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Molecular Characterization and First Report of Entomopathogenic Nematodes from Carob Forests in Tumbes, Peru

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Abstract

Given their ability to attack and eliminate insects that impact various ecosystems, entomopathogenic nematodes (EPNs) are considered effective biological pest control agents. However, there are few studies focused on their identification or their association with forest species such as the carob tree. The objective of the present study was to isolate and molecularly identify entomopathogenic nematodes from carob tree (*Neltuma* spp.) forests in Tumbes, Peru. One-kilogram soil samples were collected from ten localities located across four districts in the Tumbes region. In each soil sample, *Galleria mellonella* Linnaeus 1758 (Lepidoptera: Pyralidae) larvae were exposed as bait for the detection and isolation of EPNs. Exposure times were controlled at 12, 24, 48, 72, and 84-96 hours. The isolated nematodes were morphologically characterized, and their genomic DNA was extracted. The 18S ribosomal DNA (rDNA) gene was amplified via polymerase chain reaction (PCR). Six of the ten evaluated soils showed *G. mellonella* infection, with a positive correlation between exposure time and infection rate. However, due to marked infectivity divergences (where samples 02 and 06 exhibited the highest percentages), three representative strains were selectively isolated from soil samples 02, 05, and 06. Subsequent morphological characterization and molecular identification determined their taxonomic identity as *Heterorhabditis bacteriophora* and *Heterorhabditis indica.* This finding represents the first report of EPNs associated with carob tree plantations in northern Peru and highlights their pathogenic potential against insect larvae. It is recommended that the nematodes identified at the species level be considered in integrated pest management (IPM) programs.

Keywords: biological control, dry forest, *Heterorhabditis* spp., integrated pest management, rDNA sequencing

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Caracterización molecular y primer informe de nematodos entomopatógenos en bosques de algarrobos en Tumbes, Perú

Resumen

Dada su capacidad para atacar y eliminar insectos que impactan diversos ecosistemas, los nematodos entomopatógenos (EPN) son considerados agentes eficaces de control biológico de plagas. Sin embargo, existen pocos estudios centrados en su identificación o su asociación con especies forestales como el algarrobo. El objetivo del presente estudio fue aislar e identificar molecularmente nematodos entomopatógenos de bosques de algarrobo (*Neltuma* spp.) en Tumbes, Perú. Se recolectaron muestras de suelo de un kilogramo en diez localidades distribuidas en cuatro distritos de la región de Tumbes. En cada muestra de suelo, se expusieron larvas de *Galleria mellonella* Linnaeus 1758 (Lepidoptera: Pyralidae) como cebo para la detección y el aislamiento de los EPN. Los tiempos de exposición se controlaron a las 12, 24, 48, 72 y 84-96 horas. Los nematodos aislados se caracterizaron morfológicamente y se extrajo su ADN genómico. El gen del ADN ribosomal 18S (rADN) se amplificó mediante la reacción en cadena de la polimerasa (PCR). Seis de los diez suelos evaluados presentaron infección por *G. mellonella*, mostrando una correlación positiva entre el tiempo de exposición y la tasa de infección. No obstante, debido a marcadas divergencias de infectividad (donde las muestras 02 y 06 exhibieron los mayores porcentajes), se aislaron selectivamente tres cepas representativas a partir de las muestras de suelo 02, 05 y 06. La posterior caracterización morfológica e identificación molecular determinó su identidad taxonómica como *Heterorhabditis bacteriophora* e *Heterorhabditis indica.* Este hallazgo representa el primer reporte de EPN asociados a plantaciones de algarrobo en el norte del Perú y resalta su potencial patogénico contra larvas de insectos. Se recomienda que los nematodos identificados a nivel de especie sean considerados en los programas de manejo integrado de plagas (MIP).

Palabras clave: control biológico, bosque seco, *Heterorhabditis* spp., manejo integrado de plagas, secuenciación de ADNr

Caracterização molecular e primeiro relato de nematoides entomopatogênicos em florestas de alfarrobeira em Tumbes, Peru

Resumo

Dada sua capacidade de atacar e eliminar insetos que impactam diversos ecossistemas, os nematoides entomopatogênicos (EPNs) são considerados agentes eficazes de controle biológico de pragas. No entanto, há poucos estudos focados em sua identificação ou associação com espécies florestais, como a alfarrobeira. O objetivo do presente estudo foi isolar e identificar molecularmente nematoides entomopatogênicos de florestas de alfarrobeiras (*Neltuma* spp.) em Tumbes, Peru. Amostras de solo de um quilograma foram coletadas de dez localidades distribuídas em quatro distritos da região de tumbes. Em cada amostra de solo, larvas de *Galleria mellonella* Linnaeus 1758 (Lepidoptera: Pyralidae) foram expostas como isca para a detecção e isolamento de EPNs. Os tempos de exposição foram controlados entre 12, 24, 48, 72 e 84–96 horas. Os nematoides isolados foram caracterizados morfologicamente, e seu DNA genômico foi extraído. O gene do DNA ribossomal 18S (rDNA) foi amplificado por reação em cadeia da polimerase (PCR). Seis dos dez solos avaliados apresentaram infecção por *G. mellonella*, mostrando uma correlação positiva entre o tempo de exposição e a taxa de infecção. No entanto, devido a marcantes divergências de infectividade (onde as amostras 02 e 06 exibiram as maiores porcentagens) três cepas representativas foram isoladas seletivamente a partir das amostras de solo 02, 05 e 06. A posterior caracterização morfológica e identificação molecular determinou sua identidade taxonômica como *Heterorhabditis bacteriophora* e *Heterorhabditis indica*. Essa descoberta representa o primeiro relato de EPNs associadas a plantações de alfarrobeiras no norte do Peru e destaca seu potencial patogênico contra larvas de insetos. Recomenda-se que os nematoides identificados no nível da espécie sejam considerados em programas de manejo integrado de pragas (MIP).

Palavras-chave: controle biológico, floresta seca, *Heterorhabditis* spp., manejo integrado de pragas, sequenciamento de rDNA

1. Introduction

The continuous use of chemical pesticides in agricultural systems not only promotes resistance in pests but also leads to adverse effects on human health, environmental contamination, and negative impacts on natural enemies, potentially resulting in ecological imbalance (Abd-Elgawad, 2025; Ruiz et al., 2021). In response to this issue, the use of living organisms such as fungi, bacteria, viruses, and parasites including entomopathogenic nematodes (EPNs) has emerged as an effective alternative for the biological control of insect pests (Pacheco Hernández et al., 2019; Tarasco et al., 2023). Although EPN-based products have recently achieved significant progress in their application, their market remains largely limited to a small number of agricultural crops (Abd-Elgawad, 2025).

EPNs have been recognized for several decades as promising biological control agents for the management of insect pests such as *Lycoriella auripilla*, *Bradysia coprophila*, *Agrotis* spp., *Amathes* spp., and *Prodenia* spp. in both agricultural and natural ecosystems (Lacey & Georgis, 2012; Shapiro-Ilan et al., 2016; Sharghi et al., 2025). These nematodes primarily belong to the families Heterorhabditidae and Steinernematidae, and they maintain a mutualistic relationship with entomopathogenic bacteria: *Photorhabdus* spp. in Heterorhabditids and *Xenorhabdus* spp. in Steinernematids. Thanks to this symbiosis, EPNs are capable of killing their insect hosts within 24 to 48 hours, making them valuable tools for integrated pest management (Půža & Machado, 2024; Sharghi et al., 2025). However, one of the main limitations to their commercial use is their short shelf life during storage (Sharghi et al., 2025).

In northern Peru, the carob tree (*Neltuma* spp. (Fabaceae)), formerly known as *Prosopis pallida* (Humb. & Bonpl. ex Willd.) Kunth (Fabaceae), is recognized as a representative species of the equatorial dry forest (Cruzado-Jacinto et al., 2019; Núñez Gamboa, 2022). Its ecological importance lies in its role as a habitat for a wide range of biological diversity, including natural enemies such as predators, parasitoids, and entomopathogenic nematodes. However, little is known about the diversity, distribution, and ecological functions of native EPNs associated with *Neltuma* forests in this region. This lack of information limits our understanding of their potential roles in natural pest regulation and their applicability in sustainable pest management programs.

Based on the foregoing and to update knowledge about the ecological characteristics of carob forests in northern Peru, specifically in the Tumbes region, the present study was conducted. This work is significant because it uses molecular techniques to identify native EPN species, enabling their genetic characterization and the selection of strains with high potential for use in biological control strategies against insect pests, a matter of both national and international concern.

2. Materials and Methods

2.1 Design and Study Site

A non-experimental, descriptive-level, qualitative, and cross-sectional study was conducted, based on the exploratory search for entomopathogenic nematodes (EPNs) in carob tree forests across 10 locations situated in the districts of Corrales, Pampas de Hospital, San Juan de la Virgen, and San Jacinto, in the department of Tumbes (Figure 1).

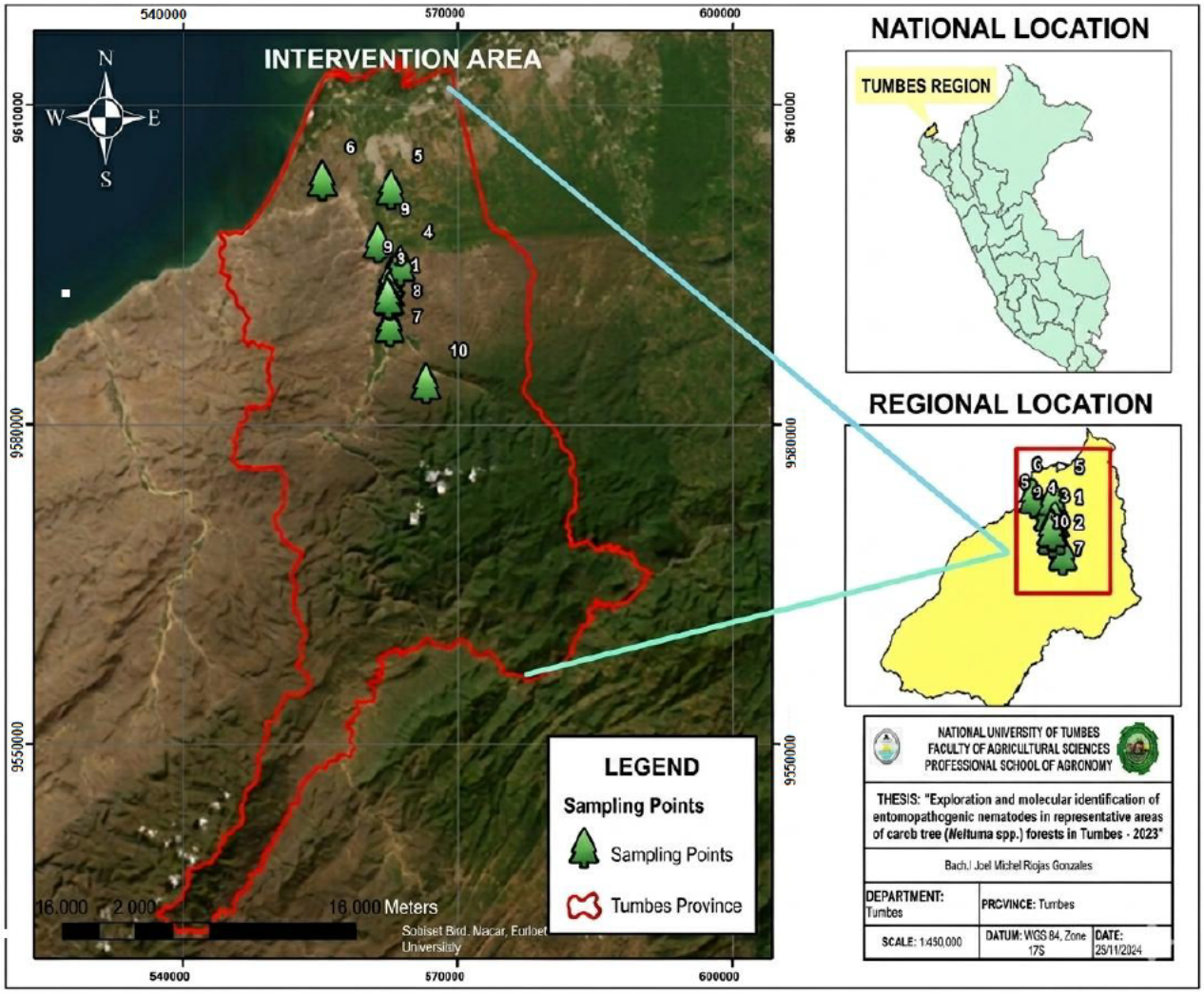

Figure 1. Geographic location of the sampling points

2.2 Sampling

On March 24, 2024, 10 soil samples were collected from carob tree forests in each locality. Sampling was performed by excavating pits measuring 20 × 20 in surface area and 30 cm in depth, located 30 cm away from the trunk of each tree, using a straight shovel previously disinfected with a 3% sodium hypochlorite solution. The soil was then placed on a 1 m² plastic sheet, and representative subsamples were selected using the quartering method. After collection, the soil samples were homogenized and 1 kg was placed in a polyethylene bag. Sampling was performed considering proximity to water sources, as these provide favorable conditions for the presence of EPNs. Each bag was labeled with location, geographic coordinates, and collection date, then stored in a cooler with gel packs to maintain storage conditions during transportation to the Genetic Resources Laboratory of the Agrarian Experimental Station (EEA) of the National Institute of Agricultural Innovation (INIA), where the samples were kept and analyzed.

2.3 Rearing of *G. mellonella*

In order to obtain an adequate number of larvae of *G. mellonella* Linnaeus, 1756 (Lepidoptera: Pyralidae) for the isolation and maintenance of EPNs, 100 g of larvae (F1 generation) were obtained as a donation from the Entomological Museum of the Faculty of Agricultural Sciences (FCA) of the National University of Tumbes. Subsequently, egg masses corresponding to an F2 generation were obtained, which were placed in disposable plastic containers covered with thin black cloth, held with elastic bands, and kept at 26 °C for 10 days. Once the eggs hatched and the larvae emerged, they were fed *Triticum aestivum* flour, *Zea mays*, and *Avena sativa* mixed with honey for 35 days until they reached the fifth instar (L5), at which point they were used in traps.

2.4 *G. mellonella* Infection and Isolation of EPNs

The EPNs were isolated using the insect bait technique, which consisted of exposing 500 g of soil in a plastic container with 10 fifth-instar *G. mellonella* larvae to be infected. Exposure times were monitored at 12, 24, 48, 72, and 84-96 hours. The containers were inverted to encourage larval movement and infection by EPNs present in the soil (Ali et al., 2023; Sturhan et al., 2005). All soil samples were kept at room temperature in the dark for 10 days. Larvae with signs of infection (flaccidity and reddish coloration) were selected and placed in plastic containers. The dead larvae were then washed with 0.5% sodium hypochlorite and placed in white traps (White, 1927), which were stored in the dark for 10 to 12 days. The appearance of EPNs was recorded (Kaya, 1985; Stock & Hunt, 2005).

Subsequently, a purification process was conducted by multiplying a single EPN collected from dead *G. mellonella* larvae using a tuberculin syringe. The collected EPN was inoculated into a new larva of *G. mellonella*. This process was repeated 5 times over a 4-month period to obtain sufficient purified EPNs for further analysis.

2.5 Morphological Characterization of EPNs

After isolation, morphological characteristics were evaluated using the taxonomic keys proposed by Rizvi (2010), Eliceche (2019), Grifaldo-Alcantara et al. (2020), and Nguyen and Hunt (2007). The nematodes were observed under a microscope at 100× magnification.

2.6 DNA Extraction from EPNs

Pools of EPN strains previously characterized morphologically were used for genomic DNA extraction following the manufacturer's instructions provided with the PowerSoil DNeasy® Kit (QIAGEN, Germany).

2.7 PCR Targeting the 18S rDNA Gene

The 18S rDNA gene was amplified using primers described by Blaxter et al. (1998) (SSU18A: AAAGATTAA-GCCATGCATG and SSU26R: CATTCTTGGCAAATGCTTTCG), the product has a size of ~1000 base pairs. The final reaction volume was 25 µL, consisting of 15.4 µL of ultrapure water, 2.5 µL of 10X buffer, 1.5 µL of $MgCl_2$ (25 mM), 0.5 µL of dNTPs, 0.5 µL of each primer (SSU18A and SSU26R), 0.1 µL of Taq DNA polymerase, and 4 µL of DNA template. Thermal cycling conditions included an initial denaturation at 95 °C for 5 minutes, followed by 35 cycles of denaturation at 94 °C for 1 minute, annealing at 52 °C for 1 minute and 30 seconds, and extension at 68 °C for 2 minutes, with a final extension at 68 °C for 10 minutes, and holding at 4 °C for up to 24 hours. A negative control was included to prevent contamination.

2.8 DNA Electrophoresis

PCR products (amplicons) were visualized by electrophoresis on a 1.5% agarose gel in 1X TAE buffer (Tris-Acetate-EDTA) stained with ethidium bromide at 0.5 ng/mL. For electrophoresis, 4 µL of 6X DNA loading dye, 8 µL of amplicon, and 2 µL of 1 kb molecular weight marker were used. Electrophoresis was conducted at 70 volts for 30 minutes.

2.9 DNA Sequencing

The PCR amplicons were sent to the biotechnology company Macrogen (https://dna.macrogen.com/) for DNA sequencing using the double-stranded Sanger sequencing method. The resulting sequences were delivered in FASTA format for analysis.

2.10 Molecular Identification

The DNA sequences obtained from the sequenced amplicons were analyzed using the Molecular Evolutionary Genetics Analysis software version 11 (MEGA, 2021), where reverse consensus sequences were generated from the forward and reverse strands of each strain's amplicon. Each consensus sequence was then analyzed using the Basic Local Alignment Search Tool (BLAST) (National Center for Biotechnology Information [NCBI], n.d.a), which searches for similarities with gene sequences stored in the database of the National Center for Biotechnology Information (NCBI, n.d.b).

2.11 Data Recording and Statistical Analysis

The data obtained from the trials of infection of *G. mellonella* with nematodes were recorded and analyzed, the information was collected in spreadsheets using Microsoft Excel software. Subsequently, the data were processed and analyzed using the R Studio v.4.5.1. software, with the support of tools based on artificial intelligence, which allowed the elaboration of frequency graphs.

On the other hand, for the recording and analysis of molecular data, the consensus sequences were compiled in documents prepared with Microsoft Word v.2021. Likewise, the accession numbers and scientific names of the matching sequences were organized and systematized in tables using Microsoft Excel v.2021.

3. Results

3.1 Infection of *G. mellonella* Larvae Exposed to Entomopathogenic Nematodes Present in Carob Soils

As shown in Table 1, out of the 10 soil samples in which *G. mellonella* larvae were exposed, only 6 yielded positive results for the presence of entomopathogenic nematodes. This was evidenced by a wine-red coloration by the larvae, which is a characteristic indicator of nematode infection.

Table 1. Record of infection in *G. mellonella* larvae exposed to forest soils of carob forests in Tumbes, Peru

N°	Localities	Districts	(+) / (-)	Soil sample
1	Tacural	San Juan de la Virgen	(+)	01
2	Cerro Blanco		(+)	02
3	Garbanzal		(+)	03
4	Los Cedros	Corrales	(+)	04
5	Facultad de Ciencias Agrarias - UNTumbes		(+)	05
6	Pampas de Hospital	Pampas de Hospital	(+)	06
7	Cabuyal		(-)	07
8	Cabeza de Lagarto		(-)	08
9	San Jacinto	San Jacinto	(-)	09
10	Higuerón		(-)	10

(+): Soil sample with a positive infection result. (−): Soil sample with a negative infection result.

Regarding the infection process over the evaluation period, soil sample 06 exhibited the highest infectivity in *G. mellonella* larvae, recording infection rates of 30% at 12 h, 50% at 24 h, 80% at 48 h, and 100% at 72 h. Soil sample 02 showed intermediate infectivity, with infection rates of 10%, 25%, 60%, and 90% at the respective evaluation times. In contrast, the remaining soil samples exhibited the lowest infectivity, reaching only 5% at 12 h, 15% at 24 h, 45% at 48 h, and 75% at 72 h (Figure 2). Based on the infection levels observed in *G. mellonella* larvae, a marked divergence among treatments was evident; soil samples 02 and 06 exhibited the highest infectivity percentages, contrasting with the attenuated and homogeneous response of samples 01, 03,

04, and 05. In light of this behavior, only three representative strains were selected and isolated from soil samples 02, 05, and 06, designated as strain 02, strain 05, and strain 06, respectively. Subsequently, these strains were subjected to morphological and molecular analyses to establish their taxonomic identity at the species level.

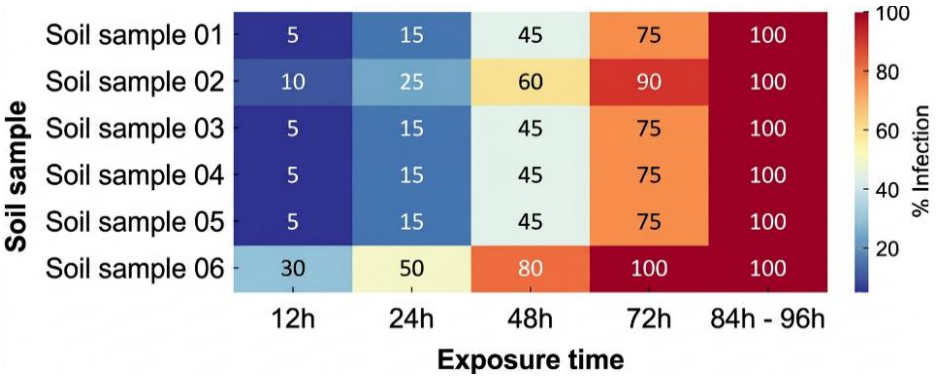

Figure 2. Percentages of infection of *G. mellonella* by EPNs present in the soils of carob forests in Tumbes, Peru

3.2 Morphological Characterization of EPNs

Following the isolation of strains 02, 05, and 06, morphological characterization was performed, allowing the preliminary identification of the specimens at the genus level (Figure 3). Based on the taxonomic keys described by Nguyen and Hunt (2007), the isolates were assigned to the genus *Heterorhabditis* Poinar, 1976 (Rhabditida: Heterorhabditidae).

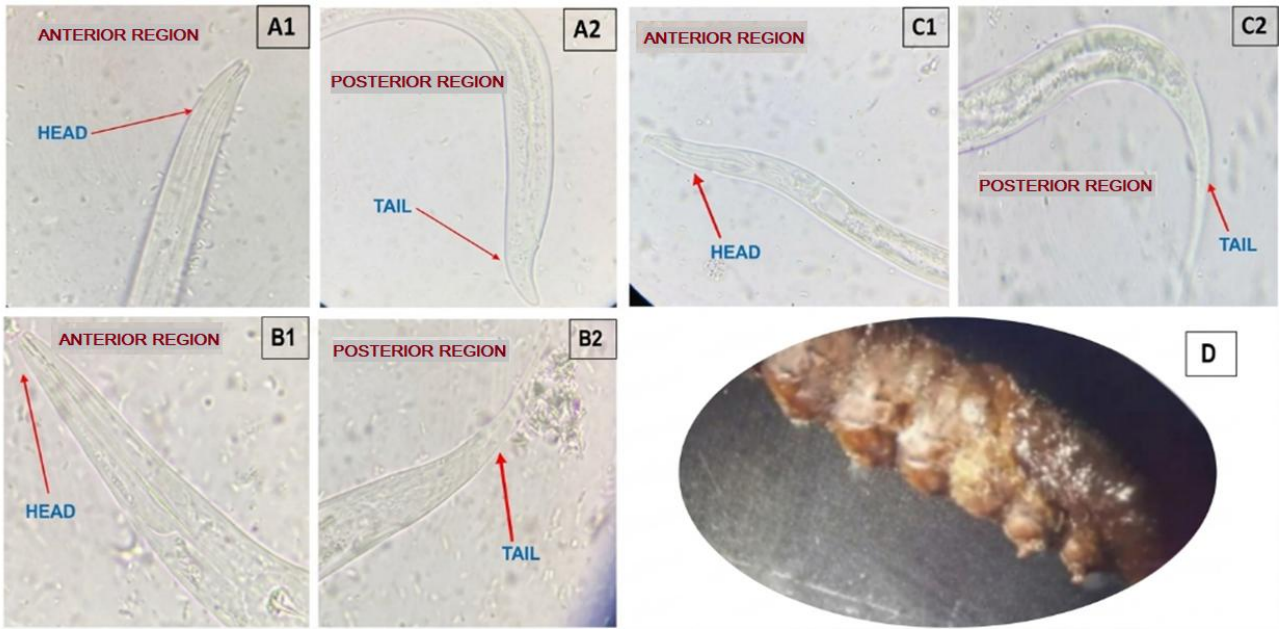

Figure 3. Nematode strains isolated and morphologically characterized as belonging to the genus *Heterorhabditis*. A1) Posterior head region of strain 06; A2) Anterior tail region of strain 06; B1) Posterior head region of strain 02; B2) Anterior tail region of strain 02; C1) Posterior head region of strain 05; C2) Anterior tail region of strain 05; D) *G. mellonella* larvae infected with nematode strains

Red arrows indicate the head and tail regions of the nematodes.

3.3 Molecular Identification of EPNs

Copies of the 18S rDNA gene were amplified from the extracted total DNA using the end-point PCR technique. All three isolated strains showed an amplicon size of ~1000 base pairs (Figure 4).

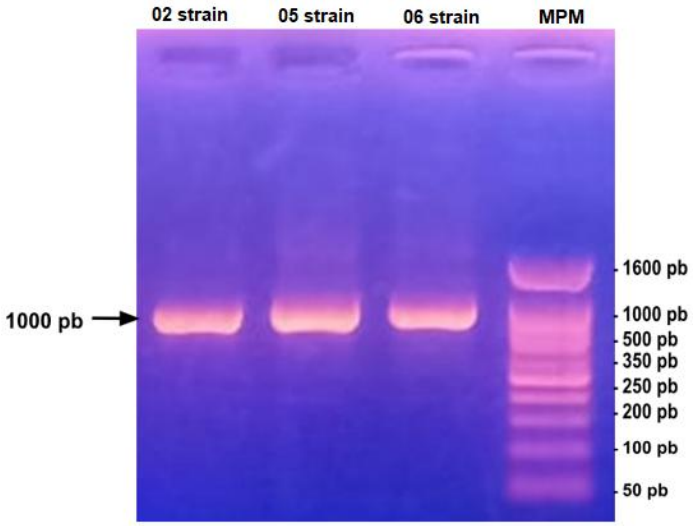

Figure 4. PCR products targeting the 18S rDNA gene from strains 02, 05, and 06 isolated from forest soils

Using consensual sequences of the 18S rDNA gene as a molecular marker, the three isolated strains (strain 02, strain 05, and strain 06) were successfully identified, confirming the classic taxonomic identification at the genus level (Table 2). Strain 02 was identified as *H. bacteriophora* and was recorded in the town of Los Cedros, whereas strains 05 and 06 corresponded to *H. indica*, isolated from the Faculty of Agricultural Sciences of the National University of Tumbes, located in the district of Corrales, and from the town of Pampas de Hospital, respectively. Furthermore, sequence alignment analysis revealed that strains 05 and 06 of *H. indica* showed high similarity to strain OL470132.1 reported in India (NCBI, 2021), whereas strain 02 of *H. bacteriophora* aligned with isolate JN996475.1, also reported in India, indicating a close phylogenetic relationship with isolates previously described worldwide (NCBI, 2012; Seenivasan et al., 2012). These findings are supported by recent studies in which the isolation and morphological and molecular characterization of entomopathogenic nematodes have been carried out in the same country (Singh et al., 2025).

Table 2. Accession codes and similarity parameters of the DNA sequences of the identified strains using the 18S rDNA gene

Strain	Identity in GenBank	E-Value	Identity (%)	Accession Number
02 strain	*H. bacteriophora*	0.0	100	JN996475.1
05 strain	*H. indica*	0.0	98.72	OL470132.1
06 strain	*H. indica*	0.0	99.20	OL470132.1

E-value (Expect value): Extremely high similarity (Unequivocal identity): $E \leq 10^{-50}$ (0.0). High similarity (Strong homology): $10^{-50} < E \leq 10^{-10}$. Moderate-to-low similarity: $10^{-10} < E \leq 10^{-3}$. No similarity (By chance): $E > 10^{-3}$.

4. Discussion

The findings of this study demonstrate that *G. mellonella* larvae were infected at varying rates and exposure durations in six out of the ten carob tree soil samples analyzed. In farm soils in Colombia, nematode isolates belonging to the genus *Steinernema* Travassos (Rhabditida: Steinernematidae) have been identified (Caro-Arias et al., 2021). Likewise, in sandy soils of Argentina, nematode species belonging to the genus *Cervidellus* Thorne, 1937 (Rhabditida: Cephalobidae) have been reported (Holovachov et al., 2011). According to Moscol et al.

(2022), the soil of a dry forest is characterized as sandy loam, and in some areas, sandy clay loam, consistent with the soil texture analyzed in the present study. Porazinska et al. (2009) and Ferris et al. (2012) state that EPNs are widely distributed in soils across all continents and represent one of the most abundant animal groups, due to their phenotypic plasticity that allows them to inhabit a wide range of ecosystems. However, Matuska-Łyżwa et al. (2024) point out that some EPNs have greater mobility and survival in sandy and sandy loam soils, while clay soils limit their displacement and infectivity. This supports the above.

Regarding the wine-red coloration observed in the infected larvae, this is consistent with the studies by Wattanachaiyingcharoen et al. (2021) and Nthenga et al. (2021), who reported the same symptoms in *G. mellonella* larvae when infected with nematodes of the *Heterorhabditis* genus. According to Chandrakasan et al. (2024), *G. mellonella* larvae allow for the recovery of EPNs belonging to the genera *Steinernema* and *Heterorhabditis*. This is supported by Valencia et al. (2022), who state that using larvae of this insect makes it possible to isolate nematodes belonging to the genus *Heterorhabditis*. These findings align with studies conducted by Monteiro et al. (2024), who reported *H. amazonensis* (Rhabditida, Heterorhabditidae) as a highly effective EPN in causing mortality in *Stomoxys calcitrans* Linneo, 1758 (Diptera: Muscidae) larvae.

However, other authors note that *G. mellonella* can also be infected by nematodes of the genus *Steinernema*, which have subsequently demonstrated the same lethal effect in *Premnotrypes vorax,* Hustache, 1933 (Coleoptera: Curculionidae), another pest insect with significant agricultural impact (Caro-Arias et al., 2021). Sánchez Jara et al. (2019) documented that the nematode *H. bacteriophora* exhibits over 50% efficacy in the biological control of *Spodoptera frugiperda* Walker (Lepidoptera: Noctuidae), a pest of economic importance in maize crops. Similar results were reported by Tesén et al. (2023) and Abbasipour et al. (2023). Other authors, such as Restrepo-García et al. (2022), indicate that other entomopathogenic nematode species, including *Steinernema* and *Heterorhabditis*, have demonstrated virulence against *Eurhizococcus colombianus* Jakubski, 1965 (Hemiptera: Margarodidae), a pest of economic relevance. Therefore, the results of the present study can be interpreted within this broader scientific context.

Regarding the morphological characterization, which revealed that the genus of the analyzed nematode strains was *Heterorhabditis*, previous studies, such as those conducted by Sáenz and López (2011), documented the presence of nematodes from the same genus in soils associated with crops of *Brachiaria* spp. (Poaceae), *Coffea arabica* L. (Rubiaceae), *Musa* spp. (Musaceae), and *Guadua angustifolia* Kunth (Poaceae), following the exposure of *G. mellonella* larvae. Other authors, such as Grifaldo-Alcantara et al. (2020), describe that *Heterorhabditis* exhibits distinctive morphological features throughout its life cycle. For instance, the infective juvenile stage is characterized by an elongated body, non-functional mouth and anus, longitudinal striations, and a pointed tail. In adults, males have a rounded cephalic region and differentiated sexual structures, while females display a C-shaped curved body and a conical tail ending in a prominent anal lip, traits that were also observed in the purified strains of the present study.

According to Johnigk and Ehlers (1999), approximately 14 hours after host penetration, nematodes such as *Heterorhabditis* begin a progressive opening of the stoma, accompanied by expansion of the esophagus and basal bulb, as well as hypertrophy of the excretory glands. After 48 hours, the molt to the hermaphroditic preadult stage occurs, preceded by the separation and rupture of the previous cuticle, allowing the nematode to emerge. At this stage, body width increases more significantly than length, until proportions similar to those of the adult are reached. Furthermore, several studies have reported that, in later stages of the life cycle, amphimictic adults (males and females) of the second generation emerge around 185 hours post-infection, and are smaller than the primary hermaphrodites (Ogier et al., 2023; Sáenz & Luque, 2000). According to Abdel-Razek et al. (2018), such variations in body length are closely linked to the morphological changes that nematodes undergo throughout their ontogenetic development.

On the other hand, the identification of *H. bacteriophora* and *H. indica* at the species level highlights the usefulness of the SSU18A and SSU26R primers for identifying and differentiating EPN species, particularly those belonging to the genera *Heterorhabditis* and *Steinernema* (Torrini et al., 2014). However, Vélez Zambrano and Guzmán Cedeño (2022) note that among the genes and/or molecular markers used for the identification of agriculturally important nematode species are specific regions of mtDNA (mitochondrial DNA), rDNA (ribosomal DNA), and IGS (intergenic spacer), which complement the 18S, 5.8S, and 28S regions of rDNA.

In this context, Chaves-Velasquez et al. (2023) state that the partial ITS1-5.8S-ITS2 region, along with the 18S rDNA gene, enables the molecular identification of nematodes affecting banana and plantain crops. Other authors, such as Palomares-Rius et al. (2017), mention that the 18S rDNA gene, a small subunit of ribosomal DNA, is useful for studying relationships among genera of plant-parasitic nematodes. Nevertheless, researchers like Chacón Marcheco et al. (2022) argue that the ITS-2 rDNA gene should be used for nematode identification.

5. Conclusions

The present study confirmed the presence of entomopathogenic nematodes (EPNs) in soils from carob tree (*Neltuma* spp.) forests in the Tumbes region, where six native strains were isolated and three species were successfully identified through morphological and molecular analyses. Sequencing of the 18S rDNA gene identified the species as *H. bacteriophora* and *H. indica*. All evaluated strains showed pathogenicity against *G. mellonella* larvae, demonstrating their potential as biological control agents for sustainable pest management.

This study constitutes the first report of native EPNs associated with *Neltuma* spp. forest soils in Tumbes, contributing valuable information for biodiversity records and supporting future applications in integrated pest management strategies.

Transparency of Data

Available data: The entire data set that supports the results of this study was published in the article itself.

Author Contribution Statement

	JM R-G	PS C-C	RE C-H	CA M-F	SM G-G	VT T-S	AA R-P	DL G-R	E F-H	JS C-C
Conceptualization	■	■								■
Data curation				■	■		■			■
Formal analysis	■	■					■			■
Funding acquisition								■		
Investigation	■	■	■	■	■	■	■		■	■
Methodology	■			■	■		■			■
Project administration	■	■						■		■
Resources	■	■	■	■	■	■	■		■	■
Software							■			■
Supervision		■	■					■		■
Validation		■		■		■		■	■	■
Visualization					■	■	■	■	■	■
Writing – original draft	■	■	■	■	■	■	■	■	■	■
Writing – review and editing	■	■	■	■	■	■	■	■	■	■

References

Abbasipour, F., Saeedizadeh, A., & Shapiro-Ilan, D. I. (2023). Efficacy of three local isolates of entomopathogenic nematodes against the tomato leafminer, *Tuta absoluta* (Meyrick). *Revista de la Sociedad Entomológica Argentina, 82*(1), 24-30. https://doi.org/10.25085/rsea.820103

Abd-Elgawad, M. M. M. (2025). Transformational technologies for more uptakes of entomopathogenic nematodes. *Egyptian Journal of Biological Pest Control, 35*, Article 1. https://doi.org/10.1186/s41938-024-00838-9

Abdel-Razek, A. S., Hussein, M., & Shehata, I. (2018). Isolation and identification of indigenous entomopathogenic nematode (EPN) isolate from Egyptian fauna. *Archives of Phytopathology and Plant Protection, 51*(3-4), 197-206. https://doi.org/10.1080/03235408.2018.1445080

Ali, M., Allouf, N., & Ahmad, M. (2023). Isolation, identification of entomopathogenic nematodes with insights into their distribution in the Syrian coast regions and virulence against *Tuta absoluta*. *Journal of Nematology*, *55*(1), Article 20230056. https://doi.org/10.2478/jofnem-2023-0056

Blaxter, M. L., De Ley, P., Garey, J. R., Liu, L. X., Scheldeman, P., Vierstraete, A., Vanfleteren, J. R., Mackey, L. Y., Dorris, M., Frisse, L. M., Vida, J. T., & Thomas, W. K. (1998). A molecular evolutionary framework for the phylum Nematoda. *Nature*, *392*(6671), 71-75. https://doi.org/10.1038/32160

Caro-Arias, A. X., Yépez-Bolaños, D. A., & Soto-Giraldo, A. (2021). *Premnotrypes vorax* Hustache (Coleoptera: Curculionidae) y su control con nematodos entomopatógenos nativos. *Boletín Científico: Centro de Museos: Museo de Historia Natural, 25*(2), 33-42.

Chacón Marcheco, E., Toro Molina, B., Atamba Yépez, M., Milán Chariguaman, M., & Silva Déley, L. (2022). Identificación molecular del *Toxocara canis* en caninos del cantón Salcedo, Ecuador. *Revista Científica y Tecnológica UPSE, 9*(1), 66-74. https://doi.org/10.26423/rctu.v9i1.679

Chandrakasan, G., Trejo, J. F. G., Jácome, O. A., & Rico-García, E. (2024). Abundancia, caracterizaciones moleculares y producción masiva de comunidades microbianas benéficas (nematodos entomopatógenos) del suelo. *Perspectivas de la Ciencia y la Tecnología*, (Edición Especial), 83-103. https://revistas.uaq.mx/index.php/perspectivas/article/view/1068

Chaves-Velasquez, Á. M., Guzmán-Piedrahita, Ó. A., Valencia-Jiménez, A., & Villegas-Estrada, B. (2023). Caracterización morfométrica y molecular de poblaciones de *Radopholus similis* [(Cobb) Thorne (Rhabditida: Pratylenchidae)] en Colombia. *Boletín Científico: Centro de Museos: Museo de Historia Natural, 27*(2), 97-116. https://doi.org/10.17151/bccm.2023.27.2.6

Cruzado-Jacinto, L., Chávez-Villavicencio, C., & Charcape-Ravelo, M. (2019). Uso y selección de las partes aéreas del algarrobo *Prosopis pallida* (Fabaceae) por reptiles, aves y mamíferos en Sechura (Piura-Perú). *Revista Peruana de Biología, 26*(1), 81-86. https://doi.org/10.15381/rpb.v26i1.15417

Eliceche, D. P. (2019). *Estudio de las características bio-ecológicas de un aislamiento autóctono del entomonemátodo Heterorhabditis bacteriophora (Nematoda: Heterorhabditidae) y su potencialidad como agente biorregulador de insectos* [Doctoral dissertation, Universidad Nacional de La Plata]. Naturalis. https://naturalis.fcnym.unlp.edu.ar/handle/628872547/49505

Ferris, H., Griffiths, B. S., Porazinska, D. L., Powers, T. O., Wang, K. H., & Tenuta, M. (2012). Reflections on plant and soil nematode ecology: Past, present and future. *Journal of Nematology*, *44*(2), 115-126. https://journals.flvc.org/jon/article/view/79265

Grifaldo-Alcantara, P. F., Alatorre-Rosas, R., Silva-Rojas, H. V., Stock, S. P., Hernández-Rosas, F., Vargas-Madriz, H., & Durán-Trujillo, Y. (2020). Caracterización molecular y morfométrica de *Heterorhabditis indica* (cepa CP13JA) aislado en el cultivo de caña de azúcar. *Tropical and Subtropical Agroecosystems, 23*(2), Article 64. http://dx.doi.org/10.56369/tsaes.3278

Holovachov, O., Boström, S., & De Ley, P. (2011). Description of *Cervidellus caucetensis* sp. n. (Rhabditida: Cephalobidae) from Argentina. *Journal of Nematode Morphology and Systematics, 14*(1), 21-26.

Johnigk, S. A., & Ehlers, R.-U. (1999). Juvenile development and life cycle of *Heterorhabditis bacteriophora* and *Heterorhabditis indica* (Nematoda: Heterorhabditidae). *Nematology, 1*(3), 251–260.

Kaya, H. K. (1985). Entomogenous nematodes for insect control in IPM systems. In M. A. Hoy & D. C. Herzog (Eds.), *Biological control in agricultural IPM systems* (pp. 283-302). Academic Press.

Lacey, L. A., & Georgis, R. (2012). Entomopathogenic nematodes for control of insect pests above and below ground with comments on commercial production. *Journal of Nematology*, *44*(2), 218-225. https://europepmc.org/article/med/23482993

Matuska-Łyżwa, J., Duda, S., Nowak, D., & Kaca, W. (2024). Impact of abiotic and biotic environmental conditions on the development and infectivity of entomopathogenic nematodes in agricultural soils. *Insects*, *15*(6), Article 421. https://doi.org/10.3390/insects15060421

MEGA. (2021). *MEGA11: Molecular Evolutionary Genetics Analysis* (Version 11) [Software]. https://mega.io

Monteiro, A. C., de Souza, A. C. F., da Silva, D. P., de Almeida, G. P. S., de Souza, V. T., Monteiro, J. L. L., Chambarelli, M. C. M. D. C., & Bittencourt, A. J. (2024). Assessment of infection of *Stomoxys calcitrans* larvae by entomopathogenic nematodes *Heterorhabditis amazonensis* NEPT11. *Brazilian Journal of Veterinary Medicine*, *46*, Article e000424. https://doi.org/10.29374/2527-2179.bjvm000424

Moscol, J., Puescas, M., & Herrera, E. (2022). Estructura y diversidad arbórea y su relación con el suelo forestal en un ecosistema de bosque seco. *Manglar, 19*(4), 323-330. https://doi.org/10.57188/manglar.2022.041

National Center for Biotechnology Information. (n.d.a). *BLAST: Basic Local Alignment Search Tool* [Software]. National Library of Medicine. https://blast.ncbi.nlm.nih.gov/Blast.cgi

National Center for Biotechnology Information. (n.d.b). *GenBank* [Database]. National Library of Medicine. https://www.ncbi.nlm.nih.gov/genbank/

National Center for Biotechnology Information. (2012). Heterorhabditis bacteriophora isolate TtcK KKMH2 18S ribosomal RNA gene, partial sequence (GenBank JN996475.1) [Database record]. GenBank. https://www.ncbi.nlm.nih.gov/nuccore/JN996475.1

National Center for Biotechnology Information. (2021). Heterorhabditis indica isolate O1 small subunit ribosomal RNA gene, partial sequence (GenBank OL470132.1) [Database record]. GenBank. https://www.ncbi.nlm.nih.gov/nuccore/OL470132.1/

Nguyen, K. B., & Hunt, D. J. (Eds.). (2007). *Entomopathogenic nematodes: Systematics, phylogeny and bacterial symbionts*. Brill.

Nthenga, I., Knoetze, R., & Malan, A. P. (2021). Distribution and diversity of entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) in a South African nature reserve. *Koedoe, 63*(1), Article a1661. https://doi.org/10.4102/koedoe.v63i1.1661

Núñez Gamboa, L. J. (2022). Reforestación del bosque seco para la prevención de desbordes fluviales en el Perú: El caso del distrito de Íllimo, provincia y región Lambayeque (2018–2019). *Kawsaypacha: Revista Kawsaypacha: Sociedad y Medio Ambiente,* (9), 108-123. https://doi.org/10.18800/kawsaypacha.202201.005

Ogier, J. C., Akhurst, R., Boemare, N., & Gaudriault, S. (2023). The endosymbiont and the second bacterial circle of entomopathogenic nematodes. *Trends in Microbiology*, *31*(6), 629-643. https://doi.org/10.1016/j.tim.2023.01.004

Pacheco Hernández, M. de L., Reséndiz Martínez, J. F., & Arriola Padilla, V. J. (2019). Organismos entomopatógenos como control biológico en los sectores agropecuario y forestal de México: Una revisión. *Revista Mexicana de Ciencias Forestales, 10*(56), 4–32. https://doi.org/10.29298/rmcf.v10i56.496

Palomares-Rius, J. E., Cantalapiedra-Navarrete, C., Archidona-Yuste, A., Subbotin, S. A., & Castillo, P. (2017). The utility of mtDNA and rDNA for barcoding and phylogeny of plant-parasitic nematodes from Longidoridae (Nematoda, Enoplea). *Scientific Reports*, *7*(1), Article 10905. https://doi.org/10.1038/s41598-017-11085-4

Porazinska, D. L., Giblin-Davis, R. M., Faller, L., Farmerie, W., Kanzaki, N., Morris, K., Powers, T. O., Tucker, A. E., Sung, W., & Thomas, W. K. (2009). Evaluating high-throughput sequencing as a method for metagenomic analysis of nematode diversity. *Molecular Ecology Resources*, *9*(6), 1439-1450. https://doi.org/10.1111/j.1755-0998.2009.02611.x

Půža, V., & Machado, R. A. R. (2024). Systematics and phylogeny of the entomopathogenic nematobacterial complexes Steinernema-Xenorhabdus and Heterorhabditis-Photorhabdus. *Zoological Letters*, *10*(1), Article 13. https://doi.org/10.1186/s40851-024-00235-y

Restrepo-García, A. M., Bernal-Arias, L., & Soto-Giraldo, A. (2022). *Eurhizococcus colombianus* Jakubski (1965) (Hemiptera: Margarodidae): Evaluación de su control biológico utilizando una mezcla de los nematodos entomopatógenos *Steinernema* (Rhabditida: Steinernematidae) y *Heterorhabditis* (Rhabditida: Heterorhabditidae). *Boletín Científico: Centro de Museos: Museo de Historia Natural, 26*(1), 155-168. https://doi.org/10.17151/bccm.2022.26.1.11

Rizvi, A. N. (2010). First record of three species of soil nematodes of the suborder Cephalobina from Ladakh region, Jammu & Kashmir, India. *Journal of Threatened Taxa, 2*(11), 1286-1290. https://doi.org/10.11609/JoTT.o2259.1286-90

Ruiz, J. K. Z., Osorio, O. R., Hernández, H. L. U., Ochoa, F. A. A., Silva, V. R., & Méndez, Z. G. (2021). Actividad acaricida de extractos de plantas contra el ácaro rojo de las palmeras *Raoiella indica* (Acari: Tenuipalpidae). *Revista Sociedad Entomológica Argentina*, *80*(1), 33-39. https://dx.doi.org/https://doi.org/10.25085/rsea.800104

Sáenz, A., & López, J. C. (2011). Ciclo de vida y patogenicidad del aislamiento nativo *Heterorhabditis* sp. SL0708 (Rhabditida: Heterorhabditidae). *Revista Colombiana de Entomología, 37*(1), 43-47. https://doi.org/10.25100/socolen.v37i1.9036

Sáenz, A. A., & Luque, J. E. (2000). Ciclo de vida del entomonematodo nativo *Steinernema feltiae* Filipjev. *Agronomía Colombiana, 17*(1), 17-24. https://revistas.unal.edu.co/index.php/agrocol/article/view/21540

Sánchez Jara, J., Valle Delgado, J., Pérez Tesén, E., Neira de Perales, M., & Calderón Arias, C. (2019). Control biológico de *Spodoptera frugiperda* en cultivo de *Zea mays*: Uso de nematodos entomopatógenos. *Scientia Agropecuaria, 10*(4), 551-557. https://doi.org/10.17268/sci.agropecu.2019.04.12

Seenivasan, N., Prabhu, S., Makesh, S., & Sivakumar, M. (2012). Natural occurrence of entomopathogenic nematode species (Rhabditida: Steinernematidae and Heterorhabditidae) in cotton fields of Tamil Nadu, India. *Journal of Natural History, 46*(45-46), 2829-2843. https://doi.org/10.1080/00222933.2012.727216

Shapiro-Ilan, D. I., Morales-Ramos, J. A., & Rojas, M. G. (2016). In vivo production of entomopathogenic nematodes. In T. Glare & M. Moran-Diez (Eds.), *Microbial-based biopesticides: Methods and protocols* (pp. 137-158). Humana Press. https://doi.org/10.1007/978-1-4939-6367-6_11

Sharghi, H., Eivazian Kary, N., & Mohammadi, D. (2025). Enhancing the shelf life of entomopathogenic nematodes formulation: The impact of super absorbent polymer and infective juveniles concentration. *Crop Protection, 190*, Article 107091. https://doi.org/10.1016/j.cropro.2024.107091

Singh, A. K., Patil, J., Nekkanti, A., Paschapur, A. U., Bhat, S., Gouri, V., Verma, G., Jaiman, M., Pankaj, Mishra, K. K., & Kant, L. (2025). Isolation, morphological, and molecular characterization of a native *Heterorhabditis indica* strain from the Mid-Indian Himalayas with insights into biocontrol potential. *Frontiers in Plant Science*, *16*, Article 1576159. https://doi.org/10.3389/fpls.2025.1576159

Stock, S. P., & Hunt, D. J. (2005). Nematode morphology and systematics. In P. S. Grewal, R. U. Ehlers & D. I. Shapiro-Ilan (Eds.), *Nematodes as biological control agents* (pp. 3-43). CABI Publishing.

Sturhan, D., Spiridonov, S., & Mráček, Z. (2005). *Steinernema silvaticum* sp. n. (Rhabditida: Steinernematidae), a new entomopathogenic nematode from Europe. *Nematology*, *7*, 227-241.

Tarasco, E., Fanelli, E., Salvemini, C., El-Khoury, Y., Troccoli, A., Vovlas, A., & De Luca, F. (2023). Entomopathogenic nematodes and their symbiotic bacteria: From genes to field uses. *Frontiers in Insect Science*, *3*, Article 1195254. https://doi.org/10.3389/finsc.2023.1195254

Tesén, E. D. P., Adrianzén, J. E. R., Cabrera, A. C., & de Perales, M. E. N. (2023). Pathogenicity of *Heterorhabditis bacteriophora* in ultraviolet light–irradiated agar suspension on *Spodoptera frugiperda* (Lepidoptera: Noctuidae) larvae. *Journal of Entomological Science, 58*(4), 412-422. https://doi.org/10.18474/JES22-48

Torrini, G., Landi, S., Benvenuti, C., De Luca, F., Fanelli, E., Troccoli, A., & Bazzoffi, P. (2014). Morphological and molecular characterization of a *Steinernema carpocapsae* (Nematoda: Steinernematidae) strain isolated in Veneto region (Italy). *Redia, 97*, 89-94. https://ricerca.uniba.it/handle/11586/178433

Valencia, E. E. A., Tixe, L. O. V., Samaca, C. P. H., Gallegos, P., Cifuentes, M. P. C., & Alcazar, J. (2022). Etología de nematodos entomopatógenos del género *Heterorhabditis* aislados de larvas de gusano blanco (*Premnotrypes vorax*) plaga de la papa (*Solanum tuberosum*) en Ecuador. *Boletín Técnico, Serie Zoológica, 17*, 47-61. https://journal.espe.edu.ec/ojs/index.php/revista-serie-zoologica/es/article/view/2725

Vélez Zambrano, S. M., & Guzmán Cedeño, A. M. (2022). Técnicas de identificación del nematodo agallador *Meloidogyne*. *Manglar, 19*(2), 209-215. https://doi.org/10.17268/manglar.2022.026

Wattanachaiyingcharoen, W., Lepcha, O., Vitta, A., & Wattanachaiyingcharoen, D. (2021). Efficacy of Thai indigenous entomopathogenic nematodes for controlling fall armyworm (*Spodoptera frugiperda*) (J. E. Smith) (Lepidoptera: Noctuidae). *Egyptian Journal of Biological Pest Control, 31*(1), Article 149. https://doi.org/10.1186/s41938-021-00497-0

White, G. F. (1927). A method for obtaining infective nematode larvae from cultures. *Science*, *66*(1709), 302-303. https://doi.org/10.1126/science.66.1709.302-a