 2009; 99% with *H. taysearae*; 98% with *H. mexicana* Nguyen, Shapiro-Ilan, Stuart, McCoy, James & Adams, 2004 and *H. floridensis* Nguyen, Gozel, Koppenhöfer & Adams, 2006; and 97% with *H. amazonensis* Andaló, Nguyen & Moino, 2006 and *H. baujardi*. The remainder of the selected isolates shared sequence similarity of 99% with *H. indica.*

In the phylogenetic tree inferred from the ITS sequences (sum of branch length = 660.72; Fig. 1), the 29 aforementioned isolates clustered with *H. sonorens*is (bootstrap value = 63%). The pairwise distance comparison (showed below branches) revealed the 29 isolates to differ from *H. taysearae* and *H. mexicana* by one and four bases, respectively. No sequence difference was found between these isolates and *H. sonorensis*. Hence, we concluded that these 29 isolates belonged to *H. sonorens*is. Likewise, three other isolates (Ayogbe1, Ayogbe2 and Dodji) clustered in a separate group (bootstrap value = 96%). They formed a monophyletic clade with *H. indica* supported by a high bootstrap value of 99% (Fig. 1). They therefore were considered conspecific with *H. indica*.

The morphology and morphometrics of the isolates conformed to the original description of the species to which they were assigned. Both species are easily separated from each other by the position of the excretory/secretory pore of the male, which in *H. indica* is located posterior to the basal bulb, whereas in *H. sonorensis* it is usually posterior to the nerve ring at the level of the basal bulb (data not shown). Further, the gubernaculum of *H. indica* is flat, about half the spicule length in size, whereas the gubernaculum of *H. sonorensis* is slightly curved ventrally and about 60% of spicule length (data not shown).

Spatial distribution of EPNs and interrelated variables

Diversity patterns of sampled sites. The correlation between features characterising the environment and major components 1 and 2 (Table 2) revealed that the first three components expressed 73.15% of data variability and are sufficient to describe reliably diversity patterns among sampled sites. The first component represented a gradient of decreasing organic matter content (−0.61), pH (−0.64), longitude (−0.89) and latitude (−0.89) with habitat (−0.60) changing progressively from semi-closed habitats (woodland, fruit fields and citrus orchards) to open habitats (crop fields) from left to the right. The second component represented increasing contents of sand (0.94) and decreasing contents of silt (−0.67) and clay (−0.83) from the bottom to the top. The third component represented soil texture (0.89) changing from sand, sandy loam, sandy clay loam and sandy clay from the bottom to the top.

When soil samples were projected on the plane of components 1 and 2 (Fig. 2A), samples from the more western longitude and the more southern latitude, with the highest pH and the lowest organic matter, silt, sand and clay content collected in open habitat (crop field) were located at the top left of the

quadrant I. Samples from the more eastern longitude and the more north latitude, with the lowest pH, organic matter, silt and clay content, and the highest sand content collected in semi-closed habitat (citrus orchards, fruit fields and woodland) were located at the top right of the quadrant II. Quadrant III contained samples from the more western longitude and western latitude, with the highest pH, organic matter, silt and clay content and the lowest sand content collected in opened habitat (crop field). When soil samples were projected on the plane of components 1 and 3 (Fig. 2B), samples from lighter-textured soils were located in quadrants I and II.

Occurrence of isolates with associated ecological parameters. In terms of recovery frequency, an unequal separation of EPN isolates was observed (Table 3). Entomopathogenic nematodes occurred in all the studied ecosystems, with the exception of crop fields and soils with more than 40% silt or clay content, or less than 50% sand content.

In terms of species diversity, *H. sonorensis* was the most prevalent species found in 90.6% of the positive samples located between latitudes 1°48′ and 2°37′, longitudes 6°25′ and 7°30′ and altitudes between 8 and 252 m. The soil samples positive for *H. sonorensis* had a pH ranging from 4 to 7, a silt content from 1.3 to 30.5%, a sand content from 53.6 to 89.5%, a clay content from 4.5 to 36.4%, and an organic matter content between 0.1 and 4.7%. This species was found at sites with a sandy, sandy clay, sandy loam, or sandy clay loam soil texture and occurred in semi-closed habitats, such as citrus orchards, fruit fields and woodland.

Heterorhabditis indica was found in 9.4% of the positive samples. This species was located between latitude 2°15′ and 2°00′ and longitude 6°41′ and 7°17′. It was found associated with citrus orchards and fruit field habitats, at altitudes between 12-102 m, in soils with pH ranging from 5.4 to 6.4, silt content from 2.3 to 2.7%, sand content from 77.1 to 83.4%, clay content from 20.6-13.9% and organic

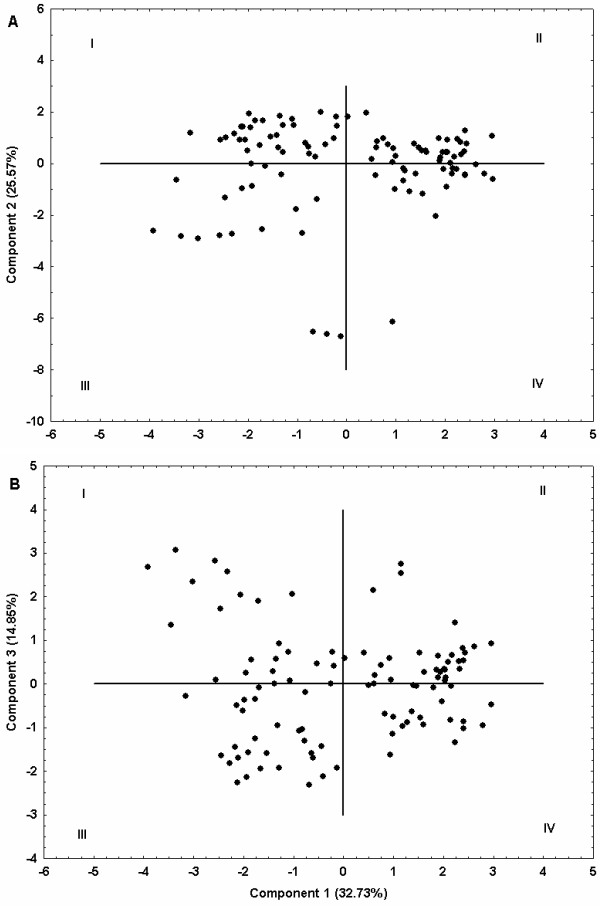

Fig. 2. Projection of soil samples on the planes defined by the component 1 and 2 (A) and the components 1 and 3 (B) of the principal component analysis of soil samples involving environmental variables (latitude, longitude, altitude, habitat, pH, silt, sand, clay and organic matter content and texture). Dots represent more than one soil sample.

Table 2. Correlation between variables characterising the environment of sampled sites in south Benin and the first three components of the principal component analysis (bold values indicate parameters dominating principal components 1, 2 or 3).

Variables	Component 1	Component 2	Component 3
Silt	−0.46	**−0.67**	0.51
Sand	0.29	**0.94**	−0.06
Clay	0.01	**−0.83**	−0.45
Soil texture	−0.24	−0.06	**0.89**
Organic matter	**−0.61**	−0.45	−0.32
pH	**−0.64**	0.24	0.14
Altitude	0.45	0.04	0.10
Habitat	**−0.60**	0.18	−0.28
Longitude	**−0.89**	0.35	−0.08
Latitude	**−0.89**	0.35	−0.08
Eigen values variance	3.27	2.56	1.48
Percentage of variance	32.73	25.57	14.85
Cumulative percentage of variance (%)	32.73	58.30	73.15

matter content from 2.4 to 2.9%. The soil textures were sand and sandy clay. *Heterorhabditis sonorensis* was found in all sub-ecosystems where *H. indica* was found. However, *H. indica* was not present in sub-ecosystems with sandy loam and sandy clay loam soil types, with pH < 5 located, and altitude > 150 m and latitude below 2°00′, where *H. sonorensis* was present (Table 3).

Discriminant analysis. Five variables (longitude, organic matter, silt, sand, and clay content) discriminated, as best, positive soil samples from negative soil samples (Table 4).

Redundancy analysis. An overall test of significance showed that the canonical relationship between EPN presence and selected environmental variables is highly significant ($P = 0.016$ after 1000 permutations; *Pseudo F* = 0.115). The RDA axis 1 explains 96.3% of the total variance of the data, which is sufficient to describe the relation between environmental variables and EPN presence. Standardised canonical coefficients for explanatory variables are given in Table 5. Magnitudes of these coefficients represent their relative contributions to the related axis. All variables for the canonical axis 1 have negative coefficients while coefficient of sand, silt and clay demonstrate the highest contribution of these characteristics to the canonical axis in absolute value.

When the variables and species were projected on the plane of axis 1 and 2, EPN-positive soil samples, the arrows of the variables longitude, sand and organic matter pointed at the left direction, whereas the arrows of the variables clay and silt pointed at the opposite direction. Arrows indicate the direction of increasing of either the values of the significant environmental variables or species presence. As a result, the presence of *H. sonorensis* and *H. indica* is correlated with increases of longitude, sand and organic matter content and a decrease of clay and silt content (Fig. 3). However, no difference in individual ecological preferences was observed between the two species.

DISCUSSION

The present study reports for the first time the occurrence of EPN in Benin. During the surveys conducted in 2010 and 2011, only the genus *Heterorhabditis* was recorded. This could be due to: *i*) the low prevalence of *Steinernema* genus as compared with *Heterorhabditis* genus; *ii*) the difference in biology of the two genera. One heterorhabditid is sufficient to multiply after invasion while at least two steinernematids (IJ to develop in male and female) must invade before reproduction can occur (Downes & Griffin, 1996); *iii*) the restricted sampling; a more intensive sampling might have yielded additional species; and *iv*) the use of *G. mellonella* only as the baiting insect; several different hosts might have yielded additional species. As a consequence, the *Galleria*-bait method may not have allowed us to identify all EPN present. Occasionally, the method does not detect EPN in positive samples (Spiridonov & Moens, 1999).

Out of 280 samples, 32 were EPN-positive (11.4%). Prevalence of EPN varies widely between different surveys, and ranges between 2% and 45% (Hominick, 2002). However, the recovery frequency

Table 3. Distribution of heterorhabditids in south Benin at ranges of different environmental variables.

Categories (total samples)	Recovery frequency (%)[a]	Positive samples	
		No[b]	Percentage[c]
Latitude			
[1°39 - 2°00] (74)	5.4	4 (4-0)	12.5
[2°00 - 2°38] (206)	13.6	28 (25-3)	87.5
Longitude			
[6°24 - 7°00] (171)	9.4	16 (15-1)	50
[7°00 - 7°30] (109)	14.7	16 (14-2)	50
Altitude (m)			
[7 - 50] (146)	11.6	17 (15-2)	53.1
[50 - 150] (115)	12.2	14 (13-1)	43.8
[150 - 252] (19)	5.3	1 (1-0)	3.1
Habitat			
Citrus orchards (167)	9.6	16 (15-1)	50
Fruit fields (56)	17.9	10 (8-2)	31.3
Woodland (44)	13.6	6 (6-0)	18.8
Crop fields (13)	0	0	0
pH			
[4 - 5] (24)	4.2	1 (1-0)	3.1
[5 - 6] (127)	9.5	12 (11-1)	37.5
[6 - 7] (106)	16	17 (15-2)	53.1
[7 - 8] (23)	8.7	2	6.3
Silt content % (0.002-0.05mm)			
[0 - 10] (214)	12.2	26 (23-3)	81.3
[10 - 20] (36)	11.1	4 (4-0)	12.5
[20 - 30] (21)	4.8	1 (1-0)	3.1
[30 - 40] (6)	16.7	1 (1-0)	3.1
[40 - 50] (3)	0	0	0.00
Sand content % (0.05 - 2mm)			
[30 - 40] (8)	0	0	0.00
[40 - 50] (12)	0	0	0.00
[50 - 60] (13)	15.4	2 (2-0)	6.3
[60 - 70] (29)	13.8	4 (4-0)	12.5
[70 - 80] (82)	13.4	11 (11-0)	34.4
[80 - 90] (136)	11	15 (12-3)	46.9
Clay content % (<0.002mm)			
[4 - 10] (37)	13.5	5 (5-0)	15.6
[10 - 20] (189)	12.2	23 (22-1)	71.9
[20 - 30] (40)	7.5	3 (1-2)	9.4
[30 - 40] (6)	16.7	1 (1-0)	3.1
[40 - 50] (8)	0	0	0
Organic matter content %			
[0 - 1] (37)	10.8	4 (4-0)	12.5
[1 - 2] (85)	5.9	5 (5-0)	15.6
[2 - 3] (94)	14.9	14 (11-3)	43.8
[3 - 4] (37)	8.1	3 (3-0)	9.4
[4 - 5] (15)	26.7	4 (4-0)	12.5
[5 - 6] (12)	16.7	2 (2-0)	6.3
Soil texture			
sand (144)	11.1	16 (15-1)	50
sandy clay (100)	13	13 (11-2)	40.6
sandy loam (23)	8.7	2 (2-0)	6.3
sandy clay loam (13)	7.7	1 (1-0)	3.1

[a] Recovery frequency (number of positive samples/total number of samples).
[b] Number of positive samples (number of isolate of *H. sonorensis* – number of isolate of *H. indica*)
[c] Percentage positive samples (number positive samples per category variable/number total positive samples).

value recorded in south Benin is within the ranges reported in African countries: Egypt (10%) (Shamseldean & Abd-Elgawad, 1994), Ethiopia (6.9%) (Mekete *et al*., 2005), Central Kenya (24%) (Waturu, 1998), and south Cameroon (10.4%) (Kanga *et al*., 2012).

This is the second record of *H. sonorensis* originally described from the Sonoran desert in Mexico (Stock *et al*., 2009) and so the first record for Africa, expanding its currently known geographic range. The presence of *H. sonorensis* in these two different parts of the world is probably

because they both prefer a tropical climate. South Benin has a subtropical climate with two rainy seasons, whereas the Sonoran Desert has a dry tropical climate also with two rainy seasons. Moreover, in the Sonoran desert, about half of the biota is tropical in origin. The finding of *H. indica* in south Benin demonstrates its prevalence in tropical and subtropical zones (Hominick, 2002). The species was first isolated in Coimbatore, India (Poinar *et al*., 1992); later its wide distribution in the Asian continent was demonstrated by its detection in Malaysia (Mason *et al*., 1996), Japan (Yoshida *et al*., 1998), Indonesia (Griffin *et al*, 2000); Palestine (Sansour & Iraki, 2000), Vietnam (Phan *et al*., 2001), Saudi Arabia (Saleh *et al*., 2001), Pakistan (Anis *et al*., 2000; Shahina *et al*., 2001; Shahina & Mahreen, 2010). On the American continent, *H. indica* was reported from Cuba (Mráček *et al*., 1994), Guadeloupe, Jamaica, Dominican Republic, Martinique, Puerto Rico, and Trinidad (Constant *et al*., 1998; Fisher-Le Saux *et al*., 1998), Venezuela (Rosales & Suarez, 1998), the Virgin Islands and Florida (Stack *et al*., 2000). In Africa, it was only detected in Kenya (Waturu, 1998) and Egypt (Grenier *et al*., 1996; Stack *et al*., 2000). The species was also reported in Australia (Akhurst, 1987).

Although the majority of the soil samples were taken from citrus orchards (164/280), the principal component analysis has revealed high diversity patterns among sampled sites. The area surveyed covered wide ranges of pH of the soil (4-8), silt (0-50%), sand (30-90%), clay (4-40%) and organic matter (0-6%) content, soil texture (sand, sandy loam, sandy clay loam and sandy clay), habitat (citrus orchards, fruit fields, woodland and crop fields), and altitude (7-252 m). However, no EPN were recovered from arable crop fields. This is in contrast to Akhurst & Brooks (1984), Hominick & Briscoe (1990) and Griffin *et al*. (1991) who observed more EPN in agricultural fields. Our observations might be biased by the survey periods, when fields were greatly disturbed by farmers' practices, including use of fertilisers and pesticides.

Like many other surveys (Kung *et al*., 1990b; Griffin *et al*., 1991; Hara *et al*., 1991; Rueda *et al*., 1993; Choo *et al*., 1995; Liu & Berry, 1995; Miduturi *et al*., 1996; Hazir *et al*., 2003; Kanga *et al*., 2012) our study showed that sandy soil is the preferred soil texture for EPN. Earlier it was shown that both mobility and survival of EPN are favoured in soils with a high sand content, whereas soils with high clay content restrict nematode movements (Molyneux & Bedding, 1984; Kung *et al*., 1990b). In terms of pH-tolerance, *H. indica* was only isolated from slightly acidic (pH = 5.4-6.4) soils; *H. sonorensis* was isolated from acidic (pH = 5) to neutral (pH = 7.1) soils. This agrees with other studies in which the pH of *Heterorhabditis*-positive

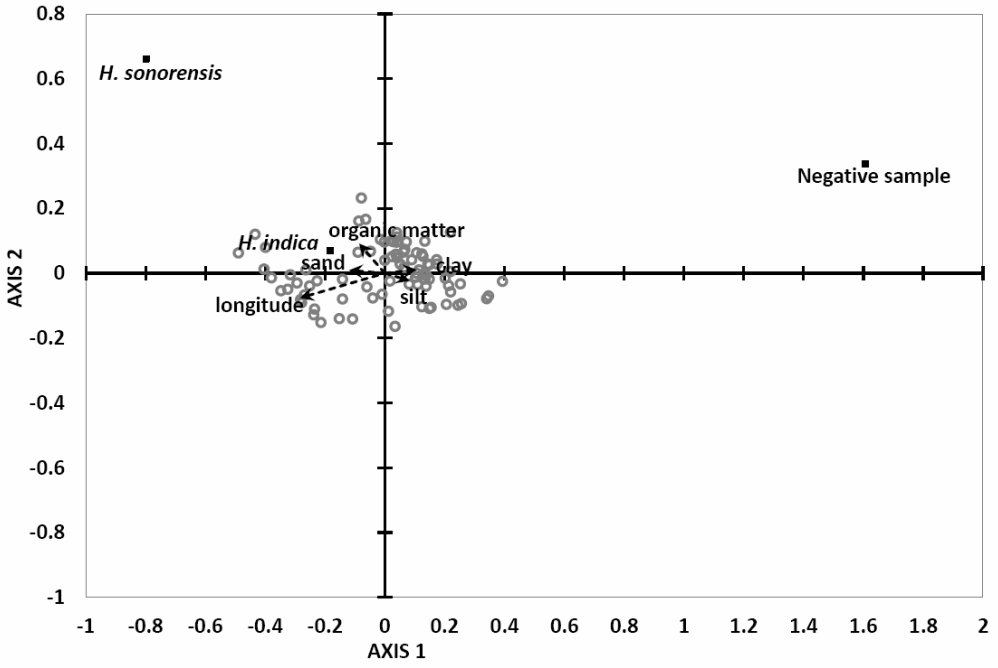

Fig. 3. Redundancy analysis triplot showing correlation between *Heterorhabditis*-positive samples, *Heterorhabditis*-negative samples and environmental variables (longitude, silt, sand, clay and organic matter content). Dots represent more than one sample.

soil samples varied from 4.3 to 7.0 (Canhilal & Carner, 2006), and between 4.6 and 8 (Hara *et al*., 1991; Griffin *et al*., 1994). In most agro-ecosystems the pH (range: 4-8) is not likely to have any significant

Table 4. Discriminant analysis of environmental variables predicting presence of entomopathogenic nematodes in soil samples (bold values indicate variables in the model).

Parameters	Wilks' Lambda	Partial Lambda	*P*-level	1-R-square
Longitude	0.961002	0.968678	0.012987	0.211826
Silt	0.950930	0.978939	0.054721	0.980739
Organic matter	0.942605	0.987585	0.181723	0.200838
Sand	0.945439	0.984624	0.120621	0.991339
Clay	0.943223	0.986937	0.166158	0.977789
pH	0.927415	0.996254	0.600206	0.181189
Altitude	0.925494	0.994190	0.452738	0.328629
Habitat	0.930714	0.999798	0.972891	0.141064
Latitude	0.926365	0.995126	0.514501	0.319167

effect on EPN presence; however, pH ≥ 10 is likely to be detrimental (Kung *et al.*, 1990a). *Heterorhabditis indica* was isolated from soils with 2.4-2.9% organic matter content, whereas *H. sonorensis* was isolated from soils with a larger range (0.1-4.7%). Canhilal and Carner (2006) reported that the organic matter content of *Heterorhabditis*-positive soils collected in South Carolina averaged 3% (0.7-7.8). In heterorhabditid-positive soil samples collected in the Central Rift Valley Region of Kenya a range of 2-3% was reported by Mwaniki *et al.* (2008). Earlier reports suggest that *H. indica* is not restricted by vegetation type (Griffin *et al*., 2000); however, during the present survey, *H. indica* was only observed from citrus orchards and fruit production fields, whereas *H. sonorensis* was observed from citrus orchards, fruit production fields and woodland. This suggests that the influence of agricultural activities on *H. sonorensis* seems to be less than on *H. indica* in our survey. Moreover, the differences in the distribution of these two species could be the result of the availability or differences in the distribution of suitable host insects.

Table 5. Standardised canonical coefficients, eigenvalues and percentages of inertia from redundancy analysis.

Variables	AXIS 1	AXIS 2	AXIS 3
Silt	–21.854	10.938	–2.916
Sand	–33.342	16.551	–4.125
Clay	–19.859	9.924	–2.410
Organic matter	–0.243	0.118	0.060
Longitude	–0.460	0.010	0.092
Eigenvalue	0.128	0.004	0.001
Constrained inertia (%)	96.254	2.802	0.944
Cumulative %	96.254	99.056	100.000

Discriminant analysis identified longitude, organic matter content and soil texture (silt, sand and clay content) to be the most important abiotic factors determining the occurrence of EPN in soil from south Benin. Using these parameters, redundancy analysis results revealed *H. sonorensis* and *H. indica* to prefer soil with high sand or organic matter content located in the more eastern longitude; in other words, soil with decreasing silt and clay content. However, no significant difference was observed in ecological trends of EPN species taken individually. Soil texture is essential for the existence of some *Heterorhabditis* species and accordingly plays an important role in the EPN dispersal and persistence (Georgis & Poinar, 1983; Kung *et al*., 1990b; Sturhan, 1999).

This was the first attempt to study the occurrence and diversity of EPN in Benin. In comparison with other studies the diversity is rather low with no record of *Steinernema*. Within the on-going project, we expect further surveys in other areas to increase the number of EPN species of both genera.

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Резюме. Впервые проведено обследование почв пяти департаментов южной (Гвинейской) зоны в Южном Бенине на присутствие почвенных энтомопатогенных нематод (ЭПН). Из 84 исследованных природных и агроэкосистем из 280 собранных проб почвы, в 26 (31.0%) и 32 (11.4%), соответственно, были обнаружены ЭПН. Определение ЭПН было основано на анализе последовательностей ITS rDNA, а также морфологических и морфометрических исследованиях. Было обнаружено два вида ЭПН: *Heterorhabditis sonorensis* и *H. indica.* Это первое сообщение о *H. sonorensis* с момента первого обнаружения в пустыне Сонора в Мексике. *Heterorhabditis sonorensis* оказался наиболее распространенным видом, показывая предпочтение к полузакрытым пространствам, вроде цитрусовых плантаций, фруктовых посадок или древесных насаждений, при содержании песка и органического вещества в пределах 53.6-89.5% и 0.1-4.7%, соответственно, и pH от кислого (4) до нейтрального (7.1). ЭПН не обнаруживались в культивируемой почве полей (под кукурузой, кассавой, арахисом и бобами) и образцах почвы с содержанием песка менее 50%. *Heterorhabditis indica* встречался в цитрусовых садах и фруктовых посадках на кислых песчаных почвах или песчаных суглинках (pH = 5.4-6.4), но не покрытых лесом площадях. Дискриминантный анализ выявил несколько основных факторов среды, определяющих распределение ЭПН в почвах Южного Бенина: географическая долгота местности, содержание в почве органического вещества, текстура почвы (содержание песка, глины). По результатам анализа этих факторов было показано, что *H. sonorensis* и *H. indica* предпочитают почвы с высоким содержанием песка или органического вещества и тяготеют к восточных регионам страны. Не было выявлено видоспецифичных особенностей распределения каждого из двух выявленных видов.