

Article

Molecular Identification and Phylogenetic Diversity of Native Entomopathogenic Nematodes, and Their Bacterial Endosymbionts, Isolated from Banana and Plantain Crops in Western Colombia

Jorge Mario Londoño-Caicedo ^{1,2,*} , Miguel Uribe-Londoño ^{1,3}, María Angélica Buitrago-Bitar ¹, Andrés J. Cortés ^{4,*}  and Jaime Eduardo Muñoz-Flórez ¹

- ¹ Grupo de Investigación en Diversidad Biológica, Facultad de Ciencias Agropecuarias, Universidad Nacional de Colombia, Palmira 763531, Colombia; miguribelon@unal.edu.co or director@jardinbotanicodelmagdalena.com (M.U.-L.); mabuitragob@unal.edu.co (M.A.B.-B.); jemunozf@unal.edu.co (J.E.M.-F.)
- ² Grupo de Investigación en Biodiversidad y Biotecnología (GIBUQ), Universidad del Quindío, Armenia 630004, Colombia
- ³ Jardín Botánico del Magdalena, Vereda la Atarraya Km 31, La Dorada 175037, Colombia
- ⁴ Corporación Colombiana de Investigación Agropecuaria (AGROSAVIA)—C.I. La Selva, Rionegro 054048, Colombia
- * Correspondence: jmlondono@uniquindio.edu.co or jomlondonoca@unal.edu.co (J.M.L.-C.); acortes@agrosavia.co (A.J.C.)
- † Secondary Address: Facultad de Ciencias Agrarias, Departamento de Ciencias Forestales, Universidad Nacional de Colombia, Sede Medellín, Medellín 050034, Colombia.
- ‡ Secondary Address: Department of Plant Breeding, Swedish University of Agricultural Sciences, 23053 Alnarp, Sweden.



Citation: Londoño-Caicedo, J.M.; Uribe-Londoño, M.; Buitrago-Bitar, M.A.; Cortés, A.J.; Muñoz-Flórez, J.E. Molecular Identification and Phylogenetic Diversity of Native Entomopathogenic Nematodes, and Their Bacterial Endosymbionts, Isolated from Banana and Plantain Crops in Western Colombia.

Agronomy **2023**, *13*, 1373. <https://doi.org/10.3390/agronomy13051373>

Academic Editors: Letian Xu, Zhanghong Shi and Jacob D. Wickham

Received: 15 April 2023

Revised: 8 May 2023

Accepted: 8 May 2023

Published: 14 May 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Abstract: With the increasing negative impacts on worldwide food production caused by pests, the recovery of native entomopathogenic nematodes (EPNs) is relevant, since they are adapted to local environments, entomofauna, and significant virulence. Therefore, the present study was designed to recover and understand the phylogenetic diversity of EPNs and their associated bacterial endosymbionts, from banana and plantain crops, as alternatives for the control of weevil species. An extensive sampling of western Colombia covered 325 ha, yielding the recovery of three EPNs' isolates (0.49% of the samples). The molecular characterization included four mitochondrial and nuclear loci, which, after merging with the sequences of 48 species, confirmed the presence of *Steinernema carpocapsae*, the first report of *S. costaricense* in South America, and monophyly in most of the *Steinernema* clades. The tree topologies were consistent for the nuclear loci but not for mitochondrial, probably due to the high nucleotide substitution rate, deficit in the number of species available for these loci, and incomplete lineage sorting. The endosymbiotic bacteria associated with *S. carpocapsae* were identified as *Xenorhabdus nematophila*. However, the *S. costaricense* bacterial symbiont presented a genetic similarity to *X. koppenhoferi* and *X. khoisanae*, which are still uncertain in their classification. The identification of *S. costaricense* in South America indicates the wide range distribution of this species in the Americas and its ability to persist in different soil types. For the first time, EPN isolation and phylogenetic characterization are directed to plantain and banana crops. Leveraging EPNs' diversity promises novel applications for crop protection, while the genetic resources from the bacterial endosymbionts may provide metabolites with a wide spectrum of uses, either for agricultural or medicinal purposes.

Keywords: biological control agents; endosymbiotic bacteria; integrated pest management; weevil species

1. Introduction

Worldwide food production is affected by approximately 10,000 insect and 30,000 beetle weevil species [1,2], putting the world food supply at risk and representing an estimated crop loss of 18–20% of annual production, reaching an economic value of USD 470 billion [3]. These agricultural losses are more accurate in developing countries [4]. For instance, current crop losses in Latin America account for >14% [3].

Together, bananas and plantains are one of the most important crops worldwide, classified as essential for food security due to their high nutritional value [5–8]. These crops are grown in more than 135 countries, and the main producers are located in Central and South America, and the Philippines, totalizing 20 million tons of exports [9]. Colombia is one most important banana producers after Ecuador, the Philippines, Guatemala, and Costa Rica. In 2018, this fruit was the fourth main product in the global export trade (8.4% and USD 914,937,000) [10]. However, banana and plantain crops are severely affected by weevil species, generating losses of 2,600,000 tons/yield cycle [11,12]. Five banana weevil species have been reported so far. *Cosmopolites sordidus* (Germar) is a species with tropical and subtropical distribution around the world, with its center of origin in Southeast Asia [13,14], from where it spread widely over banana-production areas [13]. *Metamasius hemipterus* (Linnaeus), *Metamasius hebetatus* (Gyllenhal), and *Metamasius submaculatus* (Champion) are all distributed in Central and South America, with the Antilles as the plausible center of origin for the genus [15]. Meanwhile, *Polytus mellerborgii* (Boheman), an Indo-Malaysian native with pantropical distribution, has recently been spread by international trading in the tropical world [16], reaching the Neotropics within the last decade [12]. *C. sordidus* was reported as the most damaging species of banana and plantain plantations in Colombia [11,12] and worldwide [17–19]. The larval stage bores in the corm, creating galleries that reduce nutrient uptake and weaken the stability of the plant [17].

Conventional methods for pest control include chemical agents, which have been used worldwide for decades, bringing negative consequences to the environment, reducing the microbiome diversity in soils, jeopardizing ground water, increasing the flux of greenhouse gases, and accounting for 9.3 billion tons of CO₂ [4]. Besides the high toxicity of these chemicals, the resistance of weevil species (i.e., *M. hemipterus*) to a broad spectrum of synthetic insecticides has been reported [13], where endosymbiotic bacterial associations could be playing an important role in detoxification mechanisms [20–22], meaning the chemical method for pest control will become obsolete over time. Alternative strategies such as biological control are currently capturing the attention as methods to decrease soil pollution, while favoring costs for banana and plantain production. Several studies have applied this method by using insect enemies [23], fungal species such as *Bauveria bassiana* [24], and entomopathogenic nematodes to control *C. sordidus* populations [25]. However, these approaches still remain incipient.

Entomopathogenic nematodes (EPNs) are soil-dwelling, obligate insect-parasitic organisms with a cosmopolitan distribution (except the Arctic and Antarctic). Up to now, 96 *Steinernema*, one *Neosteinerema*, and 21 *Heterorhabditis* species have been described [2]. Their efficiency as biological control agents has targeted agricultural pests [26] in many different crops, with outstanding efficiency against Coleoptera [27], Lepidoptera [28], and Diptera [29]. The obligate parasitic nature of EPNs makes them optimal for integrated pest management (IPM) programs, as they are safe for the environment and other organisms compared to insect pests [1,30]. They are symbiotically associated with enterobacteria of the species *Xenorhabdus* for *Steinernema* spp. and *Photorhabdus* for *Heterorhabditis* spp., which have received increasing interest due to the antibiotic and antifungal properties of their secondary metabolites [2,31–34]. EPNs are classified into two major families: Heterorhabditidae and Steinernematidae. All of their species are considered excellent biological control agents against the insect pests affecting many crops worldwide [30]. Based on their high entomopathogenic efficiency [26,28,35–37], EPNs are commercially available as an alternative method to reduce soil and water pollution in agricultural systems [30].

The infection efficiency of EPNs relies on the enhanced pathogenicity of their bacterial symbiont [31,33]. Initially, the nematode enters into the insect's body via natural openings, with a posterior release of the symbiotic bacteria into the host hemocoel, causing septic shock and killing the insect in 24–48 h [26,38–40]. This enables the optimal conditions for EPNs' reproduction and multiplication before the insect's death. The new generation of EPNs feed on the insect's cadaver and acquire the bacterial symbiont [26,30]. Once the resources are depleted, the infective juveniles (IJ) exit the cadaver in search of a new host to infect [26,41–43].

Some attempts of pest control were carried out by incorporating non-native EPNs in major crops worldwide [44,45]. However, these strains failed to parasitize local entomofauna, probably due to environmental conditions or the presence of pollutants in soils [44]. Therefore, it is paramount to isolate and characterize native EPNs, which may be locally adapted to the regional entomofauna. Local EPNs may be more infectious and competent in parasitism at either the adult or larval stage of development [46]. Particularly interesting are the strains that could tolerate intervened soils. In this sense, those EPN strains could provide potential applicability for in situ pest control.

The identification and evaluation of local EPN strains have partly been studied in Colombia with potential application in plantain and banana crop pest control [47,48], directed at the main banana beetle species found in the country [49]. These previous studies contemplated potential negative impacts on the crop [50] as well as the monitoring of reproductive cycles in order to develop target strategies for biological pest control [51]. Nevertheless, EPN molecular identification in the region remains incipient. Given this research gap, the present study aimed to:

- Isolate and characterize native entomopathogenic nematodes that are locally adapted to plantain and banana crops, as options for weevil pest biological control;
- Identify the symbiotic bacteria associated with EPNs;
- Explore EPNs and their bacterial symbionts' phylogenetic diversity.

2. Materials and Methods

2.1. Sampling Area, EPN Isolation, and Propagation

Sampling area comprised banana and plantain plantations from 67 farms distributed in 12 municipalities located in the north, center, and south of Valle del Cauca province, in southwestern Colombia, which is a major hub of banana and plantain production in the Americas. Altitudes ranged from 7 to 1734 m asl. A total of 603 soil samples were obtained (nine from each farm), covering 325.7 hectares. Using a soil bore corer, the soil samples were taken at a depth of 20 cm and at a distance of 50 cm from the plant corm (rhizome).

The strategies followed for sampling included nine randomly chosen banana or plantain plants per farm. Three soil samples were taken around each plant in a triangle-shaped fashion, mixed into a single bag, and transported to the laboratory. To evaluate the presence of entomopathogenic nematodes (EPNs), 1 kg per soil sample was separated into three subsamples using plastic containers. Ten last instar *Galleria mellonella* larvae (Lepidoptera: Pyralidae) were employed as hosts and were placed alive into each container, following the method proposed by [52] with minor modifications. The containers were flipped over every 24 h and kept at room temperature (22 °C) for 3–7 days. All larvae cadavers were separated based on the color patterns drawn by the nematodes' infection (brownish for Steinernematids and reddish for Heterorhabditids). Afterward, they were rinsed with distilled water and placed in modified white traps [53,54].

The nematodes that were recovered were stored at 8 °C in a concentration of 2000 EPNs/mL and were reactivated by using a concentration of 200 EPNs/mL for each host, including *G. mellonella*, *Metamasius hemipterus*, *Cosmopolites sordidus*, and *Tenebrio molitor* larvae, for further analyses. The isolation and laboratory methods were carried out following the conditions described by Stock and Goodrich-Blair [55]. All these processes were conducted at Universidad Nacional de Colombia, Palmira campus.

2.2. DNA Extraction and PCR Amplification from EPN

The three obtained *Steinernema* isolates (coded as UNPR52, UNPR70, and UNPR73) were reared in vivo using last instar *G. mellonella* larvae. Afterward, a single F1 female was picked for DNA extraction using Quiagen® DNeasy blood and tissue kit, in accordance with the instructions of the manufacturer. A final volume of 25 µL of DNA solution per isolate was obtained with an average concentration of 20 ng/µL, from which all the downstream molecular analyses were carried out. Four standard invertebrates' barcoding loci (COI and 12S mtDNA and ITS and LSU rDNA) were considered for phylogenetic and DNA pattern analyses; two of them were from the nuclear genome (ribosomal regions ITS and LSU), and two were from mitochondrial origin (COI and 12S). PCR amplifications were performed for each locus, mixing 2 µL of DNA 20 ng/µL, 2.5 µL of PCR Buffer 10× (NH₄)₂SO₄, 2 µL of MgCl₂ 25 mM, 1 µL of dNTP's 20 mM, 0.4 µL of each forward and reverse primer 10 mM, 0.5 µL of BSA 5×, 1 µL of Trehalose 10%, 0.16 µL of Taq DNA polymerase 5 U/µL, and 15 µL of ultra-pure water for a final volume of 25 µL. All sets of primers used are listed in Table 1. The PCR profile was as follows: 95 °C × 5 min, 95 °C × 1 min, 42 °C × 45 s, and 72 °C × 1 min, followed by 32 cycles at 95 °C × 1 min, 40 °C × 45 s, and 72 °C × 1 min, plus a final extension at 72 °C × 10 min. These amplification profiles varied in the annealing temperature: 42 °C for COI, 52 °C for 12S, 55 °C for ITS, and 54 °C for LSU. The PCR products were observed in 1.5% agarose gel and electrophoresed at 120 volts for 45 min in TBE solution 0.5×. All PCR products were purified following the protocol proposed by [56], and 1 µL was electrophoresed to verify the concentration.

Table 1. Set of primers used for PCR and sequencing reactions for EPNs and bacterial symbionts. Ambiguities are indicated according to the IUPAC nucleotide code.

Organism	Primer	Region	Primer Sequence	Orientation	Author
Nematode	AB28	ITS	ATATGCTTAAGTTCAGCGGGT	Forward	Stock and Goodrich-Blair, 2012 [55]
	TW81	ITS	GTTTCCGTAGGTGAACCTGC	Reverse	
	533	ITS internal	CAAGTCTTATCGGTGGATCAC	Forward	
	534	ITS internal	GCAATTCACGCCAAATAACGG	Reverse	
	391	LSU	AGCGGAGGAAAAGAACTAA	Forward	Stock et al., 2001 [57]
	501	LSU	TCGGAAGGAACCAGCTACTA	Reverse	
	502	LSU internal	CAAGTACCGTGAGGGAAAGTTGC	Forward	
	503	LSU internal	CCTTGGTCCGTGTTCAAGACG	Reverse	
	COX1F	COI	AGTTCTAATCATAARGATATYGG	Forward	Nadler et al., 2006 [58]
	COX1R	COI	TAAACTTCAGGGTGACCAAAAATCA	Reverse	
	12SF	12S	GTTCCAGAATAATCGGCTAGAC	Forward	
	12SR	12S	TCTACTTTACTACAACTTACTCCCC	Reverse	
Bacteria	27F	16S	AGAGTTTGATCMTGGCTCAG	Forward	Gurtler and Stanisich, 1996 [59]
	1492R	16S	TACGGYTACCTTGTTACGACTT	Reverse	
	518F	16S internal	CCAGCAGCCGCGGTAATACG	Forward	
	800R	16 internal	TACCAGGGTATCTAATCC	Reverse	

2.3. DNA Sequencing and Data Analysis

DNA sequencing was performed in both directions (forward and reverse) for all loci. Yet, for ITS and LSU rDNA, internal primers were used to obtain high-quality sequence data. For ITS rDNA, the first primer combination AB28–534 flanked the 5' end of the sequence (~500 bp), whereas the second primer combination 533–TW81 flanked the 3' end (~600 bp). For LSU rDNA, the primer combinations 391–503 (~600 bp) and 502–501 (~670 bp) flanked the 5' and 3' ends of the sequences, respectively (Table 1). Raw sequences were initially assembled following each primer combination separately and were manually edited.

PCR primers were anchored and removed from the sequences prior to the whole assembly of 5' and 3' end fragments. For ITS rDNA, a final sequence of ~780 bp was obtained after contigs were assembled with an overlapping region of ~300 bp, whereas for LSU the sequence comprised ~900 bp with an overlapping region of ~400 bp. All steps were carried out in Geneious R10[®] [60]. Multiple alignment was performed using Bioedit[®] v.7.1.11 [61], including sequences from *Steinernema* species reported by Hunt and Nguyen [62] for ITS rDNA, LSU rDNA, 12S mtDNA, and COI mtDNA.

2.4. EPN Phylogenetic Analysis

Phylogenetic inferences were executed for each locus independently, following the best model of nucleotide substitution as obtained in Mega v.7.0 [63]. Maximum Parsimony (MP) and Maximum Likelihood (ML) analyses were performed using a bootstrap of 1000 replicates, based on General Time Reversible (GTR + G + I) model [64], including invariable sites (I) [65] and rate of variations across sites (G) [66]. A concatenated analysis was also performed, comprising sequences from 48 species reported for both ITS and LSU [62]. These MP and ML analyses comprised 1000 bootstrap replicates based on (GTR + G + I) model. Additionally, Bayesian analyses were carried out in MrBayes v.3.2 [67] under the GTR + G + I model with 10 million generations, sampling every 100 trees.

Caenorhabditis elegans was used as outgroup to root all phylogenies. Codon assignment, reading frames, and amino acid identification for COI mtDNA were performed in Geneious[®] R10 [60], considering the genetic code for mitochondrial invertebrates. Each sample was individually translated to verify the correct reading frame. No stop codons were present. *S. carpocapsae* (AY944007) [68] was used as reference sequence to identify all substitutions at nucleotide and amino acid levels. The quantification of genetic diversity between EPN species relative to the isolates from this study was carried out in Mega 7 [63]. Meanwhile, the number of segregating sites based on the average number of polymorphic nucleotides between sequences and G + C content was computed in DnaSP 5.1 [69]. All nucleotide sequences for EPN isolates were deposited in the GenBank NCBI database (Table 2).

Table 2. NCBI accession numbers for each locus and georeferenced strain voucher isolated in the present study. Species names' assignment was based on molecular approaches. Some loci could not be sequenced for *S. costaricense* and are indicated by dashes (-).

Species	Specimen Voucher	Municipality	Lat Lon	ITS rDNA	LSU rDNA	12S mt rDNA	COI mtDNA
<i>S. carpocapsae</i>	UNPR52	Obando	4587 N 75,916 W	MH231235	MH231232	MH231121	MH252432
<i>S. carpocapsae</i>	UNPR73	Buenaventura	3481 N 76,185 W	MH231236	MH231233	MH231122	MH252433
<i>S. costaricense</i>	UNPR70	Palmira	3742 N 76,965 W	-	MH231234	-	-

2.5. Bacterial Symbionts Isolation and DNA Extraction

Bacterial recovery was executed four days after nematodes infection (using 200 IJs/isolates/*G. mellonella* larvae), using a drop of hemolymph from infected larvae, which was then isolated and plated in NBTA agar incubated at 27 °C, following the method described by [55].

These isolates were coded as UNPB52, UNPB70, and UNPB73. Additional purification process was performed by choosing an individual colony per plate and placing it into a new culture medium, following the conditions described by Stock and Blair [55]. Afterward, a single colony per isolate was picked up and transferred into 15 mL of liquid culture medium (Luria Bertani LB), using oscillating shaking conditions and at 27 °C for 24 h. Bacterial cells were recovered by centrifugation. For the DNA extraction, DNeasy Blood & Tissue kit QUIAGEN[®] was used, as in the manufacturer's guide.

2.6. Molecular Identification and Phylogenetic Analysis of EPNs' Bacterial Symbionts

Ribosomal 16S rDNA region was employed for molecular characterization using a set of universal primers (Table 1). External primers 27F and 1492R were employed for initial PCR reactions by mixing 2 µL of DNA 20 ng/µL, 2.5 µL of PCR Buffer 10× (NH₄)₂SO₄, 2 µL of MgCl₂ 25 mM, 1 µL of dNTP's 20 mM, 0.4 µL of each forward and reverse primer 10 mM, 0.5 µL of BSA 5×, 1 µL of Trehalose 10%, 0.16 µL of Taq DNA polymerase 5 U/µL, and 15 µL of ultra-pure water, for a final volume of 25 µL. The thermal profile included an initial denaturing step at 94 °C × 4 min, followed by 30 cycles at 94 °C × 1 min, 55 °C × 45 s, and 72 °C × 1.30 min plus a final extension of 72 °C × 7 min. This locus generated a fragment of 1400 bp. However, internal primers were employed for better sequencing results. The primer combination of 27F and 800R flanked the 5' end of the sequence, generating a partial fragment of 760 bp, while the primer combination of 518F and 1492R flanked the 3' end and generated a sequence of 940 bp. Sequence edition and contig assembly was manually and independently carried out in Geneious R10[®] [60] for each isolate. PCR primers (external and internal) were removed prior to fragment assembly. For nucleotide pattern identification and phylogenetic analysis, the conditions described above in Section 2.4. were applied for 16S rDNA region, based on the GTR + G + I model [64] for phylogenetic inferences. All nucleotide sequences from bacterial isolates were deposited in the GenBank NCBI database (Table 3).

Table 3. Endosymbiotic bacteria strain vouchers with their host of origin and NCBI accession ID.

Species	Specimen Voucher	Organism Source	16S rDNA Accession Number
<i>X. nematophila</i>	UNPB52	<i>S. carpocapsae</i>	MH249989
<i>X. nematophila</i>	UNPB73	<i>S. carpocapsae</i>	MH249990
<i>Xenorhabdus</i> sp.	UNPB70	<i>S. costaricensis</i>	MH249991

3. Results

3.1. Sampling Variation

From the extensive sampling strategy carried out in banana and plantain plantations, covering 12 municipalities of Valle del Cauca province (Figure 1), three soil samples out of 603 (0.49%) were positive for entomopathogenic nematodes (EPNs). These isolates were recovered from the municipalities of Obando (UNPR52), Palmira (UNPR70), and Buenaventura (UNPR73). All samples and isolates were properly coded and stored, following the conditions previously defined at the molecular biology laboratory for the project BPIN 2014000100014. Based on the characteristics of *G. mellonella* larvae cadavers and the morphology of infective juveniles (IJ) and adults, all isolates were identified as *Steinernema* spp. Isolates UNPR52 and UNPR73 showed a good performance of pathogenicity on either *G. mellonella*, *M. hemipterus*, *C. sordidus*, or *Tenebrio molitor* larvae (≥90% mortality), while UNPR70 failed to parasitize after a couple of infection cycles and could not be reared under laboratory conditions for any detailed morphological evaluations. All evaluations of infection, multiplication of EPNs, and downstream molecular analyses were performed in the laboratory of molecular biology at the Universidad Nacional de Colombia, Palmira campus.

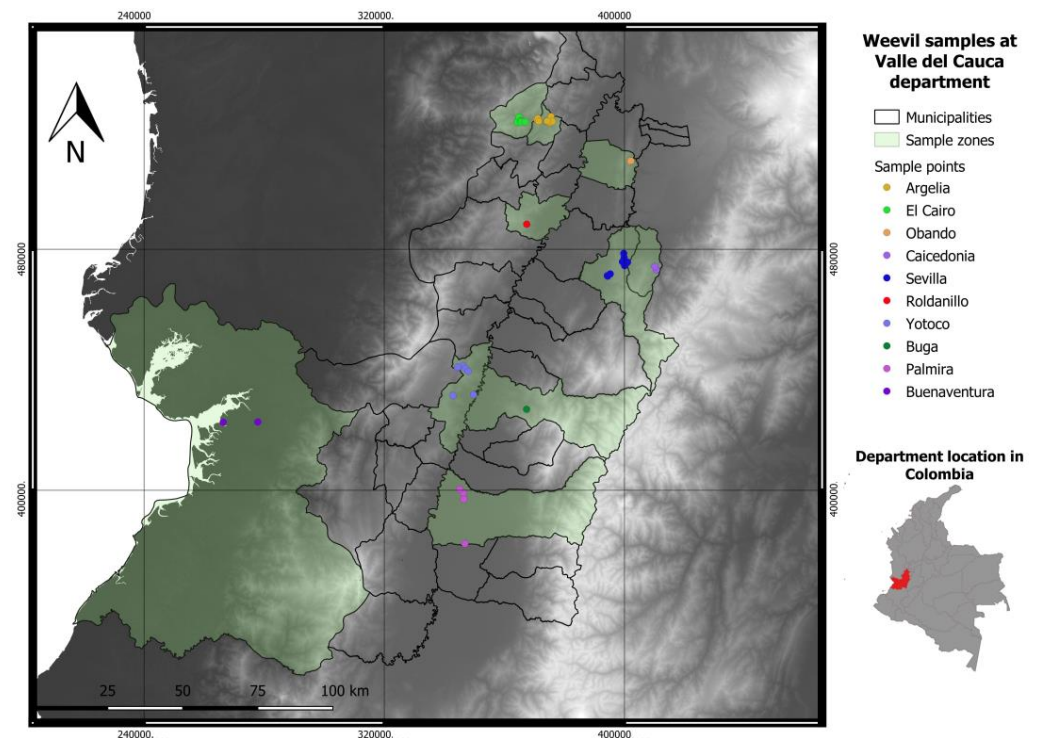


Figure 1. Geographical representation of the sampling area, with conventions on the right for specific municipalities. Shaded area represents the political division map for every municipality. EPN isolates were recovered in Obando (orange), Palmira (magenta), and Buenaventura (violet).

3.2. EPN Nucleotide Diversity

Four loci were sequenced, two ribosomal (LSU and ITS) and two mitochondrial (12S and COI) regions. For isolates UNPR52 and UNPR73, the sequencing covered 2918 bp, whereas, for the isolate UNPR70, only the LSU region was sequenced, generating 882 bp. Comparisons among the three isolates based on the LSU region showed 115 polymorphic sites. Two nucleotides varied between UNPR52 (accession number MH231232) and UNPR73 (accession number MH231233), whilst most of the nucleotide variation was exclusive to UNPR70 (accession number MH231234). In order to identify the genetic similarity of these isolates to the species reported so far for the *Steinernema* genus, the analysis included 60 LSU sequences from Hunt and Nguyen [62], generating a total of 1130 sites, of which 190 were polymorphic and 149 were parsimony-informative. The genetic distance indicated no variation between UNPR52 and UNPR73, with respect to the *S. carpocapsae* isolates reported by Mráček et al. [70] (KJ950293 and KJ950292). On the other hand, UNPR70 showed genetic proximity (0.034) to *S. costaricense* isolate “Bush-Augusta” (NCBI accession number JX493015), as reported by Ivanova et al. [71], with 14 nucleotide changes. The ITS locus analysis included isolates UNPR52 (accession number MH231235) and UNPR73 (accession number MH231236), without any nucleotide substitution in between. The whole set of sequences reported by Hunt and Nguyen [62] for the ITS region included 85 *Steinernema* spp. and generated 1293 sites, of which 127 were polymorphic and 78 were parsimony-informative. The genetic distances showed high similarity (0.001) to *S. carpocapsae* “Breton” isolate (under NCBI accession number AF121049, [72]) and to isolate “NCR” (with accession number KJ950291, [70]), showing a genetic distance of 0.006 (five nucleotide changes). The mitochondrial loci (12S and COI) exhibited contrasting results, with respect to the information obtained for the ribosomal loci. For the 12S mtDNA, 507 site nucleotide positions were considered, of which 210 were polymorphic and 147 were parsimony-informative. The 27 *Steinernema* spp. sequences reported for this locus by Nadler et al. [68] were included.

UNPR52 (accession number MH231121) and UNPR73 (accession number MH231122) were genetically similar (0.004) to *S. carpocapsae* isolate “N2” (accession number AY944007, [68]), *S. anatoliense* isolate “Al-Jubiha” (accession number GU569024, [73]), and *S. websteri* isolate “Peru” (accession number GU569039, [73]). *S. anatoliense* and *S. websteri* were reported as synonyms of *S. carpocapsae* [62]. For the COI mtDNA analyses, the final dataset considered 31 species. A total of 676 nucleotide positions were included, of which 211 showed polymorphism and 191 were parsimony-informative. The nucleotide composition comprised Thymine (T; 45.6%), Cytosine (C; 14.3%), Adenine (A; 22.9%), and Guanine (G; 17.2%), with a strong A + T bias (68.5%). Nucleotide variation generated a genetic distance of 0.009 between the UNPR52 and UNPR73 isolates, with respect to *S. carpocapsae* isolate “N2” (accession number AY943981, [68]), 0.004 with *S. anatoliense* isolate “Al-Jubiha” (accession number GU569062, [73]), and no variation respect to *S. websteri* isolate “Peru” (GU569074, [73]). This analysis confirms the synonymy of these three species, all of them belonging to the Carpopcapsae clade [62].

Following the multispecies clades proposed by Hunt and Nguyen [62], a boxplot for F_{ST} analysis indicated no statistical differences between groups for either of the evaluated loci (Figure 2a,b). For LSU, however, there were Affine display differences from the Kushidai and Monticulum clades, when neglecting the outliers (Figure 2a), with a relatively low variation and F_{ST} values among the groups (≤ 0.5 , Figure 2a). ITS, conversely, contained more variation (≥ 0.5 , Figure 2b), with the Affine clade being more differentiated, as evidenced by the concatenated dataset (Figure 2c).

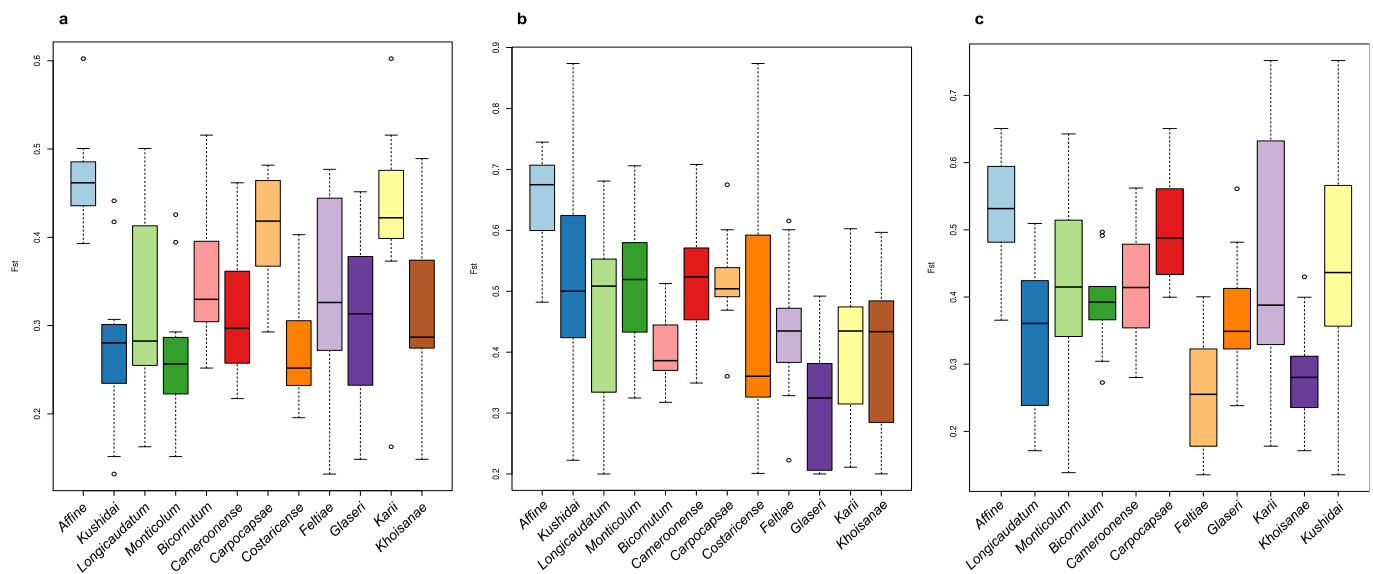


Figure 2. Boxplot of the pairwise F_{ST} distributions at (a) LSU, (b) ITS, and (c) concatenated (LSU–ITS) datasets analyzed at the clade level for EPN species. Costaricense clade was not included in the concatenated analysis, since no sequence information was found for both loci at these isolates.

A heat map analysis, based on F_{ST} values, provided an alternative perspective for clades interaction (Figure 3), where the Affine is distant for both loci and concatenated. Following the superclades classification proposed by Hunt and Nguyen [62] and Nguyen and Hunt [74], we identified that superclade II was consistent for LSU (Figure 3a) and ITS (Figure 3b) but failed when concatenated (Figure 3c). These results indicate a tendency of similar allele frequencies with possible signatures of positive selection at the LSU locus, conversely to the ITS locus (contrasting selective pressures on both loci). Despite the two markers have enough resolution for species identification, ITS rDNA has better differentiation sensitivity, since the variance in allele frequency is higher, providing additional information about the genetic diversity status at the species level optimal for the identification of new strains and their possible correlation to particular habitats.

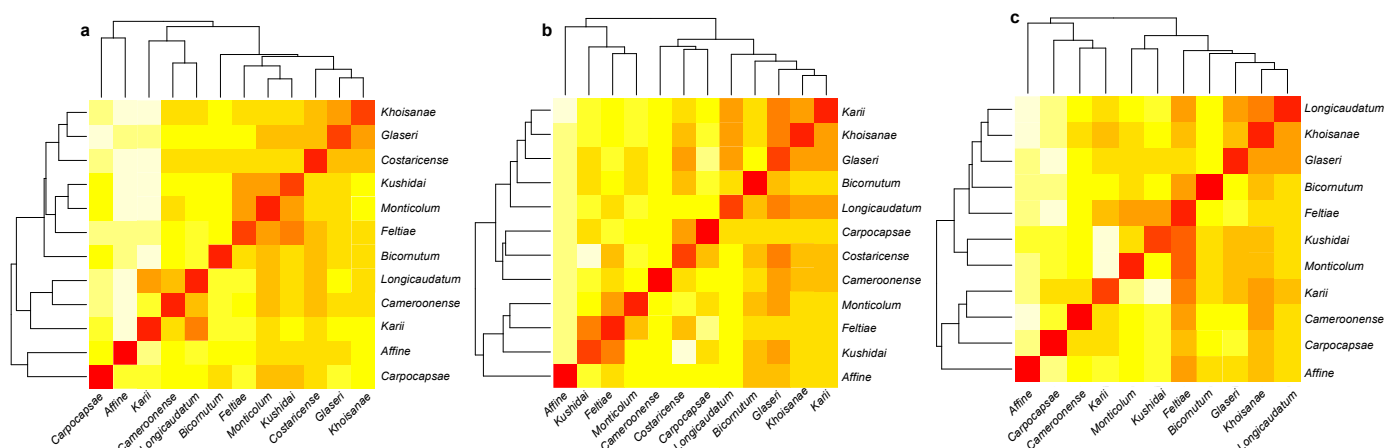


Figure 3. Clustered heatmap of the pairwise F_{ST} distributions at (a) LSU, (b) ITS, and (c) concatenated (LSU-ITS) datasets analyzed at the clade level for EPN species. Dots mark superclade nodes.

For amino acid composition analysis (at COI locus), 26 *Steinernema* spp. sequences were included, obtaining 225 amino acid residues with 15 changes among the species (Table S1). Leucine (Leu) and Serine (Ser) were the most frequent (18.1% and 10.3%, respectively), whereas Glutamine (Gln) and Lysine (Lys) represented the less-frequent amino acid residues (0.8% and 0.9%, respectively). The nucleotide substitutions that occurred among UNPR52, UNPR73, *S. carpocapsae* (AY944007), *S. anatoliense* (GU569024), and *S. websteri* (GU569074) included four transition events (A/G, T/C, C/T, and G/A) and two transversions (T/A and T/G), though all of them were synonymous mutations.

The concatenated analysis (ITS and LSU rDNA regions) included 51 nucleotide sequences, of which 50 belonged to *Steinernema* spp. in addition of *C. elegans* as the outgroup for phylogeny rooting. All the sequences were retrieved from the NCBI and reported by Hunt and Nguyen [62]. The final dataset comprised 2022 nucleotides, 361 polymorphic positions, 277 parsimony-informative sites, and a G + C content of 47.3%. There was not genetic distance between UNPR52 and UNPR73 compared to *S. carpocapsae* (KJ950291–KJ950293), supporting the classification into the latter. Concatenated analyses were unviable due to poor open data for mitochondrial loci (i.e., COI and 12S).

3.3. EPN Phylogenetic Analysis

Initially, all loci were independently analyzed to verify the congruence of phylogenetic inferences as well as the clustering of our isolates, with respect to the sequences previously reported. This approach corroborated the accuracy by each locus in a phylogenetic context. For the ribosomal locus LSU rDNA, 64 samples belonging to 58 *Steinernema* species, including the three isolates obtained here, were analyzed by the Maximum Parsimony (MP), Maximum Likelihood (ML), and Bayesian Inference (BI) methods, using *C. elegans* as the outgroup (Figure 4). The final dataset comprised 1154 positions, yielding a parsimonious tree of 1935 steps (C.I. 0.45 and R.I. 0.74). Isolates UNPR52 and UNPR73 were grouped into the Carpopcapsae clade. Although the posterior probability for this clade was not well-supported (ML 54% and MP 59%), the BI was significant (0.93). Conversely, the isolate UNPR70 was clustered with *S. costaricense* in the Costaricense clade with a strong bootstrap and posterior supports (99% ML, 100% MP, and BI 1.0), except for *S. scarabaei*, which was conflicting for all phylogenetic inferences. Following the phylogenetic hypothesis of multispecies clades proposed by Hunt and Nguyen [62], we identified Khoisanae, Feltiae, Cameroonense, Bicornutum, Carpopcapsae, and Affine as monophyletic groups for all the three analyses. With the exception of the Carpopcapsae clade, all the remaining clades were well supported, with bootstrap values $\geq 70\%$ for ML and MP, and posterior probability ≥ 0.93 for BI.

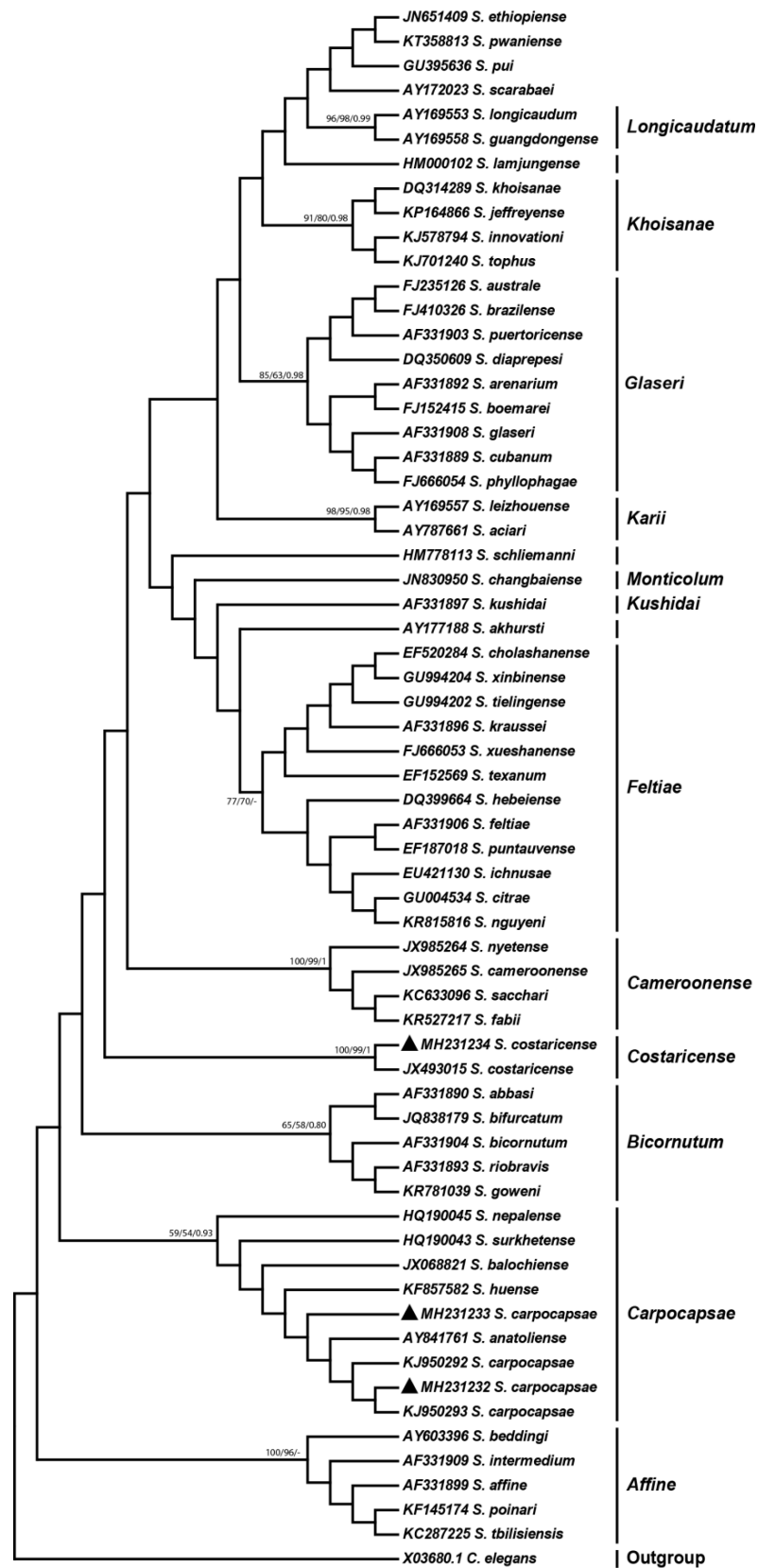


Figure 4. Phylogenetic inference for LSU rDNA locus including 63 *Steinernema* species clustered into 12 clades, as proposed by Hunt and Nguyen [62]. Triangles indicate the strains obtained in this study. From left to right: ML, MP, and Bayes scores. *Caenorhabditis elegans* used as outgroup.

For the ITS rDNA analyses, a total of 88 nucleotide sequences were considered, of which 85 belonged to the *Steinernema* species, 2 sequences were from the isolates found in this study (UNPR52 and UNPR73), and *C. elegans* was the outgroup. The final dataset contained 1691 positions, yielding a parsimonious tree of 7479 steps (C.I 0.28 and R.I 0.60). Highly congruent topologies were identified for all phylogenetic inferences (MP, ML, and BI; Figure 5), under bootstrapping resampling for most of the clades. The isolates UNPR52 and UNPR73 were clustered with *S. carpocapsae*, with high support for the branch tip (100% and 1.0) and at the *Steinernema* clade backbone (100% and 0.96). Unlike the LSU rDNA region, the ITS rDNA region revealed moderate ($\geq 75\%$) to strong (100%) support for the nodes containing the multispecies clades, which were Longicaudatum, Monticolum, Kushidai, Feltiae, Cameroonense, Bicornutum, Carpopcapsae, and Affine as the best-supported clades ($\geq 75\%$ for ML and MP and ≥ 0.68 for BI).

The tree topologies generated by these two loci demonstrated the consistency for the aforementioned clades, except for the Longicaudatum clade that was not supported by LSU rDNA. Additionally, the position of these clades, with respect to one another, was congruent with the topology proposed by Hunt and Nguyen [62], with superclade 2 (Feltiae, Kushidai, and Monticolum) and superclade 3 (Carpocapsae, Bicornutum, and Affine) being well-identified.

3.4. Concatenated Analysis for Ribosomal Loci in EPNs

A total of 51 sequences for the concatenated analysis comprised the LSU rDNA and ITS rDNA regions for the same isolates, where 48 belonged to *Steinernema* spp., two were the isolates UNPR52 and UNPR73, and one was the *C. elegans* outgroup. From the 2081 positions in the final dataset, a tree length of 5849 steps was obtained (C.I 0.41 and R.I 0.67). Eight clades were covered, five of which were highly congruent in tree topology [(Kushidai (two species), Feltiae (11), Cameroonense (4), Carpopcapsae (5), and Affine (4)], with bootstrap values $\geq 97\%$ and Bayesian posterior probabilities ≥ 0.61 .

The isolates that clustered into the Carpopcapsae clade generated a sub-cluster at the tip of the branches, being strongly supported by the bootstrap values (ML 100%, MP 99%, and BI 1.0; Figure 6). The tree comparison showed conflicting topologies for multispecies clades inferred from both the concatenated and individual datasets, where Glaseri, Khoisanae, and Karii showed unstable topologies. Additionally, the Bicornutum clade was conflicting only in the concatenated dataset, where the species were separately clustered into two clades, with one next to the other (i.e., sister clades). On the contrary, monophyletic clades were identified for either individual locus and/or concatenated loci, from weakly ($\geq 54\%$ LSU Carpopcapsae clade) to moderately ($\geq 70\%$ LSU rDNA Feltiae) to strongly ($\geq 99\%$ ITS rDNA and concatenated—Monticolum, Kushidai, Feltiae, Carpopcapsae, Cameroonense, and Affine) supported topologies. In general, ITS rDNA showed better resolution for tree topology, bootstrapping scores ($\geq 75\%$), and posterior probability values (≥ 0.68) for 10 (Khoisanae, Longicaudatum, Costaricense, Monticolum, Kushidai, Feltiae, Cameroonense, Bicornutum, Carpopcapsae, and Affine) of the 12 multispecies clades, as previously reported [62,74].

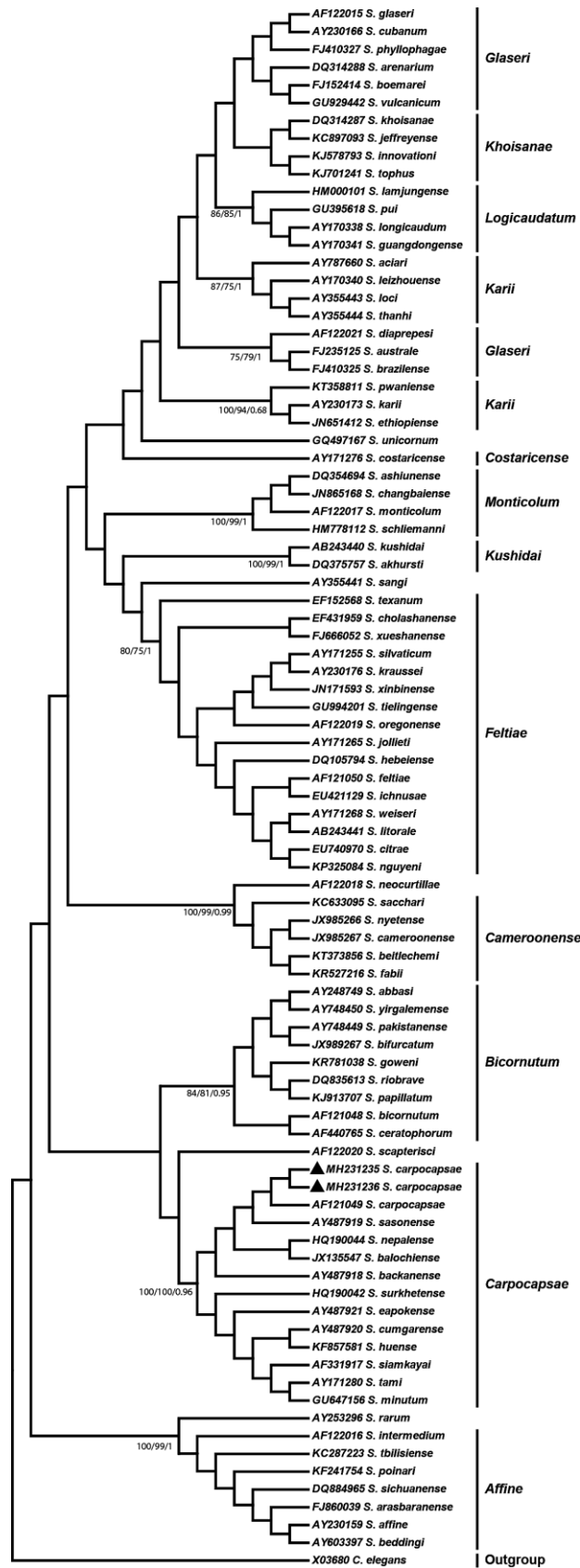


Figure 5. Phylogenetic inference for ITS rDNA locus including 87 *Steinernema* species clustered into 12 clades, as proposed by Hunt and Nguyen [62]. Triangles indicate the strains obtained in this study. From left to right: ML, MP, and Bayes scores. *Caenorhabditis elegans* used as outgroup.

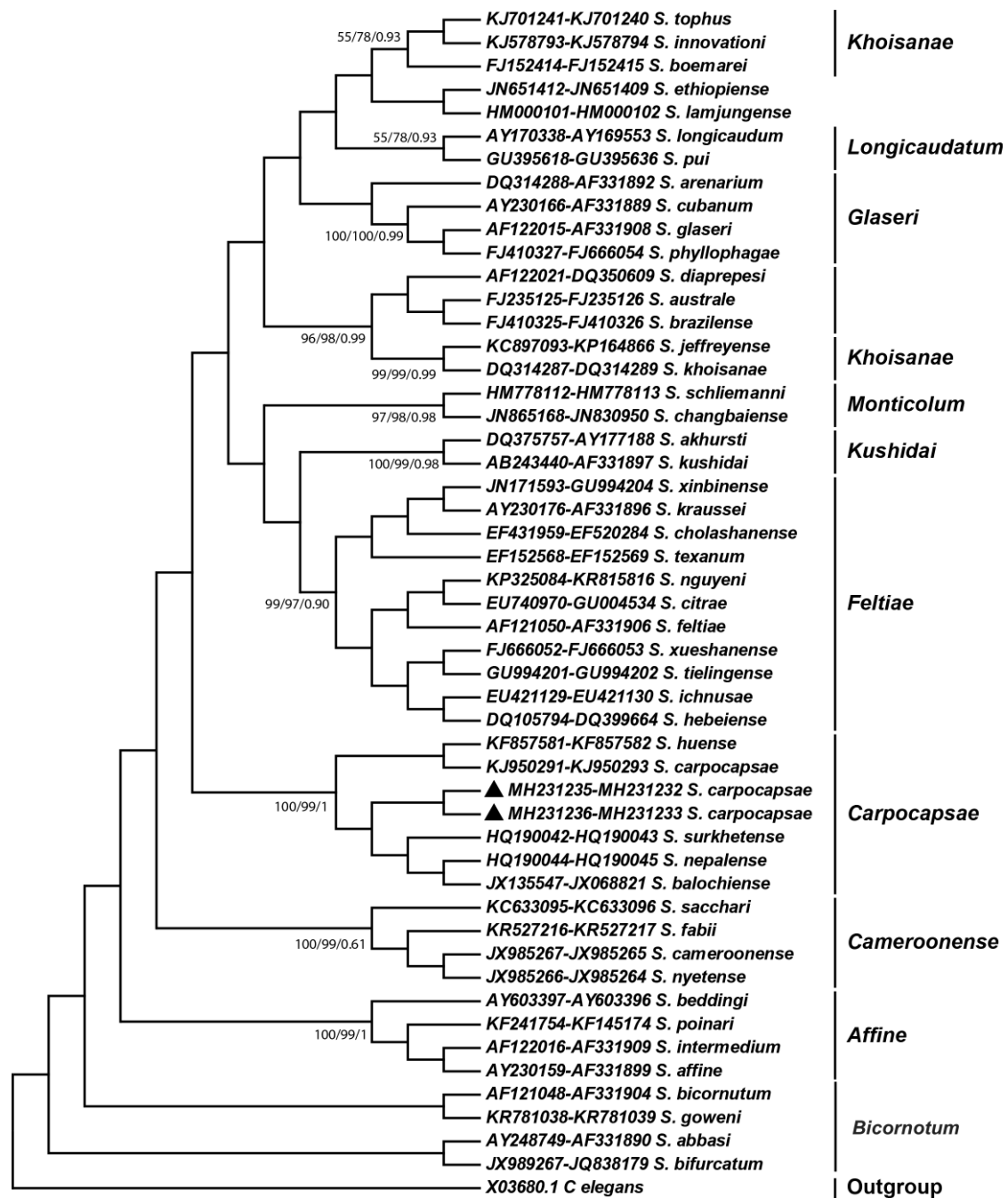


Figure 6. Phylogenetic inference for rDNA concatenated analysis (ITS–LSU) including 50 *Steineria* species clustered into 10 clades, as proposed by Hunt and Nguyen [62]. Triangles indicate the strains obtained in this study. From left to right on top of the main branches of each cluster: ML, MP, and Bayesian support scores. *Caenorhabditis elegans* was used as the outgroup.

3.5. Concatenated Analysis for Mitochondrial Loci in EPNs

Datasets for mitochondrial concatenated analysis included the 12S mtDNA and COI mtDNA. Twenty sequences included the final dataset, 17 from *Steineria* spp. retrieved from Hunt and Nguyen [62], two isolates UNPR53 and UNPR73, and the outgroup. This analysis comprised 1296 positions generating a tree length of 1585 steps (C.I 0.46 and R.I 0.50). MP, ML, and BI supported the relationships (100%, 100%, and 1.0, respectively) between our isolates and *S. carpocapsae* (Figure 7).

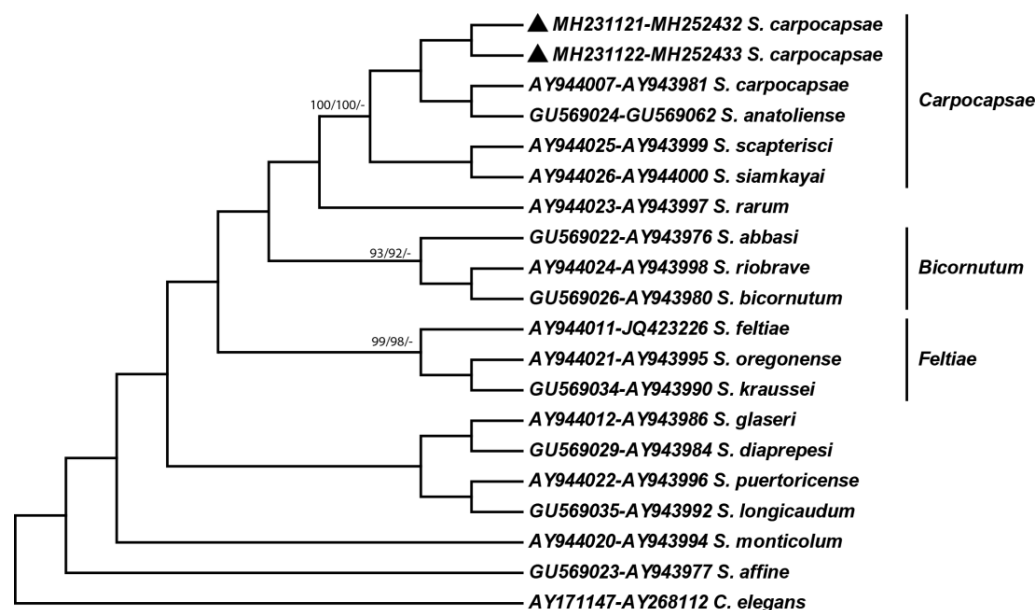


Figure 7. Phylogenetic inference for mtDNA concatenated analysis (COI–12S) including 19 *Steinerhema* species clustered into 3 clades, as proposed by Hunt and Nguyen [62]. Triangles indicate the strains obtained in this study. From left to right on top of the main branches of each cluster: ML, MP, and Bayesian support scores. *Caenorhabditis elegans* was used as the outgroup.

In general, all trees showed the same topology from weakly ($\geq 51\%$) to strongly ($\geq 93\%$) supported bootstrap values at the most recent nodes. For the Carpocapsae clade, four species (*S. carpocapsae*, *S. anatoliense*, *S. scapterisci*, and *S. siamkayai*) were considered to generate a monophyletic group with high bootstrap values and posterior probabilities support (100% and 1.0). Other monophyletic groups identified were Bicornutum and Feltiae clades, with three species each, from moderately (≥ 0.85) to strongly ($\geq 92\%$) supported nodes.

3.6. Nucleotide Diversity in EPN's Symbiotic Bacteria

Molecular characterization for the bacterial symbionts considered the whole 16S rDNA region. The isolates were coded the same way as their respective EPN sources: UNPB52, UNPB70, and UNPB73. Initial evaluations comprised 32 sequences belonging to 23 species plus the three isolated bacteria from the present study. A total of 1550 sites were included, of which 118 were polymorphic and 89 were parsimony-informative. Nucleotide composition corresponded to A 24.8%, C 23.1%, G 32.5%, and T 19.6%, with G + C content of 55.6%. Genetic distance reinforced the closeness between UNPB52 (accession number MH249989) and UNPB73 (accession number MH249990) with *Xenorhabdus nematophila* strain “DSM 3370” (accession number AY278674 [75] at 0.002, and with UNPB73 at 0.003). Additionally, only three nucleotide substitutions were identified between UNPB52 and UNPB73.

For the isolate UNPB70 (accession number MH249991), which belongs to the symbiotic bacteria isolated from *S. costaricense*, the genetic similarity was toward *X. koppenhoferi* strain “USNJ01” (accession number DQ205450, [76]) and *X. khoisanae* strain “SF362” (accession number JX623978 [77]), having 21 nucleotide substitutions for each comparison with a distance of 0.012. Overall comparisons between *Xenorhabdus* species showed 147 mutations in specific regions of the 16S rDNA, distributed mainly in three mutational hotspots at the 5' end of the sequence (from nucleotides 70 to 100 and from nucleotides 460 to 480) and at the 3' end of the sequence (from nucleotides 1000 to 1050).

3.7. Phylogenetic Analysis of EPN's Symbiont Bacteria

The phylogenetic analyses carried out for EPN's symbiotic bacteria 16S rDNA included 33 nucleotide sequences from the 16S rDNA locus covering 21 *Xenorhabdus* species, three isolates from the present study, and *Photorhabdus luminescens* strain DSM 3368 (X82248) [78] as the outgroup. From 1550 sites in the final dataset, 89 were parsimony-informative. A parsimonious tree was yielded with 609 steps (C.I 0.34 and R.I 0.62). Isolates UNPB52 (MH249989) and UNPB73 (MH249990), corresponding to the UNPR52 and UNPR73 EPN isolates, respectively, were grouped in the same cluster with *Xenorhabdus nematophila* under strong bootstrap support (96%) and Bayesian posterior probability (1.0), whereas the bacterial isolate UNPB70 (MH249991, corresponding to the EPN isolate UNPR70) was clustered with *X. koppenhoeferi* with very weak bootstrap support (40%) and Bayesian posterior probability (0.63). Tree comparisons showed instability for the most ancient nodes in each phylogenetic inference. Meanwhile, recent nodes showed monophyletic clades (*X. nematophila*, *X. beddingii*, *X. poinarii*, *X. bovienii*, and *X. japonica*) strongly supported for both bootstrap resampling ($\geq 96\%$) and Bayesian inference (≥ 0.96 ; Figure 8). The clustering of *X. nematophila* with the sequences from this study (UNPB52 and UNPB73) in all tree topologies suggests *S. carpocapsae* as the host nematode, thus clarifying the molecular taxonomic of these isolates.

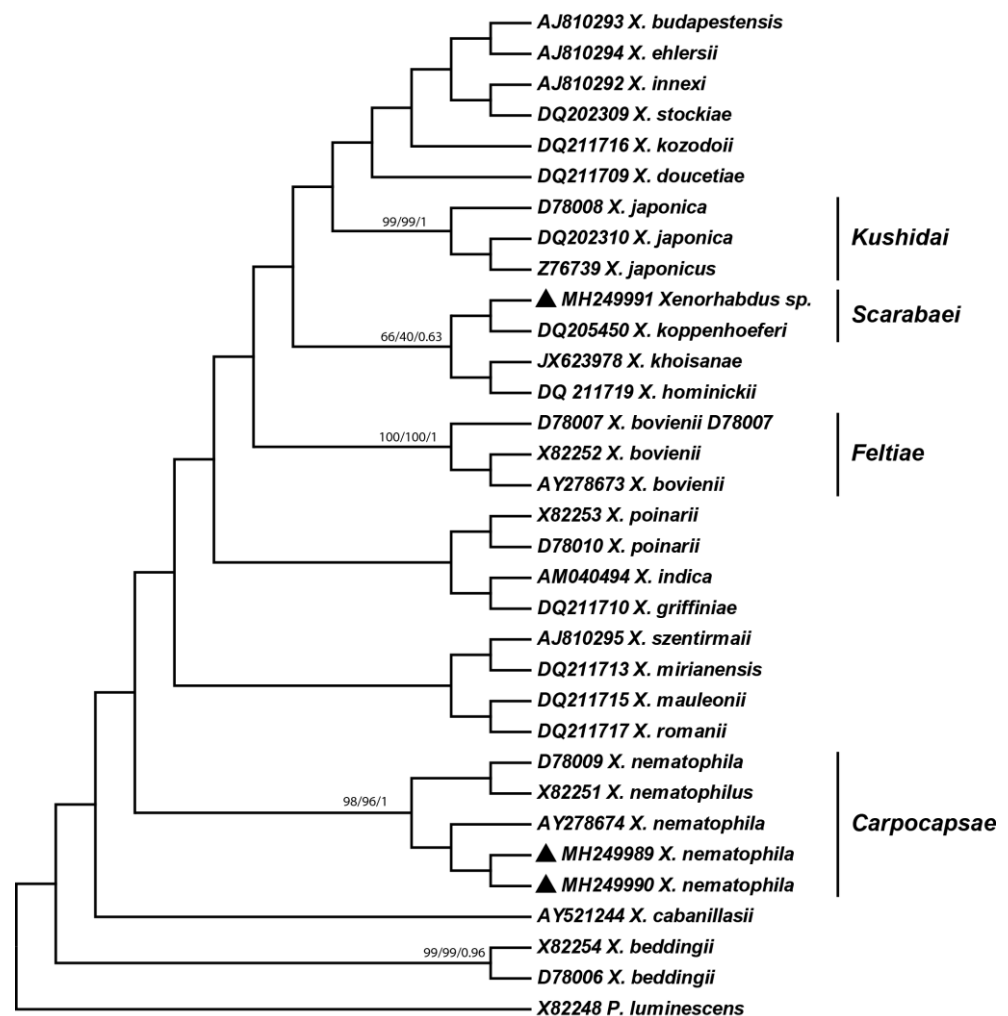


Figure 8. Phylogenetic inference for 16S rDNA including 25 *Xenorhabdus* species clustered into 4 clades following the clustering proposed for the EPN host [62]. Triangles indicate the strains obtained in this study. From left to right on top of the main branches of each cluster: ML, MP, and Bayesian support scores. *Photorhabdus luminescens* was used as the outgroup.

4. Discussion

The identification of native EPNs that are associated with the plantain and banana rhizosphere is of significant importance for biological control purposes and agricultural land use due to their adaptation to environmental conditions, soil pollution tolerance, and interaction with the native insect community, providing new insights into EPNs' efficiency. In the present study, three native EPNs (two *Steinernema carpocapsae* and one *S. costaricense*) could be recovered from soils that were destined for plantain and banana crop plantations. The low rate of EPN recovery (0.49%) could be influenced by several factors such as the high use of agrochemical pollutants in agricultural soils [27,79–82], soil sampling strategy, and failure of EPNs to parasitize insect hosts [83,84]. Laboratory assays showed differential pathogenicity responses of *S. carpocapsae* and *S. costaricense* isolates in parasitizing *G. mellonella* and weevil species (*C. sordidus* and *M. hemipterus* larval stages), with *S. carpocapsae* being the most pathogenic for all the insect larvae species evaluated, whereas *S. costaricense* failed to parasitize any of the larvae host species.

Several factors have been proposed to explain these differences in pathogenicity, including (1) the EPN body size, with *S. carpocapsae* being the smallest, which provides efficiency for host penetration [85]; (2) the pathogenicity of symbiotic bacteria types “swarming” and “stimulated” [86], where the former type has a more lethal activity and is present in the dauer juveniles, whilst the second type is a latent immobile stage that has a much-reduced lethal activity in response to environmental adversities [83]; and (3) the immune response of the host, such as the phagocytosis and humoral mechanisms against the symbiotic bacteria [42,83]. It was reported that *S. carpocapsae* possesses a higher rate of mortality on several species of Hemiptera [83], Coleoptera [27,39,87–89], Diptera [29,90], and Lepidoptera [28], which correlates with our experimental results where *S. carpocapsae* killed *G. mellonella*, *C. sordidus*, and *M. hemipterus* larvae in 48 h. However, *S. costaricense* could not parasitize after one round of infection. Shapiro-Ilan and Raymond [84] proposed that the main drawback of EPN reduction virulence is due to the need of serial propagation in vivo, which can accentuate competition and reduce the reproductive rate, leading to virulence instability. On the other hand, Adams et al. [91] argued that EPN virulence could be related to strategic dispersal, based on the host's dependence on mobility to reach other potential hosts, which is a strong selection pressure to reduce EPN virulence, while keeping the host alive long enough to provide resources for the life cycle.

In Colombia, the first report on EPN species, from forest and crop soils, was relatively recent [92]. Additionally, López-Núñez et al. [57] reported a new *Steinernema* species *S. colombiense*, which, nevertheless, was excluded from the analysis due to inconsistencies in the phylogenetic assignment, demonstrating the potential diversity of these organisms in the country. Several EPN strains were imported to Colombia for biological control strategies that unfortunately failed. Therefore, identifying native EPNs well adapted to specific environmental conditions may convey applications to a broad spectrum of crops in the region. For instance, *S. costaricense* was initially reported by Uribe-Lorío et al. [93] in forest soils from Costa Rica based on morphological and molecular characterizations (LSU rDNA). Later, Ivanova et al. [71] reported that the same species were isolated from deciduous woodland in Missouri, USA, based on morphology and molecular data (ITS rDNA).

Here, we report for the first time *S. costaricense* in Colombia, based on the LSU rDNA sequence isolated from agricultural soils, as well as its symbiotic bacteria based on molecular data (16S rDNA gene), showing close genetic similarity to *Xenorhabdus koppenhoeferi* [76] and *X. khoisanae* [77], while still being uncertain in its classification. Yet the identification of *S. costaricense* in South America indicates not only the wide range of this species in the Americas but also the ability to persist in different soil types, an ad hoc hypothesis that deserves further exploration. Also for the first time, EPN isolation is directed at plantain and banana crops as an alternative to identify the natural persistence of these entomopathogens and, eventually, to couple land agricultural practices with native organisms as potential biological control agents directed at insect pests.

Recently, similar approaches were carried out in the isolation of indigenous EPNs for pest control, targeting local adaption for biological control [27,89,94,95]. These agents provide promising results to reduce intense chemical pest management [89,96]. The isolation of environmentally adapted EPNs to cropland and, specifically, from plantain and banana cultures, is a feasible alternative in weevil-species-complex pest control, while mitigating soil, water, and environmental pollution. The strains obtained here and their symbionts showed a remarkable virulence against the two most common weevil pest species (*Cosmopolites sordidus* and *Metamasius hemipterus*), killing over 90% of the hosts (to be included in an oncoming report) and demonstrating their utility as biological control agents.

4.1. Polymorphism of Gene Barcodes in EPN and Their Bacterial Endosymbionts

Several genetic regions from eukaryotes were analyzed as potential sources of information for species identification [70,72,74,97–102]. These target barcode regions comprise nuclear ribosomal loci (ITS, SSU, and LSU) and mitochondrial genes (COI, 12S, and NAD). Among these, ribosomal loci represent a major tool for investigating and identifying nematodes species [72,74,89,95,99,101,103]. They also represent the main source of information in the databases, providing more reliability for species identification [74,99,101]. Among the ribosomal loci, the coding regions SSU and LSU play an important role in entomopathogenic nematodes' species validation and phylogenetic relationships, due to its conserved nature [74,99,101]. On the contrary, ITS is a rapidly evolving, presumably neutral, noncoding region that provides information about recent evolutionary events, which is useful for intra-species genetic diversity [72,74,99,104].

The patterns of genetic variation between EPN taxa demonstrate a high rate of nucleotide substitution in the ITS region compared to the LSU region across all the *Steinernema* species analyzed here. Nevertheless, very low genetic variation was observed at the intra-species level for all nuclear loci (ITS and LSU), even for the isolates retrieved by this study, despite the highly intervened soil conditions from which they were recovered. Entomopathogenic nematodes present a higher rate of evolution relative to other metazoans [72,74,105]. However, several factors could constrain certain genomic regions from evolving freely [99]. For rRNA loci, some gene variants have been detected as dominant in nematodes populations, which are often interpreted under purifying selection and concerted evolution [99,106]. Favored variants could in turn act against alternative versions as a mechanism to prevent rare rRNA alleles that could affect their function (e.g., structural modifications leading to folding errors in the secondary structure), maintaining in this way the fitness of the population [99]. This process may be reinforced by the pathogenicity nature of EPNs because virulence against several host species could favor slow evolution. After all, EPNs with a broad spectrum of hosts exhibit higher fitness to spread over populations and distant geographic regions. This scenario would, ultimately, maintain the gene flow over time, keeping the dominance of certain few allelic variants [74]. Given this situation, it is expected that most of the genetic variation may go undetected, limiting the quantification of biodiversity in EPNs. Most of the reported sequences for these taxa were obtained using Sanger sequencing technology, which summarizes the information from the large gene pools of PCR products enriched in abundant genomic rRNA copies, while being limited to specific barcodes [99]. Meanwhile, with the advance of sequencing technologies (high-throughput sequencing), it has been possible to detect small copy-number variants [107] in multiple eukaryotic genomes [106–111] and, particularly, in nematodes (i.e., *Caenorhabditis elegans*, [99]). Therefore, these technologies have offered a more precise approximation of EPN diversity, particularly for native species adapted to pollute environments. More recently, genomic studies on EPN [112–114], particularly in *S. carpocapsae*, found a correlation between non-coding RNA (ncRNA) and its functional role in the pathogenic lifestyle, which is completely absent in free-living nematodes [114]. These findings provide new insights into the molecular mechanisms underlying pathogenicity, as well as into the selective pressures targeting specific genomic regions such as rDNA loci, which could be under control of the gene expression by ncRNA or epigenetic factors.

The search for more precise species-identification genetic barcodes is mandatory because morphological traits are not completely satisfactory for EPN classification (e.g., *S. carpocapsae* [70]). This misclassification leads to a poor interpretation of the ecology and evolution in these complex taxa. Furthermore, the high copy number of the rDNA loci identified in nematodes [99] could distort the evolutionary hypothesis in phylogenetic studies, leading to incorrect species assignment [70]. This makes it difficult to report new strains with highly divergent rDNA sequence data, to the point that rare individuals may display a type of pre-adaptation to new environments [115] or host-associated habitats [116]. This case was identified in the present study with the *Steinernema colombiense* isolate SNI-0198 (NCBI accession number EU345420), originally reported by López-Núñez et al. [57] from a coffee plantation in Colombia. However, its correct classification remains uncertain, so it had to be removed from our analysis due to discrepancies in the phylogenies.

Analysis of the 16S rDNA sequences obtained from bacterial symbionts confirmed the species *Xenorhabdus nematophila* was associated with *S. carpocapsae*, whilst, for *S. costaricense*, it was not possible to determine its symbiont at the species level, being the closest taxa to *X. koppenhoeferi* (mutualistic to *S. scarabei*) and *X. khoisanae* (mutualistic to *S. khoisanae*, *S. jeffreyense*, and *S. saccharii*). *Steinernema costaricense* was first identified by Uribe-Lorío et al. [93], following a detailed morphological description and the sequencing of the LSU rDNA locus. However, its bacterial symbiont was not characterized. Later on, Stock and Blair [35] related *X. szentirmaii* as the mutualistic bacteria associated with *S. costaricense*; nevertheless, this association was not supported by other studies [34,74,117]. The isolation and identification of the EPN's bacterial symbionts has received special attention, not only for their entomopathogenic efficiency [27,29,31,33,39,89,94–96,118–122] but also for their potential applicability as antimicrobials [123], even targeting drug-resistant bacteria [34], their richness in metabolites with agricultural interest [32,124], and their abundance of peptides with functionality for cancer treatment [125].

Species identification based on the 16S rDNA locus has been challenging due to intra-genomic variable copy numbers, marker heterogeneity, and sequence variation within closely related taxa. Despite certain taxa harbor common 16S rDNA sequences from multiple species [58], genomes with more 16S rDNA copies are expected to carry diverse variants from unrelated origins (i.e., accounting for 2.4% [58] to 10% [126] of the copy number variants). In the present study, we did not analyze the presence of multiple 16S rDNA copy numbers in the EPN bacterial symbionts, since the similarity of this ribosomal locus between different species suggests that this gene may be coupled with multiple symbiotic interactions [74]. Espejo and Plaza [127] proposed the underlying effect of concerted evolution running at the intra- and inter-genomic levels (through horizontal gene transfer) in proteobacteria species (including endosymbionts), leading to rRNA locus homogenization, which in turn could confer advantages for environmental adaptations. Moreover, intergenomic recombination is favored and maintained within clonal populations, overcoming the mutation rate [73]. Multiple EPN species co-infecting the same host has been observed [128,129], providing close proximity in such way that possible horizontal gene transfer could be accomplished with posterior gene conversion, making the 16S rDNA locus still uncertain for species identification and diversity screening [126,127,130,131].

Similarly, microbiome studies carried out in *Steinernema* species demonstrated that several bacterial symbionts could be related with pathogenicity (not only mediated by *Xenorhabdus*) and distinctive antimicrobial activity [132], bringing a new perspective to the complexity of symbionts' interactions and the understanding of the pathobiome and its utility for pest control. Anyhow, native EPN isolates can provide a new source for pest control, directed to specific environmental conditions. For instance, the EPN isolates reported here showed high virulence (>90%) against three weevil species (*Cosmopolites sordidus*, *Metamasius hemipterus*, and *M. hebetatus*), which are persistent pests on plantain and banana plantations. EPN recoveries from polluted soils could be under strong selective pressures, favoring the dominance of specific alleles, not only in the EPN but also in the associated bacteria, which is critical for their adaptation and reproductive success.

4.2. Phylogenetic Inferences at Ribosomal Loci Enlightens Natural History of EPN

The phylogenetic studies carried out on EPN have proven to be conflicting since the application of several descriptors, morphological [133,134] and molecular [62,72–74,95,104,123], has provided alternative inferences to the understanding of EPN's natural history. Extensive exploration has focused on the search for the optimal loci applicable for species identification and delimitation. Since the studies of Szalanski et al. [135], who identified agreement between morphological and molecular phylogenies, further studies converged in the selection of the ITS and LSU ribosomal loci as the appropriate markers [70,72,95,104,116,136], currently denoted as DNA barcodes for EPN. Mitochondrial loci have tentatively been used as optional barcoding regions, yet significant inconsistencies with morphological and ribosomal hypotheses have limited the factual utilization of these loci. Against these expectations, the present study exhibited agreement of the mtDNA reconstruction with ribosomal phylogenies for Carpodapsae, Bicornutum, and Feltiae. Several drawbacks were identified for the mitochondrial genes, such as the reduced information available in the databases (making mtDNA markers less attractive for EPNs), their haplotypic-inheritance nature, and their high mutation rate. They may perform well at population levels to resolve recent evolutionary events, but they are suboptimal for deep phylogenetic studies, restricting the resolution power to infer a phylogenetic scenario due to marker saturation [137]. Due to this, the whole-genome mitochondrial sequencing of *S. carpocapsae* demonstrated genetic closeness to *Ascaris suum* and *Caenorhabditis elegans*, not supporting the phylogeny based on nuclear rDNA [138]. The present study covered the most important loci selected for EPN identification and phylogeny (nuclear and plastid DNA) to identify new EPN strains from the Neotropics.

Phylogenetic inferences provided stable tree topologies for the ITS and LSU loci at the clade level. The most comprehensive phylogeny for EPNs was provided by Hunt and Nguyen [62], in which all the extant species were included and analyzed through ribosomal loci. Following this hypothesis, our isolates were compared with this whole dataset grouping the Carpodapsae and Costaricense clades as well as the phylogeny obtained by their symbiotic bacteria, confirming the species obtained here (*S. carpocapsae* and *S. costaricense*, respectively). Despite the strong agreement and accuracy in species identification and delimitation provided by ITS and LSU, tree topologies were not completely consistent at the clade level [62,70,74]. This discrepancy was also identified in the present study, where Glaseri, Khoisane, and Bicornutum clades were the most unstable clades for both ribosomal loci. DNA sequence variability could be an important factor underlying these disagreements, since the high nucleotide variability found at ITS [74,139] is not of utility in resolving relationships at deep phylogenetic levels. However, it still may be useful in discriminating species within clades [62]. Previous studies reported the instability of tree topologies when analyzed with an ITS ribosomal locus [72]. The discrepancies related to the ITS locus were reported at the intra-individual level, where alternative haplotypes could be concurrently identified within a single genotype, increasing the differentiation of some species in the phylogenetic inferences [139]. In that sense, it is important to consider the sequencing of the ITS and LSU loci in order to achieve a more reliable phylogenetic reconstruction. At the supra clade level, our clusters were concordant with those reported by Hunt and Nguyen [62], in either the ITS, LSU or concatenated datasets, where superclades I (Glaceri, Karii, Longicaudatum, and Khoisanae) and II (Feltiae, Kushidai, and Monticolum) were in complete agreement, while superclade III (Carpocapsae and Bicornutum) genetically diverged from the others.

The limited resolution of the phylogenetic reconstruction for endosymbiotic bacteria through the 16S rDNA locus is already a well known fact [126,140]. In the present study, the endosymbionts of the Kushidai, Feltiae, and Carpodapsae clades were statistically supported ($\geq 98\%$ bootstrap posterior probability). However, most of the bacterial symbionts remain unresolved. This may be caused by several factors such as the host shift due to the dissociation of the endosymbiont and EPN in the insect cadaver, as a consequence of their amphimictic nature, leading to dissociation from the immediate ancestor [73].

Moreover, the spread of bacterial endosymbionts, naturally occurring via infected insects moving to new environments [121,140], or artificially occurring through the trade of commercially available strains for pest biocontrol, enables the spread of conservative traits that provide ecological advantages [73]. This may generate homogenization of the 16S rDNA variants via gene conversion by homologous recombination as a mechanism of concerted evolution [58,126], unleashing the observed complexity for the phylogenetic reconstruction of biotic interactions [141], including the EPNs' bacterial endosymbionts' evolutionary history. Ultimately, these effort to reconstruct the phylogenetic history of the species will prove essential to assist crop protection initiatives, prioritize multi-locality monitoring [142], envision modern plant enhancement strategies [143] for resistance using modern technique [144,145], and improve seed-delivery recommendations [146] together with commercial EPN, among other downstream steps.

5. Conclusions

- The identification of indigenous entomopathogenic nematodes in altered environments, such as agricultural soils, sources pest control for local conditions.
- The isolation and characterization of *Steinernema costaricense* is a key finding from our study, taking into account that there are only two reports worldwide, from Central and North America.
- Here, we report a new *S. costaricense* strain, which provides clues about the possible wide distribution of this species, not only in the Americas but also in highly intervened environments.
- Additionally, the molecular report of its bacterial symbiont was carried out, contributing to the knowledge about the understanding of specific interactions between EPNs and their bacterial endosymbionts.

6. Perspectives

- This study promotes the still incipient research of EPN in Colombia, where the real diversity of these organisms remains obscured.
- An extended sampling would convey more accuracy for comparisons with other studies. Still, EPNs' potential persists for new discoveries, applications for crop protection, and genetic resources from the bacterial endosymbionts that can provide metabolites with a wide spectrum of uses, for agricultural or medicinal purposes.
- New approaches should be considered in these organisms, such as next-generation technologies, in order to maximize their utility for pest control management that could be directed to specific phytosanitary issues.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/agronomy13051373/s1>. Table S1: Nucleotide codons and amino acid changes for COI mtDNA.

Author Contributions: J.M.L.-C. and M.U.-L. directed all field sampling and collected most of the soil samples for EPN detection. M.U.-L. performed all laboratory assays for EPN recovery and pathogenicity for every isolate in multiple host species, executed all microbiological methods for bacterial isolates, and prepared cultures for DNA extraction and sequencing. J.M.L.-C. and M.A.B.-B. performed all the molecular steps to obtain high-quality DNA amplicons and sequencing for both EPNs and bacterial symbionts. J.M.L.-C. and M.A.B.-B. analyzed all sequences data together with A.J.C., J.M.L.-C., M.A.B.-B. and A.J.C. performed the interpretation of the genetic results. J.M.L.-C., M.A.B.-B. and A.J.C. wrote the manuscript, with key suggestions from M.U.-L. and J.E.M.-F. All authors contributed to the overall interpretation and discussion of the results. J.E.M.-F., the director of the project, provided all the resources required to achieve the research and supervised the execution of the project. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the "Fondo de Ciencia Tecnología e Innovación" (FCTeI) of the "Sistema General de Regalías" (SGR), together with the "Ministerio del Medio Ambiente y Desarrollo Sostenible" and "Gobernación del Valle del Cauca", for the project "Desarrollo de Tecnologías Innovadoras para el Manejo Integrado de Plagas y Enfermedades Limitantes de Plátano y Banano en el

Valle del Cauca” (project number BPIN 2014000100014) awarded to J.E.M.-F., Elizabeth Álvarez, and their research teams. The Molecular Biology Lab on the Universidad Nacional de Colombia (UNAL), Palmira campus, provided the equipment and reagents for nematodes isolation, DNA extraction, PCR reactions, and data analysis. All members of the research team worked on the conceptualization, development, synthesis, or administration of the project.

Data Availability Statement: All sequence data are available in NCBI (<https://www.ncbi.nlm.nih.gov>) (accessed on 4 April 2018) under entry numbers MH231121, MH231122, MH231232, MH231233, MH231234, MH231235, MH231236, MH252432, and MH252433.

Acknowledgments: Special thanks go to the “Asociación de Productores de Plátano y Banano del Valle del Cauca” and all the farmers who kindly collaborated in the sampling. We thank Juan Gabriel Osorio for his key support during field sampling and sample transportation from all over the farms to the lab. We also acknowledge Jhon Brayan Valencia for assisting with the display of the geographical coverage of the sampling areas. Finally, we recognize the Molecular Biology Lab of the Universidad Nacional de Colombia, Palmira, for providing the materials and reagents required for making this research possible.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Varma, A.; Tripathi, S.; Prasad, R. *Plant Biotic Interactions*; Springer: Cham, Switzerland, 2019; ISBN 9783030266578.
2. Koppenhöfer, A.M.; Shapiro-Ilan, D.I.; Hiltbold, I. Entomopathogenic Nematodes in Sustainable Food Production. *Front. Sustain. Food Syst.* **2020**, *4*, 125. [[CrossRef](#)]
3. Arora, R.; Sandhu, S. *Breeding Insect Resistant Crops for Sustainable Agriculture*; Springer: Cham, Switzerland, 2017; ISBN 9789811060564.
4. FAO. *Emissions Due to Agriculture. Global, Regional and Country Trends 2000–2018. FAOSTAT Analytical Brief Series No 18*. Available online: <https://www.fao.org/policy-support/tools-and-publications/resources-details/en/c/1382716/> (accessed on 7 May 2023).
5. Molani, A.B.; Roa, V.N.; Maghuyop, M.A. *Advancing Banana and Plantain R & D in Asia and the Pacific*; International Plant Genetics Research Institute: Rome, Italy, 2000; ISBN 9719175133.
6. Ploetz, R.C.; Kepler, A.K.; Daniells, J.; Nelson, S.C. Banana and Plantain: An Overview with Emphasis on Pacific Island Cultivars. *Species Profiles Pac. Isl. Agrofor.* **2007**, *1*, 21–32.
7. Irish, B.M.; Cuevas, H.E.; Simpson, S.A.; Scheffler, B.E.; Sardos, J.; Ploetz, R.; Goenaga, R. *Musa* spp. germplasm management: Microsatellite fingerprinting of USDA-ARS national plant germplasm system collection. *Crop Sci.* **2014**, *54*, 2140–2151. [[CrossRef](#)]
8. Buitrago-Bitar, M.A.; Enríquez-Valencia, A.L.; Londoño-Caicedo, J.M.; Muñoz-Flórez, J.E.; Villegas-Estrada, B.; Santana-Fonseca, G.E. Molecular and morphological characterization of *Musa* spp. (Zingiberales: Musaceae) cultivars. *Bol. Cient. Cent. Mus.* **2020**, *24*, 33–47. [[CrossRef](#)]
9. FAO. *International Trade Banana: Market Review Preliminary Results 2021*; Banana Market Review; FAO: Rome, Italy, 2022; Volume 20.
10. Cubillos, J.P.T.; Soltész, B.; Vasa, L. Bananas, coffee and palm oil: The trade of agricultural commodities in the framework of the EU-Colombia free trade agreement. *PLoS ONE* **2021**, *16*, e0256242. [[CrossRef](#)]
11. Castrillon, C. *Distribucion de las Especies de Picudo del Platano Evaluacion de sus Entomopatogenos Nativos en el Departamento de Risaralda*; CORPOICA: Manizales, Colombia, 2000; p. 72.
12. Sepulveda-Cano, P.A.; Rubio-Gomez, J.D. Especies de dryophthorinae (Coleoptera: Curculionidae) asociadas a plátano y banano (*Musa* spp.) en Colombia. *Acta Biol. Colomb.* **2009**, *14*, 49–72.
13. Gold, C.; Messiaen, S. El picudo negro del banano *Cosmopolites Sordidus*. *Hoja Divulg.* **2000**, *4*, 1–4.
14. Shukla, A. Insect Pests of Banana with Special Reference to Weevil Borers. *Int. J. Plant. Prot.* **2010**, *3*, 387–393.
15. National, M.; Naturelle, D.H. *Bulletin du Muséum National d’Histoire Naturelle 1934*; Imprimerie Nationale; Masson et Cie/Masson et Cie; Muséum National D’histoire Naturelle/muséum National D’histoire Naturelle: Paris, France, 1934.
16. Zimmerman, E.C. Rhynchophorinae of Southeastern Polynesia (Coleoptera: Curculionidae). *Pac. Insects* **1968**, *10*, 47–77.
17. Gold, C.S.; Pena, J.E.; Karamura, E.B. Biology and integrated pest management for the banana weevil *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae). *Integr. Pest. Manag. Rev.* **2003**, *6*, 79–155. [[CrossRef](#)]
18. Gold, C.S.; Ragama, P.E.; Coe, R.; Rukazambuga, N.D.T.M. Selection of assessment methods for evaluating banana weevil *Cosmopolites sordidus* (Coleoptera: Curculionidae) damage on highland cooking banana (*Musa* spp., genome group AAA-EA). *Bull. Entomol. Res.* **2005**, *95*, 115–123. [[CrossRef](#)] [[PubMed](#)]
19. Tresson, P.; Tixier, P.; Puech, W.; Carval, D. The challenge of biological control of *Cosmopolites sordidus* Germar (Col. Curculionidae): A review. *J. Appl. Entomol.* **2021**, *145*, 171–181. [[CrossRef](#)]
20. Oliver, K.M.; Russell, J.A.; Moran, N.A.; Hunter, M.S. Facultative bacterial symbionts in aphids confer resistance to parasitic wasps. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 1803–1807. [[CrossRef](#)] [[PubMed](#)]

21. Ferrari, J.; Darby, A.C.; Daniell, T.J.; Godfray, H.C.J.; Douglas, A.E. Linking the bacterial community in pea aphids with host-plant use and natural enemy resistance. *Ecol. Entomol.* **2004**, *29*, 60–65. [[CrossRef](#)]
22. Martins, C.; Souza, R.F.; Bueno, O.C. Presence and distribution of the endosymbiont *Wolbachia* among *Solenopsis* spp. (Hymenoptera: Formicidae) from Brazil and its evolutionary history. *J. Invertebr. Pathol.* **2012**, *109*, 287–296. [[CrossRef](#)]
23. Cruz, C.; Segarra, A. Potential for Biological Control of Crop Pests in the Caribbean. *Fla. Entomol.* **1992**, *75*, 400–408. [[CrossRef](#)]
24. Akello, J.; Dubois, T.; Coyne, D.; Kyamanywa, S. Endophytic *Beauveria bassiana* in banana (*Musa* spp.) reduces banana weevil (*Cosmopolites sordidus*) fitness and damage. *Crop. Prot.* **2008**, *27*, 1437–1441. [[CrossRef](#)]
25. Bortoluzzi, L.; Alves, L.F.A.; Alves, V.S.; Holz, N. Entomopathogenic nematodes and their interaction with chemical insecticide aiming at the control of banana weevil borer, *Cosmopolites sordidus* Germar (Coleoptera: Curculionidae). *Arq. Inst. Biol.* **2013**, *80*, 183–192. [[CrossRef](#)]
26. Nurashikin-Khairuddin, W.; Abdul-Hamid, S.N.A.; Mansor, M.S.; Bharudin, I.; Othman, Z.; Jalinas, J.A. A Review of Entomopathogenic Nematodes as a Biological Control Agent for Red Palm Weevil, *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae). *Insects* **2022**, *13*, 245. [[CrossRef](#)]
27. Neira-Monsalve, E.; Wilches-Ramírez, N.C.; Terán, W.; del Pilar Márquez, M.; Mosquera-Espinosa, A.T.; Sáenz-Aponte, A. Isolation, identification, and pathogenicity of *Steinernema carpocapsae* and its bacterial symbiont in Cauca-Colombia. *J. Nematol.* **2021**, *52*, 1–16. [[CrossRef](#)]
28. Chongchitmate, P.; Somsook, V.; Hormchan, P.; Visarathanonth, N. Bionomics of entomopathogenic nematode *Steinernema siamkayai* Stock, Somsook and Reid (n. sp.) and its efficacy against *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae). *Kasetsart. J. Nat. Sci.* **2005**, *39*, 431–439.
29. Garriga, A.; Morton, A.; Ribes, A.; Garcia-del-Pino, F. Soil emergence of *Drosophila suzukii* adults: A susceptible period for entomopathogenic nematodes infection. *J. Pest. Sci. (2004)* **2020**, *93*, 639–646. [[CrossRef](#)]
30. Abd-Elgawad, M.M.M. Towards optimization of entomopathogenic nematodes for more service in the biological control of insect pests. *Egypt. J. Biol. Pest Control* **2019**, *29*, 77. [[CrossRef](#)]
31. Cao, M.; Goodrich-Blair, H. *Xenorhabdus nematophila* bacteria shift from mutualistic to virulent Lrp-dependent phenotypes within the receptacles of *Steinernema carpocapsae* insect-infective stage nematodes. *Environ. Microbiol.* **2020**, *22*, 5433–5449. [[CrossRef](#)] [[PubMed](#)]
32. Chacón-Orozco, J.G.; Bueno, C.J.; Shapiro-Ilan, D.I.; Hazir, S.; Leite, L.G.; Harakava, R. Antifungal activity of *Xenorhabdus* spp. and *Photorhabdus* spp. against the soybean pathogenic *Sclerotinia sclerotiorum*. *Sci. Rep.* **2020**, *10*, 20649. [[CrossRef](#)] [[PubMed](#)]
33. Incedayi, G.; Cimen, H.; Ulug, D.; Touray, M.; Bode, E.; Bode, H.B.; Yaylagul, E.O.; Hazir, S.; Cakmak, I. Relative potency of a novel acaricidal compound from *Xenorhabdus*, a bacterial genus mutualistically associated with entomopathogenic nematodes. *Sci Rep.* **2021**, *11*, 11253. [[CrossRef](#)]
34. Fodor, A.; Gualtieri, M.; Zeller, M.; Tarasco, E.; Klein, M.G.; Fodor, A.M.; Haynes, L.; Lengyel, K.; Forst, S.A.; Furgani, G.M.; et al. Type Strains of Entomopathogenic Nematode-Symbiotic Bacterium Species, *Xenorhabdus szentirmaii* (EMC) and *X. budapestensis* (EMA), Are Exceptional Sources of Non-Ribosomal Templated, Large-Target-Spectral, Thermotolerant-Antimicrobial Peptides (by Both), and Iodinin (by EMC). *Pathogens* **2022**, *11*, 342. [[CrossRef](#)]
35. Stock, S.P.; Blair, H.G. Entomopathogenic nematodes and their bacterial symbionts: The inside out of a mutualistic association. *Symbiosis* **2008**, *46*, 65–75.
36. Chang, D.Z.; Serra, L.; Lu, D.; Mortazavi, A.; Dillman, A.R. A core set of venom proteins is released by entomopathogenic nematodes in the genus *Steinernema*. *PLoS Pathog.* **2019**, *15*, e1007626. [[CrossRef](#)]
37. Theopold, U.; Dzierdzich, A.; Hyrsl, P. Special issue: Insects, nematodes, and their symbiotic bacteria. *Insects* **2020**, *11*, 577. [[CrossRef](#)]
38. Fenton, A.; Norman, R.; Fairbairn, J.P.; Hudson, P.J. Evaluating the efficacy of entomopathogenic nematodes for the biological control of crop pests: A nonequilibrium approach. *Am. Nat.* **2001**, *158*, 408–425. [[CrossRef](#)] [[PubMed](#)]
39. Sandhi, R.K.; Shapiro-Ilan, D.; Sharma, A.; Reddy, G.V.P. Efficacy of entomopathogenic nematodes against the sugarbeet wireworm, *Limoniuss californicus* (Mannerheim) (Coleoptera: Elateridae). *Biol. Control.* **2020**, *143*, 104190. [[CrossRef](#)]
40. Feyisa, B. Plant Pathology & Microbiology A Review on the Role of Entomopathogenic Nematodes (EPNs) in Control-ling Agricultural Insect Pest. *J. Plant Pathol. Microbiol.* **2021**, *12*, 583.
41. Poinar, J.G.O.; Leutenegger, R. Anatomy of the infective and normal third stage juveniles of *Steinernema carpocapsae* Weiser (Steinernematidae: Nematoda). *J. Parasitol.* **1968**, *54*, 340–350. [[CrossRef](#)]
42. Ows, B.; Peters, A. Virulence mechanisms. In *Entomopathogenic Nematodes: Systematics, Phylogeny and Bacterial Symbionts*, 5th ed.; Brill: Gainsville, FL, USA, 2002. [[CrossRef](#)]
43. Morton, A.; García del Pino, F. Effectiveness of different species of entomopathogenic nematodes for biocontrol of the Mediterranean flatheaded rootborer, *Capnodis tenebrionis* (Linné) (Coleoptera: Buprestidae) in potted peach tree. *J. Invertebr. Pathol.* **2008**, *97*, 128–133. [[CrossRef](#)]
44. Lacey, L.A.; Grzywacz, D.; Shapiro-Ilan, D.I.; Frutos, R.; Brownbridge, M.; Goettel, M.S. Insect pathogens as biological control agents: Back to the future. *J. Invertebr. Pathol.* **2015**, *132*, 1–41. [[CrossRef](#)]

45. Abate, B.A.; Slippers, B.; Wingfield, M.J.; Conlong, D.E.; Burger, D.A.; Hurley, B.P. Virulence and survival of native entomopathogenic nematodes for the management of white grubs in South Africa. *Biol. Control* **2019**, *137*, 104043. [[CrossRef](#)]
46. Sun, B.; Zhang, X.; Song, L.; Zheng, L.; Wei, X.; Gu, X.; Cui, Y.; Hu, B.; Yoshiga, T.; Abd-Elgawad, M.M.; et al. Evaluation of indigenous entomopathogenic nematodes in Southwest China as potential biocontrol agents against *Spodoptera litura* (Lepidoptera: Noctuidae). *J. Nematol.* **2021**, *53*, 1–17. [[CrossRef](#)]
47. Uribe-Londoño, M.; Muñoz-Florez, J.; Riascos-Ortíz, D. *Nematodos Entomopatígenos: Una Alternativa para el Control de los Picudos del Plátano*; Primera ed. Palmira Valle Del Cauca; Universidad Nacional de Colombia: Palmira, Colombia, 2020; Volume 1.
48. Uribe-Londoño, M.; Muñoz-Florez, J.; Riascos-Ortíz, D. *Los Nematodos: Microorganismos con Múltiples Funciones en los Cultivos*; Primera ed. Palmira Valle Del Cauca; Universidad Nacional de Colombia: Palmira, Colombia, 2020; Volume 1.
49. Solarte-Quintero, A.; Muñoz-Florez, J.; Riascos-Ortíz, D. *Picudos del Plátano y Banano: Cosmopolites sordidus, Metamasius hemipterus, Metamasius hebetatus, Metamasius submaculatus y Polytus mellerborgii*; Primera ed. Palmira Valle Del Cauca; Universidad Nacional de Colombia: Palmira, Colombia, 2020; Volume 1.
50. Angulo-Angulo, W.; Osorio, J.; Muñoz-Florez, J.; Riascos-Ortíz, D. *Evaluación de Plagas y su Impacto Sobre la Producción de Plátano y Banano*; Primera ed. Palmira Valle Del Cauca; Universidad Nacional de Colombia: Palmira, Colombia, 2020; Volume 1.
51. Angulo-Angulo, W.; Osorio, J.; Muñoz-Florez, J.; Riascos-Ortíz, D. *Monitoreo y Captura de Picudos del Plátano y Banano*; Primera ed. Palmira Valle Del Cauca; Universidad Nacional de Colombia: Palmira, Colombia, 2020; Volume 1.
52. Bedding, R.A.; Akhurst, R.J. A simple technique for the detection of insect *Paristic rhabditid* nematodes in soil. *Nematologica* **1975**, *21*, 109–110. [[CrossRef](#)]
53. White, G.F. A method for obtaining infective nematode larvae from cultures. *Science* **1927**, *66*, 302–303. [[CrossRef](#)]
54. Orozco, R.A.; Lee, M.M.; Patricia Stock, S. Soil sampling and isolation of Entomopathogenic nematodes (Steinernematidae, Heterorhabditidae). *J. Vis. Exp.* **2014**, *89*, e52083. [[CrossRef](#)]
55. Stock, S.P.; Goodrich-Blair, H. Nematode parasites, pathogens and associates of insects and invertebrates of economic importance. In *Manual of Techniques in Invertebrate Pathology*, 2nd ed.; Lacey, L.A., Ed.; Academic Press: Cambridge, MA, USA, 2012; pp. 373–426. [[CrossRef](#)]
56. Schmitz, A.; Riesner, D. Purification of nucleic acids by selective precipitation with polyethylene glycol 6000. *Anal. Biochem.* **2006**, *354*, 311–313. [[CrossRef](#)] [[PubMed](#)]
57. López-Núñez, J.C.; Plichta, K.; Góngora-Botero, C.E.; Stock, P.S. A new entomopathogenic nematode, *Steinernema colombiense* n. sp. (Nematoda: Steinernematidae), from Colombia. *Nematology* **2008**, *10*, 561–574. [[CrossRef](#)]
58. Větrovský, T.; Baldrian, P. The Variability of the 16S rRNA Gene in Bacterial Genomes and Its Consequences for Bacterial Community Analyses. *PLoS ONE* **2013**, *8*, e57923. [[CrossRef](#)] [[PubMed](#)]
59. Gürtler, V.; Stanisich, V.A. New Approaches to Typing and Identification of Bacteria Using the 16S-23S rDNA Spacer Region. *Microbiology* **1996**, *142*, 3–16. [[CrossRef](#)]
60. Kearse, M.; Moir, R.; Wilson, A.; Stones-Havas, S.; Cheung, M.; Sturrock, S.; Buxton, S.; Cooper, A.; Markowitz, S.; Duran, C.; et al. Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* **2012**, *28*, 1647–1649. [[CrossRef](#)]
61. Hall, T.A. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp. Ser.* **1999**, *41*, 95–98.
62. Hunt, D.J.; Nguyen, K.B. *Advances in Entomopathogenic Nematode Taxonomy and Phylogeny*; Brill: Boston, MA, USA, 2016; Volume 12. [[CrossRef](#)]
63. Kumar, S.; Stecher, G.; Tamura, K. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Mol. Biol. Evol.* **2016**, *33*, 1870–1874. [[CrossRef](#)]
64. Tavaré. Some probabilistic and statistical problems in the analysis of DNA sequences. *Lect. Math Life Sci. (Am. Math Soc.)* **1986**, *17*, 57–86.
65. Shoemaker, J.S.; Fitch, W.M. Evidence from nuclear sequences that invariable sites should be considered when sequence divergence is calculated. *Mol. Biol. Evol.* **1989**, *6*, 270–289. [[CrossRef](#)]
66. Yang, Z.; Goldman, N.; Friday, A. Comparison of models for nucleotide substitution used in maximum-likelihood phylogenetic estimation. *Mol. Biol. Evol.* **1994**, *11*, 316–324. [[CrossRef](#)]
67. Ronquist, F.; Teslenko, M.; Van Der Mark, P.; Ayres, D.L.; Darling, A.; Höhna, S.; Larget, B.; Liu, L.; Suchard, M.A.; Huelsenbeck, J.P. MrBayes 3.2: Efficient bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* **2012**, *61*, 539–542. [[CrossRef](#)] [[PubMed](#)]
68. Nadler, S.A.; Bolotin, E.; Stock, S.P. Phylogenetic relationships of *Steinernema travassos*, 1927 (Nematoda: Cephalobina: Steinernematidae) based on nuclear, mitochondrial and morphological data. *Syst. Parasitol.* **2006**, *63*, 161–181. [[CrossRef](#)] [[PubMed](#)]
69. Librado, P.; Rozas, J. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* **2009**, *25*, 1451–1452. [[CrossRef](#)]
70. Mráček, Z.; Půža, V.; Nermut, J. Addendum to the description of *Steinernema carpocapsae* (Weiser, 1955) Wouts, Mráček, Gerdin & Bedding, 1982. *Russ. J. Nematol.* **2014**, *22*, 102–120.
71. Ivanova, E.S.; Shepeleva, N.S.; Spiridonov, S.E. Morphological and molecular characterisation of *Steinernema costaricense* Uribe-Lorío, Mora & Stock, 2007 (Panagrolaimorpha: Steinernematidae) isolate from Bush Augusta State Park, Missouri, USA. *Syst. Parasitol.* **2013**, *85*, 219–234. [[CrossRef](#)]

72. Nguyen, K.B.; Maruniak, J.; Adams, B. Diagnostic and Phylogenetic Utility of the rDNA Internal Transcribed Spacer Sequences of *Steinernema*. *J. Nematol.* **2001**, *33*, 73–82.
73. Lee, M.M.; Stock, S.P. A multilocus approach to assessing co-evolutionary relationships between *Steinernema* spp. (Nematoda: Steinernematidae) and their bacterial symbionts *Xenorhabdus* spp. (γ -Proteobacteria: Enterobacteriaceae). *Syst. Parasitol.* **2010**, *77*, 1–12. [[CrossRef](#)]
74. Nguyen, K.; Hunt, D. *Entomopathogenic Nematodes: Systematics, Phylogeny and Bacterial Symbionts*; Brill: Leiden, The Netherlands, 2010. [[CrossRef](#)]
75. Marokhazi, J.; Waterfield, N.; LeGoff, G.; Feil, E.; Stabler, R.; Hinds, J.; Fodor, A.; French-Constant, R.H. Using a DNA microarray to investigate the distribution of insect virulence factors in strains of *Photorhabdus* bacteria. *J. Bacteriol.* **2003**, *185*, 4648–4656. [[CrossRef](#)]
76. Tailliez, P.; Pagès, S.; Ginibre, N.; Boemare, N. New insight into diversity in the genus *Xenorhabdus*, including the description of ten novel species. *Int. J. Syst. Evol. Microbiol.* **2006**, *56*, 2805–2818. [[CrossRef](#)]
77. Ferreira, T.; van Reenen, C.A.; Endo, A.; Spröer, C.; Malan, A.P.; Dicks, L.M.T. Description of *Xenorhabdus khoisanae* sp. nov., the symbiont of the entomopathogenic nematode *Steinernema khoisanae*. *Int. J. Syst. Evol. Microbiol.* **2013**, *63*, 3220–3224. [[CrossRef](#)] [[PubMed](#)]
78. Rainey, F.A.; Stackebrandt, E. Inability of the Polyphasic Approach to Systematics to Determine the Inability of the Polyphasic Approach to Systematics to Determine the Relatedness of the Genera *Xenorhabdus* and *Photorhabdus*. *Int. J. Syst. Evol. Microbiol.* **1995**, *45*, 379–381. [[CrossRef](#)]
79. Campos-Herrera, R.; Pathak, E.; El-Borai, F.E.; Stuart, R.J.; Gutiérrez, C.; Rodríguez-Martín, J.A.; Graham, J.H.; Duncan, L.W. Geospatial patterns of soil properties and the biological control potential of entomopathogenic nematodes in *Florida citrus* groves. *Soil Biol. Biochem.* **2013**, *66*, 163–174. [[CrossRef](#)]
80. Campos-Herrera, R.; El-Borai, F.E.; Ebert, T.E.; Schumann, A.; Duncan, L.W. Management to control citrus greening alters the soil food web and severity of a pest-disease complex. *Biol. Control.* **2014**, *76*, 41–51. [[CrossRef](#)]
81. Valadas, V.; Laranjo, M.; Mota, M.; Oliveira, S. A survey of entomopathogenic nematode species in continental Portugal. *J. Helminthol.* **2014**, *88*, 327–341. [[CrossRef](#)]
82. De Brida, A.L.; Rosa, J.M.O.; Oliveira, C.M.G.D.; Serrão, J.E.; Zanuncio, J.C.; Leite, L.G.; Wilcken, S.R.S. Entomopathogenic nematodes in agricultural areas in Brazil. *Sci. Rep.* **2017**, *7*, 45254. [[CrossRef](#)]
83. Berkvens, N.; Van Vaerenbergh, J.; Maes, M.; Beliën, T.; Viaene, N. Entomopathogenic nematodes fail to parasitize the woolly apple aphid *Eriosoma lanigerum* as their symbiotic bacteria are suppressed. *J. Appl. Entomol.* **2014**, *138*, 644–655. [[CrossRef](#)]
84. Shapiro-Ilan, D.; Raymond, B. Limiting opportunities for cheating stabilizes virulence in insect parasitic nematodes. *Evol. Appl.* **2016**, *9*, 462–470. [[CrossRef](#)]
85. Nguyen, K. Methodology, morphology and identification. In *Entomopathogenic Nematodes: Systematics, phylogeny and bacterial symbionts*, 5th ed.; Nguyen, K., Hunt, D., Koninklijke Brill, N., Eds.; Brill: Gainsville, FL, USA, 2007; pp. 59–119.
86. Hirao, A.; Ehlers, R.U. Influence of cell density and phase variants of bacterial symbionts (*Xenorhabdus* spp.) on dauer juvenile recovery and development of biocontrol nematodes *Steinernema carpocapsae* and *S. feltiae* (Nematoda: Rhabditida). *Appl. Microbiol. Biotechnol.* **2009**, *84*, 77–85. [[CrossRef](#)]
87. Sankaranarayanan, C.; Somasejhar, N.; Singaravelu, B. Biocontrol Potential of Entomopathogenic Nematodes Heterorhabditis and Steinernema against Pupae and Adults of White grub *Holotrichia serrata* F. *Sugar Tech.* **2006**, *8*, 2–5. [[CrossRef](#)]
88. Williams, C.D.; Dillon, A.B.; Ennis, D.; Hennessy, R.; Griffin, C.T. Differential susceptibility of pine weevil, *Hyllobius abietis* (Coleoptera: Curculionidae), larvae and pupae to entomopathogenic nematodes and death of adults infected as pupae. *BioControl* **2015**, *60*, 537–546. [[CrossRef](#)]
89. Torrini, G.; Paoli, F.; Mazza, G.; Simoncini, S.; Benvenuti, C.; Strangi, A.; Tarasco, E.; Barzanti, G.P.; Bosio, G.; Cutino, I.; et al. Evaluation of indigenous entomopathogenic nematodes as potential biocontrol agents against *Popillia japonica* (Coleoptera: Scarabaeidae) in Northern Italy. *Insects* **2020**, *11*, 804. [[CrossRef](#)] [[PubMed](#)]
90. Karagoz, M.; Gulcu, B.; Hazir, C.; Kaya, H.K.; Hazir, S. Biological control potential of Turkish entomopathogenic nematodes against the Mediterranean fruit fly *Ceratitis capitata*. *Phytoparasitica* **2009**, *37*, 153–159. [[CrossRef](#)]
91. Adams, B.; Peat, S.; Dillman, A. Phylogeny and evolution. In *Molecular Parasitology*; Springer: Vienna, Austria, 2016; pp. 693–733. [[CrossRef](#)]
92. López-Núñez, J.C.; Cano, L.; Góngora-BCE; Stock, S.P. Diversity and evolutionary relationships of entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) from the Central Andean region of Colombia. *Nematology* **2007**, *9*, 333–341. [[CrossRef](#)]
93. Uribe-Lorío, L.; Mora, M.; Stock, S.P. *Steinernema costaricense* n. sp. and *S. puntauvense* n. sp. (Rhabditida: Steinernematidae), two new entomopathogenic nematodes from Costa Rica. *Syst. Parasitol.* **2007**, *68*, 167–182. [[CrossRef](#)]

94. Mokrini, F.; Laasli, S.E.; Benseddik, Y.; Joutei, A.B.; Blenzar, A.; Lakhal, H.; Sbaghi, M.; Imren, M.; Özer, G.; Paulitz, T.; et al. Potential of Moroccan entomopathogenic nematodes for the control of the Mediterranean fruit fly *Ceratitis capitata* Wiedemann (Diptera: Tephritidae). *Sci. Rep.* **2020**, *10*, 19204. [[CrossRef](#)]
95. Flores, P.; Alvarado, A.; Lankin, G.; Lax, P.; Prodan, S.; Aballay, E. Morphological, molecular and ecological characterization of a native isolate of *Steinernema feltiae* (Rhabditida: Steinernematidae) from southern Chile. *Parasites Vectors* **2021**, *14*, 45. [[CrossRef](#)]
96. Bruno, P.; Machado, R.A.; Glauser, G.; Köhler, A.; Campos-Herrera, R.; Bernal, J.; Toepfer, S.; Erb, M.; Robert, C.A.; Arce, C.C.; et al. Entomopathogenic nematodes from Mexico that can overcome the resistance mechanisms of the western corn rootworm. *Sci. Rep.* **2020**, *10*, 8257. [[CrossRef](#)]
97. Hebert, P.D.N.; Cywinska, A.; Ball, S.L.; deWaard, J.R. Biological identifications through DNA barcodes. *Proc. Biol. Sci.* **2003**, *270*, 313–321. [[CrossRef](#)]
98. Hurst, G.D.; Jiggins, F.M. Problems with mitochondrial DNA as a marker in population, phylogeographic and phylogenetic studies: The effects of inherited symbionts. *Proc. R. Soc. B Biol. Sci.* **2005**, *272*, 1525–1534. [[CrossRef](#)]
99. Bik, H.M.; Fournier, D.; Sung, W.; Bergeron, R.D.; Thomas, W.K. Intra-Genomic Variation in the Ribosomal Repeats of Nematodes. *PLoS ONE* **2013**, *8*, e78230. [[CrossRef](#)] [[PubMed](#)]
100. Sharma, P.; Kobayashi, T. Are “universal” DNA primers really universal? *J. Appl. Genet.* **2014**, *55*, 485–496. [[CrossRef](#)] [[PubMed](#)]
101. Avó, A.P.; Daniell, T.J.; Neilson, R.; Oliveira, S.; Branco, J.; Adão, H. DNA barcoding and morphological identification of benthic nematodes assemblages of estuarine intertidal sediments: Advances in molecular tools for biodiversity assessment. *Front. Mar. Sci.* **2017**, *4*, 66. [[CrossRef](#)]
102. Bolaños, A.C.; Bononi, V.L.R.; Londoño, J.M.; Castillo, A.; Vitali, V.M.; Gugliotta, A.D.M.; Muñoz, J.E. Detecting Manganese Peroxidase (MnP) Gene in *Ganoderma* Species. *Cryptogamie* **2018**, *39*, 325–340. [[CrossRef](#)]
103. Bhat, A.H.; Chaubey, A.K.; Askary, T.H. Global distribution of entomopathogenic nematodes, *Steinernema* and *Heterorhabditis*. *Egypt. J. Biol. Pest Control* **2020**, *30*, 31. [[CrossRef](#)]
104. Stock, S.P.; Campbell, J.F.; Nadler, S.A. Phylogeny of *Steinernema travassos*, 1927 (Cephalobina: Steinernematidae) inferred from ribosomal DNA sequences and morphological characters. *J. Parasitol.* **2001**, *87*, 877–889. [[CrossRef](#)] [[PubMed](#)]
105. Wray, G.A.; Levinton, J.S.; Shapiro, L.H. Molecular evidence for deep Precambrian divergences among metazoan phyla. *Science* **1996**, *274*, 568–581. [[CrossRef](#)]
106. Averbeck, K.T.; Eickbush, T.H. Monitoring the mode and tempo of concerted evolution in the *Drosophila melanogaster* rDNA locus. *Genetics* **2005**, *171*, 1837–1846. [[CrossRef](#)]
107. Ganley, A.R.D.; Kobayashi, T. Highly efficient concerted evolution in the ribosomal DNA repeats: Total rDNA repeat variation revealed by whole-genome shotgun sequence data. *Genome Res.* **2007**, *17*, 184–191. [[CrossRef](#)]
108. Long, E.; Dawid, B. *Drosophila* of Ribosomal *melanogaster* DNA Insertions in *Drosophila melanogaster*. *Cell* **1979**, *18*, 1185–1196. [[CrossRef](#)]
109. Keller, I.; Chintauan-Marquier, I.C.; Veltsos, P.; Nichols, R.A. Ribosomal DNA in the grasshopper *Podisma pedestris*: Escape from concerted evolution. *Genetics* **2006**, *174*, 863–874. [[CrossRef](#)] [[PubMed](#)]
110. Eickbush, T.H.; Eickbush, D.G. Finely orchestrated movements: Evolution of the ribosomal RNA genes. *Genetics* **2007**, *175*, 477–485. [[CrossRef](#)] [[PubMed](#)]
111. McTaggart, S.J.; Dudycha, J.L.; Omilian, A.; Crease, T.J. Rates of recombination in the ribosomal DNA of apomictically propagated *Daphnia obtusa* lines. *Genetics* **2007**, *175*, 311–320. [[CrossRef](#)] [[PubMed](#)]
112. Bai, X.; Adams, B.J.; Ciche, T.A.; Clifton, S.; Gaugler, R.; Kim, K.S.; Spieth, J.; Sternberg, P.W.; Wilson, R.K.; Grewal, P.S. A Lover and a Fighter: The Genome Sequence of an Entomopathogenic Nematode *Heterorhabditis bacteriophora*. *PLoS ONE* **2013**, *8*, e69618. [[CrossRef](#)] [[PubMed](#)]
113. Dillman, A.R.; Macchietto, M.; Porter, C.F.; Rogers, A.; Williams, B.; Antoshechkin, I.; Lee, M.M.; Goodwin, Z.; Lu, X.; Lewis, E.E.; et al. Comparative genomics of *Steinernema* reveals deeply conserved gene regulatory networks. *Genome Biol.* **2015**, *16*, 9–11. [[CrossRef](#)] [[PubMed](#)]
114. Rougon-Cardoso, A.; Flores-Ponce, M.; Ramos-Aboites, H.E.; Martínez-Guerrero, C.E.; Hao, Y.J.; Cunha, L.; Rodríguez-Martínez, J.A.; Ovando-Vázquez, C.; Bermúdez-Barrientos, J.R.; Abreu-Goodger, C.; et al. The genome, transcriptome, and proteome of the nematode *Steinernema carpocapsae*: Evolutionary signatures of a pathogenic lifestyle. *Sci. Rep.* **2016**, *6*, 37536. [[CrossRef](#)]
115. Hayden, E.J.; Ferrada, E.; Wagner, A. Cryptic genetic variation promotes rapid evolutionary adaptation in an RNA enzyme. *Nature* **2011**, *474*, 92–95. [[CrossRef](#)]
116. Rooney, A.P. Mechanisms underlying the evolution and maintenance of functionally heterogeneous 18S rRNA genes in apicomplexans. *Mol. Biol. Evol.* **2004**, *21*, 1704–1711. [[CrossRef](#)]
117. Gualtieri, M.; Ogier, J.C.; Pagès, S.; Givaudan, A.; Gaudriault, S. Draft genome sequence and annotation of the entomopathogenic bacterium *Xenorhabdus szentirmaii* strain DSM16338. *Genome Announc.* **2014**, *2*, 14–16. [[CrossRef](#)]
118. Kour, S.; Khurma, U.; Brodie, G.; Hazir, S. Natural occurrence and distribution of entomopathogenic nematodes (Steinernematidae, Heterorhabditidae) in Viti Levu, Fiji Islands. *J. Nematol.* **2020**, *52*, 1–17. [[CrossRef](#)]

119. Sajnaga, E.; Kazimierczak, W. Evolution and taxonomy of nematode-associated entomopathogenic bacteria of the genera *Xenorhabdus* and *Photorhabdus*: An overview. *Symbiosis* **2020**, *80*, 1–13. [[CrossRef](#)]
120. Santhi, V.S.; Ment, D.; Faigenboim, A.; Salame, L.; Soroker, V.; Hetzroni, A.; Glazer, I. Behavioral and molecular response of the insect parasitic nematode *Steinernema carpocapsae* to cues emitted by a host, the red palm weevil, *Rhynchophorus ferrugineus*. *Mol. Biochem. Parasitol.* **2021**, *241*, 111345. [[CrossRef](#)] [[PubMed](#)]
121. Skowronek, M.; Sajnaga, E.; Pleszczyńska, M.; Kazimierczak, W.; Lis, M.; Wiater, A. Bacteria from the midgut of common cockchafer (*Melolontha melolontha* L.) larvae exhibiting antagonistic activity against bacterial symbionts of entomopathogenic nematodes: Isolation and molecular identification. *Int. J. Mol. Sci.* **2020**, *21*, 580. [[CrossRef](#)]
122. Dichusa, C.A.; Ramos, R.; Aryal, S.; Sumaya, N.P.D.; Sumaya, N.H. Survey and identification of entomopathogenic nematodes in the province of Cotabato, Philippines, for biocontrol potential against the tobacco cutworm, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae). *Egypt. J. Biol. Pest Control* **2021**, *31*, 60. [[CrossRef](#)]
123. Abate, B.A.; Slippers, B.; Wingfield, M.J.; Malan, A.P.; Hurley, B.P. Diversity of entomopathogenic nematodes and their symbiotic bacteria in south African plantations and indigenous forests. *Nematology* **2018**, *20*, 355–371. [[CrossRef](#)]
124. Böszörményi, E.; Érsek, T.; Fodor, A.; Fodor, A.M.; Földes, L.S.; Hevesi, M.; Hogan, J.S.; Katona, Z.; Klein, M.G.; Kormány, A.; et al. Isolation and activity of xenorhabdus antimicrobial compounds against the plant pathogens *Erwinia amylovora* and phytophthora nicotianae. *J. Appl. Microbiol.* **2009**, *107*, 746–759. [[CrossRef](#)]
125. Ohlendorf, B.; Simon, S.; Wiese, J.; Imhoff, J.F. Szentiamide, an N-formylated cyclic depsipeptide from *Xenorhabdus szentirmaii* DSM 16338T. *Nat. Prod. Commun.* **2011**, *6*, 1247–1250. [[CrossRef](#)] [[PubMed](#)]
126. Pei, A.Y.; Oberdorf, W.E.; Nossa, C.W.; Agarwal, A.; Chokshi, P.; Gerz, E.A.; Jin, Z.; Lee, P.; Yang, L.; Poles, M.; et al. Diversity of 16S rRNA genes within individual prokaryotic genomes. *Appl. Environ. Microbiol.* **2010**, *76*, 3886–3897. [[CrossRef](#)] [[PubMed](#)]
127. Espejo, R.T.; Plaza, N. Multiple Ribosomal RNA operons in bacteria, Their concerted evolution and potential consequences on the rate of evolution of their 16S rRNA. *Front. Microbiol.* **2018**, *9*, 1–6. [[CrossRef](#)]
128. Aydın, H.; Susurluk, A. Competitive abilities of the entomopathogenic nematodes *Steinernema feltiae* and *Heterorhabditis bacteriophora* in the same host at different temperatures. *Turk. J. Biol.* **2005**, *29*, 35–39.
129. O’Callaghan, K.M.; Zenner, A.N.R.L.; Hartley, C.J.; Griffin, C.T. Interference competition in entomopathogenic nematodes: Male *Steinernema* kill members of their own and other species. *Int. J. Parasitol.* **2014**, *44*, 1009–1017. [[CrossRef](#)] [[PubMed](#)]
130. Jaspers, E.; Overmann, J. Ecological significance of microdiversity: Identical 16S rRNA gene sequences can be found in bacteria with highly divergent genomes and ecophysologies. *Appl. Environ. Microbiol.* **2004**, *70*, 4831–4839. [[CrossRef](#)] [[PubMed](#)]
131. Caporaso, J.G.; Lauber, C.L.; Walters, W.A.; Berg-Lyons, D.; Lozupone, C.A.; Turnbaugh, P.J.; Fierer, N.; Knight, R. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 4516–4522. [[CrossRef](#)] [[PubMed](#)]
132. Ogier, J.C.; Pagès, S.; Frayssinet, M.; Gaudriault, S. Entomopathogenic nematode-associated microbiota: From monoxenic paradigm to pathobiome. *Microbiome* **2020**, *8*, 25. [[CrossRef](#)] [[PubMed](#)]
133. Wright, P.J. Morphological characterisation of the entomogenous nematodes *Steinernema* spp. and *Heterorhabditis* spp. (Nematoda: Rhabditida). *New Zeal. J. Zool.* **1990**, *17*, 577–585. [[CrossRef](#)]
134. Ngugi, C.N.; Haukeland, S.; Wachira, P.M.; Mbaka, J.N.; Okoth, S. Morphometrics and Morphological Characterization of Entomopathogenic Nematode Isolate Tk1 from Kenya. *Int. J. Agric. Environ. Biores.* **2019**, *4*, 80–90.
135. Szalanski, A.L.; Taylor, D.B.; Mullin, P.G. Assessing nuclear and mitochondrial DNA sequence variation within *Steinernema* (Rhabditida: Steinernematidae). *J. Nematol.* **2000**, *32*, 229–233.
136. Chaerani, R.; Stock, S.P. Morphological and molecular characterisation of *Steinernema hermaphroditum* n. sp. (Nematoda: Steinernematidae), an entomopathogenic nematode from Indonesia, and its phylogenetic relationships with other members of the genus. *Nematology* **2004**, *6*, 401–412. [[CrossRef](#)]
137. Nadler, S.A.; De Ley, P.; Mundo-Ocampo, M.; Smythe, A.B.; Patricia Stock, S.; Bumbarger, D.; Adams, B.J.; De Ley, I.T.; Holovachov, O.; Baldwin, J.G. Phylogeny of Cephalobina (Nematoda): Molecular evidence for recurrent evolution of probolae and incongruence with traditional classifications. *Mol. Phylogenet. Evol.* **2006**, *40*, 696–711. [[CrossRef](#)]
138. Montiel, R.; Lucena, M.A.; Medeiros, J.; Simões, N. The complete mitochondrial genome of the entomopathogenic nematode *Steinernema carpocapsae*: Insights into nematode mitochondrial DNA evolution and phylogeny. *J. Mol. Evol.* **2006**, *62*, 211–225. [[CrossRef](#)]
139. Půža, V.; Chundelová, D.; Nermuť, J.; Žurovcová, M.; Mráček, Z. Intra-individual variability of ITS regions in entomopathogenic nematodes (Steinernematidae: Nematoda): Implications for their taxonomy. *BioControl* **2015**, *60*, 547–554. [[CrossRef](#)]
140. Maneesakorn, P.; An, R.; Daneshvar, H.; Taylor, K.; Bai, X.; Adams, B.J.; Grewal, P.S.; Chandrapatya, A. Phylogenetic and cophylogenetic relationships of entomopathogenic nematodes (*Heterorhabditis*: Rhabditida) and their symbiotic bacteria (*Photorhabdus*: Enterobacteriaceae). *Mol. Phylogenet. Evol.* **2011**, *59*, 271–280. [[CrossRef](#)] [[PubMed](#)]

141. Guevara-Escudero, M.; Osorio, A.N.; Cortés, A.J. Integrative Pre-Breeding for Biotic Resistance in Forest Trees. *Plants* **2021**, *10*, 2022. [[CrossRef](#)] [[PubMed](#)]
142. Burbano-Erazo, E.; León-Pacheco, R.I.; Cordero-Cordero, C.C.; López-Hernández, F.; Cortés, A.J.; Tofiño-Rivera, A.P. Multi-Environment Yield Components in Advanced Common Bean (*Phaseolus vulgaris* L.) × Tepary Bean (*P. acutifolius* A. Gray) Interspecific Lines for Heat and Drought Tolerance. *Agronomy* **2021**, *11*, 1978. [[CrossRef](#)]
143. Arenas, S.; Cortés, A.J.; Mastretta-Yanes, A.; Jaramillo-Correa, J.P. Evaluating the Accuracy of Genomic Prediction for the Management and Conservation of Relictual Natural Tree Populations. *Tree Genet. Genomes* **2021**, *17*, 12. [[CrossRef](#)]
144. Cortés, A.J.; López-Hernández, F. Harnessing Crop Wild Diversity for Climate Change Adaptation. *Genes* **2021**, *12*, 783. [[CrossRef](#)]
145. Aggarwal, S.; Gupta, S.; Gupta, D.; Gulzar, Y.; Juneja, S.; Alwan, A.A.; Nauman, A. An Artificial Intelligence-Based Stacked Ensemble Approach for Prediction of Protein Subcellular Localization in Confocal Microscopy Images. *Sustainability* **2023**, *15*, 1695. [[CrossRef](#)]
146. Peláz, D.; Aguilar, P.A.; Mercado, M.; López-Hernández, F.; Guzmán, M.; Burbano-Erazo, E.; Denning-James, K.; Medina, C.I.; Blair, M.W.; De Vega, J.J.; et al. Genotype Selection, and Seed Uniformity and Multiplication to Ensure Common Bean (*Phaseolus vulgaris* L.) var. Liborino. *Agronomy* **2022**, *12*, 2285. [[CrossRef](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.