Entomopathogenic nematodes in agricultural areas in Brazil

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Entomopathogenic nematodes (EPNs) (Steinernematidae and Heterorhabditidae) can control pests due to the mutualistic association with bacteria that kill the host by septicemia and make the environment favorable for EPNs development and reproduction. The diversity of EPNs in Brazilian soils requires further study. The identification of EPNs, adapted to environmental and climatic conditions of cultivated areas is important for sustainable pest suppression in integrated management programs in agricultural areas of Brazil. The objective was to identify EPNs isolated from agricultural soils with annual, fruit and forest crops in Brazil. Soil samples were collected and stored in 250 ml glass vials. The nematodes were isolated from these samples with live bait traps ([Galleria mellonella L. (Lepidoptera: Pyralidae) larvae]. Infective juveniles were collected with White traps and identified by DNA barcoding procedures by sequencing the D2/D3 expansion of the 28S rDNA region by PCR. EPNs identified in agricultural areas in Brazil were Heterorhabditis amazonensis, Metarhabditis rainai, Oscheios tipulae and Steinernema rarum. These species should be considered pest biocontrol agents in Brazilian agricultural areas.

Entomopathogenic nematodes (EPNs) (Steinernematidae and Heterorhabditidae) can control pests due to the mutualistic association with bacteria of the genus Xenorhabdus (Thomas & Poinar) and Photorhabdus (Boemare, Louis & Kuhl), respectively¹². These nematodes penetrate the host through natural openings or through the cuticle transporting bacteria into the hemocele³⁴ where they reproduce and kill the host from septicemia within 24 to 48 hours⁵⁶, making the environment favorable for nematode development and reproduction⁷. Infective juveniles seek another host in the soil when the insect host resources run out⁸. Interest in these biological control agents is increasing⁹ due to the reduced efficiency of conventional chemical and cultural methods for insect soil management and the broad spectrum of EPN hosts¹⁰.

EPNs are globally distributed, with different species and groups according to geographic regions¹¹,¹². Information on EPNs and their symbiotic bacteria is scarce in many countries, including in Brazil. Heterorhabditis amazonensis Andaló and Steinernema brasilensis Nguyen were reported as native species in Brazil¹³,¹⁴ and Heterorhabditis indica and H. baujardi were reported in Rondônia state, Brazil¹⁵. Isolates of H. amazonensis, H. baujardi, H. indica and H. mexicana were found in Minas Gerais state, Brazil¹⁶,¹⁷.

Nematode species can be identified by molecular characterization based on sequencing of rDNA subunit (28S)¹⁸–²⁰, because low morphological variation and similar characteristics within this group hamper identification¹⁵. Infectivity, environmental tolerance and suitability for commercial formulations vary between EPN isolates and species²¹,²² which can be used to control pests of various orders, such as Coleoptera²³–²⁵, Hemiptera²⁶–³⁰ and Lepidoptera³¹–³³.

EPN identification, adapted to environmental and climatic conditions of cultivated areas is important for sustainable pest suppression in integrated management programs in agricultural areas of Brazil. The objective was to identify EPNs from agricultural soils with annual, fruit and perennial crops in Brazil.

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Methods

Nematode collection. Soil samples were collected in agricultural areas in Barretos, Botucatu, Garça, São Manuel (São Paulo State), and Palotina (Paraná State), Brazil from 2010 to 2013. Thirty-seven samples were collected in areas with annual crops, [Glycine max (L.) Merrill, Zea mays (L.), Avena sativa (L), Saccharum officinarum (L.) and irrigated Oryza sativa (L.)], 20 in areas with forest plantations [Anadenanthera falcata (Benth.) Spec., Peltophorum dubium (Spreng.) Taub., Khaya ivorensis, Swietenia macrophylla, Hevea brasiliensis (L.), Eucalyptus spp., Cedrela odorata (L.), Acrocarpus fraxinifolius, Azadirachta indica (A. Juss.), Cordia ecalyculata (Vell), Calophyllum brasiliense (Cambess.) and Pocciolathe parviflora (Benth.)], and 97 in areas with fruit [Litchi chinensis (Son.), Macadamia integrifolia (Maiden & Betche), Citrus reticulata (L.), Prunus persica (L.), Prunus sp., Psidium guajava (L.), Mangifera indica (L.), Citrus sinensis (L.), Citrus sp., Rubus idaeus (L., Musa spp. and Coffea arabica (L.)]. In addition, four samples were collected in forested nature, in one in a pasture and 42 in plowed soil areas, totaling 201 samples.

A zero to 25 cm deep soil sample was taken per sampling point and placed in 2L labeled plastic bags, stored in a Styrofoam box and transferred to the laboratory. The geographical coordinates of each sample were obtained with Garmin GPS device Etrex Vista H 2.8. Nematodes were isolated in the laboratory using fifth instar Galleria mellonella Linnaeus (Lepidoptera: Pyralidae) larvae. Briefly, each soil sample was packed into a 250 mL glass vial with five G. mellonella larvae. These vials were closed and stored without light at 25 ± 2 °C. After three to seven days, dead G. mellonella showing nematode infection symptoms were removed, rinsed in distilled water and transferred to White traps34. The infective juveniles (IJs) were again inoculated in G. mellonella larvae for multiplication. Five G. mellonella larvae were used in a Petri dish (9 cm diameter) containing two moistened paper filters with 1.5 mL of solution with 100 JJs/larvae, for each nematode species. The samples were covered with PVC plastic and stored at 25 ± 2 °C and RH > 95%. After 25 ± 2 °C and RH > 95%, the vials were transferred to a White trap at 25 ± 2 °C for seven days and 15 days. The IJs that left the G. mellonella carcasses were collected with distilled water every two days and stored at 18 °C. The nematode samples were named FCA 01 to 14 and stored in the Entomopathogenic Nematode Bank of the Nematology Laboratory at FCA/UNESP. Vials contained 1 M NaCl solution and were frozen at –80 °C until DNA extraction and EPN identification.

PCR. Genomic DNA was obtained for each population (FCA 01 to 14) from 13 individuals of each EPN, extracted using Lysis Buffer Hollerman [(HLB) (800 μg proteinase K/mL, 3-mercaptoethanol 1% (v/v), 0.2 M NaCl and 0.2 M Tris HCl pH 8)]36. A total of 25 μL of HLB was diluted in 25 μL of ultrapure water totaling 50 μL in a 0.2 mL Eppendorff tube. A drop of this solution (5 μL) was placed on a glass slide, where the nematodes were individually cut into three parts and placed in the same 0.2 mL tube. The 45 μL of remaining solution was used to wash the slide and added to the tube with the sectioned nematode. Samples were submitted to PCR at 65 °C for 2 h, 99 °C for five minutes and stored at −20 °C37. The universal primers D2A (5′-TTCGGAGGAGCGGTACCTC-3′) and D3B (5′-TGGCAGCTCGCTGGATGCTT-3′) and D3B (5′-TCGGGAGGAGCGGTACCTC-3′) were used to amplify the D2/D3 expansion segment of 28S rDNA by PCR38. A total of 12.5 μL of Gotaq Hot Start (Promega, São Paulo State, Brazil), with the reagents necessary for reaction: 5 U/μL Taq, 100 mM of each NTP and 25 mM MgCl2, 9.5 μL of nuclease free water (Promega), and 1 μL of each primer [10 mM] and 1 μL of cDNA from each representative population of target and non-target species, totaling 25 μL per reaction was submitted to PCR at 94 °C for seven minutes; followed by 35 cycles at 94 °C for 60 seconds, 55 °C for 60 seconds, 72 °C for 60 seconds; and 72 °C for 10 minutes39. Five μL of PCR product was used for electrophoresis in TAE buffer39 on 1% agarose gel, stained with ethidium bromide (0.02 mg/mL), visualized and photographed under UV light. The result of the amplification was compared to the molecular weight marker VIII.

The amplified fragments of D2/D3 expansion 28S rDNA were sequenced with the Big Dye Terminator kit (Applied Biosystems)40. A reagent mix containing 2 μL Big Dye, 3.2 mmol sense primers, 3.0 μL of amplified product containing 400 ng DNA and 2.0 μL of water was prepared for the product end of the PCR reaction. The reaction for sequencing was carried out according to manufacturer's instructions (Applied Biosystems) with further purification of the amplified product by precipitation with isopropanol. Samples were denatured at 95 °C for three minutes and electrophoresis performed in an ABI Prism 377 DNA Sequencer unit (Applied Biosystems).

The sequences were aligned and compared to nucleotide polymorphism identification with the aid of BioEdit Alignment Sequence Edition Program. The EPN population sequences were compared with other nematode species in the database (GenBank, http://www.ncbi.nlm.nih.gov) for identification based on genetic similarity.

For phylogenetic analysis, the multiple alignments between the sequences of the region D2/D3 of the different isolates were excluded manually with BioEdit Sequence Alignment program when phylogenetically uninformative columns were excluded from the analyses. Phylogenetic analyses were inferred using the Maximum Likelihood method based on the Kimura 2-parameter model42, considered the best fitting model for sequence evolution determined using the BIC scores (Bayesian Information Criterion) implemented in 6 MEGA program42. Initial trees for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (4 categories (+G, parameter = 2.3452)). The model variation rate allowed for some sites to be evolutionarily invariable (+I, 19.1558% sites). The tree was drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 48 nucleotide sequences, including 16 obtained in the present study (FCA01 to FCA16) and 32 from the GenBank. All positions containing gaps and missing data were eliminated. A total of 292 positions was obtained in the final dataset. Trees were sampled at intervals of 1000 generations and Caenorhabditis elegans (Brenner) was selected as outgroup. Evolutionary analyses were conducted in 6 MEGA program42.
Results
The nematodes obtained from White traps were inoculated in new *G. mellonela* larvae, which demonstrates parasitism by the isolated entomopathogen. EPNs were found in 16 soil samples, corresponding to 8% of 201 samples. Seven (35%) of 20 samples from forest plantation areas had nematodes (FCA 04, FCA 05, FCA 06, FCA 07, FCA 08, FCA 10 and FCA 15). In annual crops, three (8.1%) out of 37 samples had nematodes: isolated FCA 11, detected in sandy soil in irrigated rice in Botucatu, São Paulo State, Brazil and isolates FCA 16 and FCA 03 in soybean crops in clay soils in Palotina, Paraná State, Brazil. Among 97 samples taken from orchards, six (6.2%) were positive for EPNs, FCA 12 isolates grown in sandy soils with wild raspberry, FCA 13 in citrus soil and FCA 14 in mango soil in São Manuel, São Paulo, Brazil. FCA 01 and FCA 02 isolates were also found in clay soil with citrus in Botucatu, São Paulo State, Brazil (Fig. 1). EPN samples were not found in plowed soil, native forest and pasture areas.

Amplification of D2/D3 expansion 28S rDNA gene of EPN isolates produced 590 bp fragments, whose sequences were deposited in the GenBank under the accession codes KRO11843 and KRO11858 (Fig. 2). The technique of DNA barcode sequences showed that the expansion D2/D3 28S rDNA gene of FCA 07 were identical to *H. amazonensis* (EU099036). The sequences of isolates FCA 01, FCA 04, FCA 06, FCA 08, FCA 15 and FCA 16 were identical to the *Metarhabditis rainai* (EU195966). The sequences of FCA 02, FCA 03 and FCA 05 isolates were identical to *Oscheius tipulae* (Lam & Webster, 1971) (EU195969). FCA 09, FCA 10, FCA 11, FCA 12, FCA 13 and FCA 14 isolates were observed (99–100%) with *Steinernema rarum* (AF331905). However, these isolates formed two groups that had three polymorphisms between them. One group with FCA 09, FCA 11 and FCA 12 isolates were similar to each other, but different in 3 bp from the group comprising FCA 10, FCA 13 and FCA 14 isolates. The phylogenetic tree (Fig. 2) obtained from the 48 aligned sequences of the D2/D3 expansion 28S rDNA genes in EPNs showed four distinct groups, *H. amazonensis* (FCA 07), *S. rarum* (FCA 09, FCA 10, FCA 11, FCA 12, FCA 13 and FCA 14), *M. rainai* (FCA 01, FCA 04, FCA 06, FCA 08, FCA 15 and FCA 16) and *O. tipulae* (FCA 02, FCA 03 and FCA 05).

*Heterorhabditis amazonensis* was found in clay soil with *P. parviflora* in Garça, São Paulo State, Brazil; *S. rarum* in clay soil with *Eucalyptus* sp. and *C. reticulata* in Botucatu, São Paulo State, Brazil and in sandy soil with *R. idaeus* (FCA 12) and *C. reticulata* (FCA 13) and *M. indica* (FCA 14) in São Manuel, São Paulo State, Brazil.

Figure 1. Geographical distribution of the sampling sites (x) in São Paulo and Paraná States, Brazil. EPNs positive samples (. ) (ESC.: 1: 10000). AutoCAD SP2 (2015) [vJ.210.0.0]. http://www.Autodesk.com.br/products/autocad/overview.
Metarhabditis rainai was detected in clay soils cultivated with *A. fraxinifolius*, *C. odorata*, *C. brasiliense* and *C. ecalyculata*, in Garça, São Paulo State, Brazil and in those with *C. reticulata*, in Botucatu, São Paulo State, Brazil. *Oscheius tipulae* was found in soils with *A. indica* and *C. reticulata* in Botucatu, São Paulo State, Brazil and in clay soils cultivated with *G. max*, in Palotina, Paraná State, Brazil (Table 1).

**Discussion**

Nematology surveys with *G. mellonella* baiting technique are useful to detect Steinernematidae and Heterorhabditidae species as well as other rhabditids. The molecular technique used was adequate to identify nematode isolates, enabling knowledge of its biodiversity and contributing to the detection of new isolates that may be used in biological control programs of insect pests.

The sequence of D2/D3 expansion 28S rDNA gene analysis by DNA barcode technique was useful for the diagnosis of *H. amazonensis*, *S. rarum*, *M. rainai* and *O. tipulae*. The phylogenetic tree obtained from the 48 aligned sequences of the expansion D2/D3 EPNs with four distinct groups support the molecular identification of these nematodes isolated from soil samples. This technique has been used to diagnose plant and animal parasites and entomopathogenic nematodes with accurate and reliable results, such as one *Pratylenchus penetrans* (Cobb) specimen in potato, *Bursaphelenchus fungivorus* (Franklin & Hooper) in coconut fiber, *M. rainai* (Carta & Osbrink) in soil cultivated with soybean and *Metarhabditis blumi* (Sudhaus) parasitizing the ear canal of cattle.

The finding of *H. amazonensis* and *S. rarum* in seven (3.5%) of the 201 soil samples, shows the reduced occurrence of these organisms in Brazil compared to surveys in Western Canada (20%) and 2001 by Yoon and Kim (1998); Argentina (13.2%) and Spain (23.3%) However, the prevalence of 3.5%, of these species, is similar to surveys in Turkey (2%, 9.1%) and Portugal (3.9%) and Minas Gerais state, Brazil (9%) and Azores Archipelago, Portugal (3.9%) and Minas Gerais state, Brazil (9%). The absence of EPNs in plowed soil...
**Table 1.** Code (Code), species, sampling site (Site), soil type (S), crops, geographical coordinates (coordinates) and collection date (date) of entomopathogenic nematodes (species) in soils with annual, fruit and forest plantation crops in Brazil (2010 to 2013). Species: *Heterorhabditis* (H), *Steinernema* (S), Site: Botucatu (Bo), Palotina (Pa), Garça (Ga), São Manuel (SM), clay soil (C), Sandy soil (S). Date: October (Oct), January (Jan), September (Sept).

| Code    | Species          | Site       | S       | Crops | Coordinates          | Date     |
|---------|------------------|------------|---------|-------|----------------------|----------|
| FCA01   | M. rainai        | Bo (SP)    | C       | Citrus| S7733173; W7484343   | Oct/2011 |
| FCA02   | O. tipulacea     | Bo (SP)    | C       | Citrus| S773250; W7484319    | Oct/2011 |
| FCA03   | O. tipulacea     | Pa (PR)    | C       | G. max| S4172381; W3521906    | Jan/2013 |
| FCA04   | M. rainai        | Ga (SP)    | C       | A. fraxinfoliis     | S763062; W77469943 | Jan/2013 |
| FCA05   | O. tipulacea     | Ga (SP)    | C       | A. indica           | S640610; W7544268 | Jan/2013 |
| FCA06   | M. rainai        | Ga (SP)    | C       | C. odorata          | S640581; W7544259 | Jan/2013 |
| FCA07   | H. amazonensis   | Ga (SP)    | C       | P. parviflora       | S640551; W7544214 | Jan/2013 |
| FCA08   | M. rainai        | Ga (SP)    | C       | C. brasiliensis     | S640467; W7544384 | Jan/2013 |
| FCA09   | S. raron         | Bo (SP)    | C       | Citrus             | S766468; W7474631 | Sept/2012|
| FCA10   | S. raron         | Bo (SP)    | C       | Eucalyptus         | S764574; W7472130 | Sept/2012|
| FCA11   | S. raron         | Bo (SP)    | S       | G. sativa          | S766897; W7474367 | Sept/2012|
| FCA12   | S. raron         | SM (SP)    | S       | R. iidea           | S749498; W7479197 | Oct/2012 |
| FCA13   | S. raron         | SM (SP)    | S       | Citrus             | S749477; W7479125 | Oct/2012 |
| FCA14   | S. raron         | SM (SP)    | M. indica | S749436; W7479022 | Oct/2012 |
| FCA15   | M. rainai        | Ga (SP)    | C       | C. ecalyculata     | S640534; W7544537 | Jan/2013 |
| FCA16   | M. rainai        | Pa (PR)    | C       | G. max             | S4211166; W3491260 | Jan/2013 |

areas suggests inadequate conditions for nematode survival, but zero EPN detection in the natural forest was unexpected and may be due to the low soil samples collected in this area. This result may also indicate the need for a higher number of samples taken at different soil depths. The species habitat and soil type affected EPNs recovery\(^{25,50}\). Our samples with nematodes were obtained from, clay (75%) and sandy (25%) soils, indicating the mobility and survival of EPNs in soils rich in sand, but S. raron occurred in clay (FCA 09 and FCA 10) and sandy (FCA, 11, 12, 13 and 14) soils. Many EPNs positive samples (89.65%) were obtained in acid soils in Nepal\(^{51}\). Six EPNs were found in soils with pH < 4, which is uncommon, but this has also been reported in Belgium\(^{52}\). *Steinernema* spp. are widespread in different regions and niches, whereas *Heterorhabditis* spp. are common in forest and river banks\(^{53}\). *Heterorhabditis* and *Steinernema* species were found in Sandy soils with pH < 6, whereas representatives of *Heterorhabditis*, mainly in Sandy soils with pH > 6 were found on nine islands of Açores\(^{19}\).

*Steinernema raron* detection in sandy soil with *Rubus edaeus*, *Mangifer indica* and *Oryza sativa* crops and clay soils with *Citrus* sp. and *Eucalyptus* sp. demonstrates the habitat diversity of this nematode, which has been detected in *Olea europea* L. crops in Argentina\(^{49}\), *Carya illinoinensis* orchards (Wangen.) in Mississippi and Louisiana State, USA\(^{48}\) and in sandy soils with annual and forest cultures in China\(^{46}\). In Brazil, this nematode was detected in soils with *Nicotiana tabacum* L., *Triticum aestivum* L., *Glycine max* and in native vegetation\(^{54}\). *Steinernema raron* showed desiccation tolerance and freezing intolerance\(^{55}\), features that may be related to its adaptation to Brazilian soils. This species has shown promise in biological control programs against *Anatomus grandis* Boheman (Coleoptera: Curculionidae) and *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae)\(^{57}\). *Metarhabditis rainai* detection in fruit orchards, annual crops and forest areas shows the broad habitat diversity of this nematode. This species was reported in *Glycine max* crop in Araras, São Paulo State, Brazil\(^{49}\). Other species, such as *Metarhabditis blumi*, are considered entomopathogenic and associated with the bacteria *Alcaligenes faecalis* Castellani & Chalmers, *Flavobacterium* sp. (Bernardet & Grimont) and *Providencia vermicensa* (Somvanshi), which killed *G. mellonella* larvae\(^{46}\). Other genera, besides *Heterorhabditis* and *Steinernema* may feed on and kill insects\(^{49}\). *Rhabditidae* nematodes are usually bacteriophages, found in insect carriorns\(^{59}\). However, *M. rainai* was described from specimens isolated from the *Oscheios tipulae* termite gut\(^{40}\) detected in soil samples and may parasitize insects and was also found to be associated with *Tipula paludosa* Meigen (Diptera: Tipulidae) larvae\(^{61}\). This nematode has been frequently isolated in soil samples from around the world, enabling species population\(^{62}\). *Oscheios* genus (*Rhabditidae*) includes free-living, vertebrate and invertebrate parasitic species\(^{63}\), such as *Oscheios chongningensis* (Zhang), considered a facultative entomopathogenic nematode\(^{61}\). The bacteria symbiosis with this genus species is similar to that of *Steinernematidae* and *Heterorhabditidae*\(^{65}\).

EPNs identified in agricultural areas in Brazil were *Heterorhabditis amazonensis*, *Metarhabditis rainai*, *Oscheios tipulae* and *Steinernema raron*. These species should be considered a pest biocontrol agent.

**References**

1. Poinar, G. O. & Grewal, P. S. History of entomopathogenic nematology. *J Nematol* **44**, 153–161 (2012).
2. Batalla-Carrera, L., Morton, A. & Garcia-del-Pino, P. Virulence of entomopathogenic nematodes and their symbiotic bacteria against the hazelnut weevil *Cerculo nucum*. *J Appl Entomol* **140**, 115–123 (2016).
3. Lewis, E. E., Campbell, J., Griffin, C., Kaya, H. K. & Peters, A. Behavioral ecology of entomopathogenic nematodes. * Biol Control* **38**, 66–79 (2006).
4. Fujimoto, A., Lewis, E. E., Cobanoglu, G. & Kaya, H. K. Dispersal, infectivity and sex ratio of early- or late-emerging infective juveniles of the entomopathogenic nematode *Steinernema carpocapsae*. *J Nematol* **39**, 333–337 (2007).
5. Ciche, T. A. & Ensign, J. C. For the insect pathogen, Photorhabdus luminescens, which end of a nematode is out? Appl Environ Microb 69, 1890–1897 (2003).

6. Mekete, T., Gauget, R., Nguyen, K. B., Mandefro, W. & Tessera, M. Biogeography of entomopathogenic nematodes in Ethiopia. Nematropica 35, 31–36 (2005).

7. Kucharska, K., Kucharska, D. & Zajdel, B. Bacteria Xenorhabdus and Photorhabdus, entomopathogenic nematodes and insects - Their role in the complex symbiont-parasite-host relationship. Post. Mikrobiol 55, 159–164 (2014).

8. Rivera, M. J., Rodríguez-Saona, C., Alb orn, H. T. & Koppenhofer, A. M. Differential response of a local population of entomopathogenic nematodes to non-native herbivore induced plant volatiles (HIPV) in the laboratory and field. J Chem Ecol 42, 1259–1264 (2016).

9. Kaya, H. K. et al. Status of entomopathogenic nematodes and their symbiotic bacteria from selected countries or regions of the world. Biol Control 38, 134–155 (2006).

10. Georgis, R. et al. Successes and failures in the use of parasitic nematodes for pest control. Biol Control 38, 103–123 (2006).

11. Tarasco, E. & et al. Biodiversity of entomopathogenic nematodes in Italy. J Helminthol 89, 359–366 (2015).

12. Tumialis, D. et al. Occurrence of entomopathogenic nematodes in Polish soils. Cienc Rural 46, 1126–1129 (2016).

13. Andaló, V., Nguyen, K. B. & Moino, A. Jr. Heterorhabditis amazonensis n. sp. (Rhabditida: Heterorhabditidae) from Amazonas, Brazil. Nematology 8, 855–867 (2006).

14. Nguyen, K. B., Ginetar, C. M. A., Leite, L. G., Santos, J. M. & Harakava, R. Steinernema brasiliense n. sp. (Rhabditida: Steinernematidae), a new entomopathogenic nematode from Mato Grosso, Brazil. J Invertebr Pathol 3, 8–20 (2010).

15. Dolinski, C., Kamitani, F. L., Machado, I. R. & Winter, C. E. Molecular and morphological characterization of heterorhabditid entomopathogenic nematodes from the tropical rainforest in Brazil. Mem I Oswaldo Cruz 103, 130–159 (2008).

16. Nguyen, K. B., Shapuro, I., Fuxa, J. R., Wood, B. W., Bertolotti, M. A. & Adams, B. J. Taxonomic and biological characterization of Steinernema rari found in the Southeastern United States. J Nematol 38, 28–40 (2006).

17. Andaló, V., Moreira, G. F. & Moino, A. Jr. Studies of two new populations of Heterorhabditis amazonensis (Rhabditida: Heterorhabditidae). Nematropica 39, 199–211 (2009).

18. Thanwisai, A. et al. Diversity of Xenorhabdus and Photorhabdus spp. and their symbiotic entomopathogenic nematodes from Thailand. PLoS ONE 7, e43855 (2012).

19. Karaborklua, S., Avyaz, A., Yılmaz, S., Azižoglu, U. & Akbulut, M. Native entomopathogenic nematodes isolated from Turkey and their effectiveness on pine processionary moth, Thaumetopoea pityocampa Tams. Int J Pest Manage 61, 3–8 (2015).

20. Cimen, H., Lee, M. M., Hatting, J., Hazir, S. & Stock, S. P. Steinernema tapiro sp. n. (Nematoda: Steinernematidae), a new entomopathogenic nematode from South Africa. J Nematol 48, 148–158 (2016).

21. Hazir, S., Stock, S. P., Kaya, H. K., Koppenhofer, A. M. & Keskin, N. Developmental temperature effects on five geographic isolates of the entomopathogenic nematode Steinernema feltiae (Steinernematidae), J Invertebr Pathol 77, 243–250 (2001).

22. Koppenhofer, A. M. & Fuzy, E. M. Ecological characterization of Steinernema scabaei, a scarab-adapted entomopathogenic nematode from New Jersey. J Invertebr Pathol 83, 139–148 (2003).

23. Ramos-Rodriguez, O., Campbell, J. F. & Ramaswamy, S. B. Efficacy of the entomopathogenic nematode Steinernema riobrave against the stored-product insect pests Tribolium castaneum and Plodia interpunctella. Biol Control 40, 15–21 (2007).

24. Nagem, M. et al. Comparative virulence of strains of entomopathogenic nematodes against management of eggplant Grey Weevil, Myllocerus subfasciatus Guerin (Coleoptera: Curculionidae). Indian J Exp Biol 54, 835–842 (2016).

25. Li, X., Liu, Q., Lewis, E. & Tarasco, E. Activity of antioxidant and detoxifying enzymes in Tenebrio molitor (Coleoptera: Tenebrionidae) larvae infected by the entomopathogenic nematode Homörhabditis becherian (Rhabditida: Heterorhabditidae). Parasitol Res 115, 1–10 (2016).

26. Leite, L. G., Machado, L. A., Agullera, M. M., Rodrigues, R. C. D. & Negrisoli, A. S. Jr. Pathogenicity of Steinernema and Heterorhabditis (Nematoda: Rhabditidae) against sugarcane root stiltlebug nymphs, Mahanarvah fimbriolata (Hemiptera: Cercopidae). Revista do Agricultura 78, 139–148 (2002).

27. Leite, L. G., Machado, L. A., Goulart, R. M., Tavares, F. M. & Batista-Filho, A. Screening of entomopathogenic nematodes (Nematoda: Rhabditida) and the efficiency of Heterorhabditis sp. against the sugar cane root stiltlebug Mahanarvah fimbriolata (Fabr.) (Hemiptera: Cercopidae). Rev Colomb Entomol 37, 198–202 (2011).

28. Batista, E. S. P., Auda, A. M., Resende, T. T. & Monteiro, C. M. O. Screening of entomopathogenic nematodes to control Mahanarvah fimbriolata (Hemiptera: Cercopidae). J Invertebr Pathol 90, 615–623 (2016).

29. Malan, A. P. & Moore, S. D. Evaluation of local entomopathogenic nematodes for the control of false codling moth, Thaumatinobia leucotrotia (Meyrick, 1913), in a citrus orchard in South Africa. Afr Entomol 24, 489–501 (2016).

30. Van Damme, V. M. et al. Efficacy of entomopathogenic nematodes against larvae of Tuta absoluta in the laboratory. Pest Manag Sci 72, 1702–1709 (2016).

31. White, G. F. A method for obtaining infective nematode larvae from cultures. Science 66, 302–303 (1927).

32. Woodring, L. & Kaya, H. K. Steinernematid and heterorhabditid nematodes: a handbook of techniques. Southern Cooperative Series Bull. 331. Arkansas agric. Exp. Statn., Fayetteville, AR, USA. 39p (1988).

33. Holterman, M. et al. Phyllum-wide analysis of SSU rDNA reveals deep phylogenetic relationships among nematode and accelerated evolution toward crown clades. Mol Biol Evol 23, 1792–1800 (2006).

34. Consoli, E. A. et al. Desenvolvimento de diagnostico molecular para aidentificatione de Pratylenchus sahenii. Nematologia Brasileira 31, 103–111 (2008).

35. Al-Banna, L. et al. Discrimination of six Pratylenchus species using PCR and species-specific primers. J Nematol 36, 142–146 (2004).

36. Márquez, C. et al. Steinernema sichuanense n. sp. (Rhabditida, Steinernematidae) a new species of entomopathogenic nematode from the province of Sichuan, east Tibetan Mts., China. J Invertebr Pathol 93, 157–169 (2006).

37. Sambrook, J., Fritsch, E. F. & Maniatis, T. Composition of the electrophoresis buffer In Molecular cloning, a laboratory manual (ed. Ford, N. et al.) 66–67, Cold Spring Harbor Laboratory Press, 1982.

38. Oliveira, C. M. G. et al. Diagnose de Aphielenchoides fragariae e Pratylenchus spp. pela aplicação da tecnologia do código de barras do DNA. Nematologia Brasileira 33, 218–225 (2009).

39. Tamura, K. et al. MEGA6: Molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30, 2725–2729 (2013).

40. Oliveira, C. M. G. et al. Caracterização morfológica e molecular de Bursaphelenchus jugovirus (Nematoda: Aphelenchida), detectado pela primeira vez no Brasil. Nematologia Brasileira 35, 3–4 (2011).

41. Tomazini, D. M. et al. Análise biome trica e molecular de isolado brasileiro de Rhabditis rainai (Nematoda: Rhabditida). Nematologia Brasileira 37, 1–2 (2013).

42. Bossi P. V. et al. Molecular identification and phylogenetic analysis of Metarhabdus blumi (Nematoda: Rhabditida). Vet Parasitol 214, 1–2 (2015).
46. Mrácek, Z. & Webster, J. M. Survey of Heterorhabditidae and Steinernematidae (Rhabditida, Nematoda) in western Canada. J Nematol 25, 710–717 (1993).
47. Stock, S. P. Natural population of entomopathogenic nematodes in the Pampean region of Argentina. Nematropica 25, 143–148 (1995).
48. García del Pino, E. & Palomo, A. Natural occurrence of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) in Spanish soils. J Invertebr Pathol 68, 84–90 (1996).
49. Erbas, Z. et al. Isolation and identification of entomopathogenic nematodes (Nematoda: Rhabditida) from the Eastern Black Sea region and their biocontrol potential against Melolontha melolontha (Coleoptera: Scarabaeidae) larva. Turk J Agric For 38, 187–197 (2014).
50. Kary, N. E., Niknam, G., Griffin, C. T., Mohammadi, S. A. & Moghaddam, M. A survey of entomopathogenic nematodes of the families Steinernematidae and Heterorhabditidae (Nematoda: Rhabditida) in the north-west of Iran. Nematology 11, 107–116 (2009).
51. Khatri-Chhetri, H. B., Waeyenberge, L., Manandhar, H. K. & Moens, M. Natural occurrence and distribution of entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) in Nepal. J Invertebr Pathol 103, 73–78 (2010).
52. Miduriti, J. S., Waeyenberge, L. & Moens, M. Natural distribution of entomopathogenic nematode (Heterorhabditidae and Steinernematidae) in Belgian soils. Russ J Nematol 5, 55–63 (1997).
53. Griffin, C. T., Chaerani, K., Fallon, D., Reid, A. P. & Downes, M. J. Occurrence and distribution of entomopathogenic nematodes Steinernema spp. and Heterorhabditis indica in Indonesia. J Helminthol 74, 143–150 (2000).
54. Doucet, A. M. M. et al. Consideraciones acerca de nematodos entomófagos (Mermithidae, Heterorhabditidae, Steinernematidae) de la provincia de Córdoba. Boletín de la Academia Nacional de Ciencias 66, 73–85 (2001).
55. Wang, H. et al. Natural occurrence of entomopathogenic nematodes in Liaoning (Northeast China). J Asia-Pac Entomol 17, 399–406 (2014).
56. Negrisoli, C. R. C. B. et al. Survey of entomopathogenic nematodes (Rhabditida: Heterorhabditidae, Steinernematidae) in Rio Grande do Sul, State, Brazil. Nematologia Brasileira 34, 189–197 (2010).
57. Shapiro-Ilan, D. I., Brown, I. & Lewis, E. E. Freezing and desiccation tolerance in entomopathogenic nematodes: Diversity and correlation of traits. J Nematol 46, 27–34 (2014).
58. Park, H. W. et al. Effects of associated bacteria on the pathogenicity and reproduction of the insect-parasitic nematode Rhabditis blumi (Nematoda: Rhabditida). Can J Microbiol 57, 750–758 (2011).
59. Stock, S. P., Caicedo, A. M. & Calatayud, P. A. Rhabditis (Oscheius) colombiana n. sp. (Nematoda: Rhabditidae), a necromenic associate of the subterranean burrower bug Cyrtomenus bergi (Hemiptera: Cydnidae) from the Cauca Valley, Colombia. Nematology 7, 363–373 (2005).
60. Carta, L. K. & Oszbrink, W. Rhabditis rainai n. sp. (Nematoda: Rhabditidae) associated with the Formosan subterranean termite, Coptotermes formosanus (Isoptera: Rhinotermitidae). Nematology 7, 863–879 (2005).
61. Baille, D., Barriere, A. & Felix, M. A. Oscheius tipulae, a widespread hermaphroditic soil nematode, displays a higher genetic diversity and geographical structure than Caenorhabditis elegans. Mol Ecol 17, 1523–1534 (2008).
62. Felix, M. A. RNA interference in nematode and the chance that favored Sydney Brenner. Journal of Biology 7, 1–34 (2008).
63. Parkinson, J. et al. A transcriptomic analysis of the phylum nematode. Nat Genet 36, 1259–1267 (2004).
64. Jarosová, A., Puza, V. & Zurovcová, M. The complete mitochondrial genome of the facultative entomopathogenic nematode Oscheius chongmingnensis (Rhabditidae). Mitochondrial DNA 11, 1–2 (2015).
65. Anjus, K. M. et al. Purification and identification of an antibacterial protein from the symbiotic bacteria associated with novel entomopathogenic nematode, Rhabditis (Oscheius) sp. World J Microb Biot 31, 621–632 (2015).

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Author Contributions
A.L.B., J.M.O.R., C.M.G.O., and B.M.C.C. performed experiments, and analyzed the data; J.E.S., and J.C.Z. analyzed the data; A.L.B., J.E.S., J.C.Z., L.G.L., C.M.G.O., and S.R.S.W. designed the experiments and wrote the manuscript.

Additional Information
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