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Research Article

MORPHOBIOLOGICAL CHARACTERISTICS OF THE ENTOMOPATHOGENIC NEMATODE "HETERORHABDITIS BACTERIOPHORA" (POINAR, 1976) IDENTIFIED IN UZBEKISTAN

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Nafosat Kurbonova Sattor Kizi
Research Scientific Institute Of Plant Protection And Quarantine, Uzbekistan

ABSTRACT

The research article discusses the identification of a new local entomopathogenic nematode, "Heterorhabditis bacteriophora," in Uzbekistan. The article includes an analysis of its morphological and molecular characteristics. Additionally, the reproductive and developmental traits of this species, recognized globally as a prolific biocontrol agent, are elaborately presented, along with methods for its extraction from the soil. The future prospects of this entomopathogenic nematode's biocontrol potential and areas for further study are highlighted, making this research a valuable resource for scientists and biolaboratory specialists.

KEYWORDS

Uzbekistan, entomopathogenic nematodes, Heterorhabditis spp, in vivo, potato, biocontrol.

INTRODUCTION

The initial types of entomopathogenic nematodes in Uzbekistan were identified in the agrobiocenosis of potato crops. Notably, representatives of the Heterorhabditidae family (Poinar, 1976) are distinguished by their infectious third-stage larvae, which can develop into either hermaphroditic or amphimictic females upon reaching sexual maturity. The research was conducted during 2020-2023, with soil samples collected from potato agrobiocenoses in the Qibray district of Tashkent region. These samples were thoroughly analyzed at the "Laboratory of Biological Protection Against Pests and Diseases" of the Research scientific institute of plant protection and quarantine in Uzbekistan.

MATERIALS AND METHODS

In vivo multiplication of entomopathogenic nematodes involves removing all residues from soil samples collected from the field. If necessary, the soil

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is moistened to improve its humidity. The prepared soil sample is then placed in lidded plastic containers, and ten late-stage larvae of G. mellonella are placed on each soil sample. The containers are incubated upside down at 55% relative humidity (RH) and a temperature of 25°C in a dark place. Infected larvae showing specific signs of entomopathogenic nematode infection are removed from the soil after 7 – 10 days and placed in a "White Trap" to collect third-stage infectious nematodes.

If the larvae are infected with Heterorhabditis nematodes, they turn reddish-brown to black. The suspension containing entomopathogenic nematodes is stored in a special flask at a temperature of 10-15°C in an incubator.

For the identification of entomopathogenic nematode species, the following diagnostic key can be used:

Representatives of the Heterorhabditidae family have both hermaphroditic and amphimictic females.

Hermaphroditic female:

Nematodes at the infectious larval stage turn into hermaphroditic nematodes after entering the insect body.

The head is slightly rounded with six separate conical lips, each provided with a terminal papilla; small raised structures are sometimes visible under each lip; the amphidial opening is small.

The stoma is wide but short; Cheilorhabdions are present, forming a ring, appearing as two refractive points in lateral view.

The other parts of the stoma merge to form an expanded posterior area, directly connected to the esophagus.

The nerve ring is located in the middle of the isthmus.

The excretory opening is usually located on the posterior side of the esophagus.

The vulva is median, slit-like, bordered by elliptical rings; the ovotestis is amphidelphic, reflexed.

Ovaries are present.

The tail is pointed and long.

Amphimictic female:

Smaller than the hermaphroditic female; has labial papillae.

The sexual system is amphidelphic, the vulva is not adapted for egg-laying but rather for mating.

Male:

A single, reflexed testis.

Paired spicules, separate and slightly bent towards the abdominal cavity. The head of the spicule is short and narrowed, distinct from the rest.

The gubernaculum is usually half the length of the spicule.

Formed with a bursa peloderan with nine pairs of genital papillae.

Infectious stage nematode larvae:

Third-stage infectious larvae (IL) are usually sheathed (due to the skin of second-stage larvae). The anterior area forms a mosaic pattern and longitudinal lines with the sheath.

The IL cuticle has two ridges bordering a uniform line in lateral areas.

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The head area has dorsal teeth. Mouth and anal openings are closed.

The stoma resembles a closed chamber with parallel walls.

The esophagus and intestine are poorly developed.

The excretory opening is located behind the nerve ring.

The tail is blunt.

Identification of Heterorhabditis species: After identifying Heterorhabditis species with the help of the key, confirm the species by comparing its morphometry with the original descriptions.

The following ratios and abbreviations are used in the key: IL = Infectious larvae. EP = Distance from the anterior end to the excretory pore. T = Tail length. E% = EP/T × 100. GS% = Gubernaculum length divided by spicule length × 100. m = micrometer.

Table 1.

Key to the Species of the Heterorhabditis Genus

#	Criteria	Species
1	Average body length of IL > 700 µm (736–800)	H. megidis
	Average body length of IL < 700 µm (528–685)	Go to 2
2	IL tail short, avg. 76 µm (68–80), E% ~147	H. brevicaudis
	IL tail longer, avg. > 80 µm (84–119), E% = 127 or less	Go to 3
3	IL body length avg. > 600 µm	Go to 4
	IL body length avg. < 600 µm	Go to 6
4	IL with E% > 120, c > 7; spicular lamina with ventral expansion	H. argentinensis

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#	Criteria	Species
	IL with E% < 120, c < 7; spicular lamina without ventral expansion	Go to 5
5	IL body length avg. 654 µm, E% ~96, c ~6.1; Male body width avg. 51 µm, spicule length avg. 45 µm	H. marelatus
	IL body length avg. 685 µm, E% ~108, c ~6.6; Male body width avg. 41 µm, spicule length avg. 51 µm	H. zealandica
6	IL body length avg. 528 µm, E% ~94	H. indicus
	IL body length avg. 570 µm, E% > 100	Go to 7
7	IL's E% ~127; spicule avg. 47 µm, lamina with ventral expansion	H. hawaiiensis
	IL's E% ~112, spicule avg. 40 µm, lamina without ventral expansion	H. bacteriophora

Note: IL = Infectious Larvae, E% = Ratio of Esophagus Length to Tail Length, µm = Micrometers

(a) Morphometrics of IJS (Infectious juvenile stage) alone are insufficient for species identification. Characteristics of both male and female nematodes must also be considered.

(b) IJS produced in artificial environments (cultivated in laboratories or commercial products) tend to have a shorter lifespan compared to those produced in vivo and may not fully meet the criteria of the original species description.

(c) Male and female nematodes are collected 4 or 5 days after the demise of the infested insect host.

(d) For accurate species identification, measurements of at least 10 specimens should be taken.

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(e) For verification of identification, the morphological and morphometric characteristics of the identified nematode at various stages should be compared and checked against the original described features.

The phylogenetic relationships of entomopathogenic nematode species within the Heterorhabditidae family were studied using the mitochondrial DNA COX region analysis. For constructing the phylogenetic tree of the Heterorhabditis genus species (Heterorhabditis bacteriophora, Heterorhabditis indica, Heterorhabditis megidis), samples collected from the Surxondaryo region and ITS sequences of 16 species from this genus available in the National Center for Biotechnology Information (NCBI, GenBank) were used. The sequences were aligned using the Multiple Sequence Alignment by MAFFT tool (https://www.genome.jp/tools-bin/mafft). The data obtained from the comparison were edited and a phylogenetic tree was constructed based on maximum likelihood using the IQ-TREE web server (https://iqtree.cibiv.univie.ac.at/). The command code used was: path_to_iqtree -s Heterorhabditis.ph -st DNA -m TEST -bb 1000 -alrt 1000. The phylogenetic tree was visualized using the iTOL web tool (https://itol.embl.de/login.cgi). Heterorhabditis sp. was taken as the outgroup.

The results showed that the Heterorhabditis bacteriophora we studied, registered in the NCBI database under accession number OP578207.1 (Authors: Hassan,M., Hamad,A. and Albogame,B.), was placed in a monophyletic group sharing a common ancestor (see Image I clad), with a bootstrap support value of 97.4/100. Heterorhabditis indica, registered under accession number MK271288.1 (Authors: Rana,A. and Chaubey,A.K.), was also placed in a monophyletic group sharing a common ancestor (see Image VI clad), with a bootstrap value of 100/100. Heterorhabditis megidis, registered under accession number MZ675644.1 (Authors: Ozawa,S., Maehara,N., Takatsuka,J., Aikawa,T. and Nakamura,K.), was placed in a monophyletic group with a common ancestor (see Image VIII clad), with a bootstrap value of 100/100.

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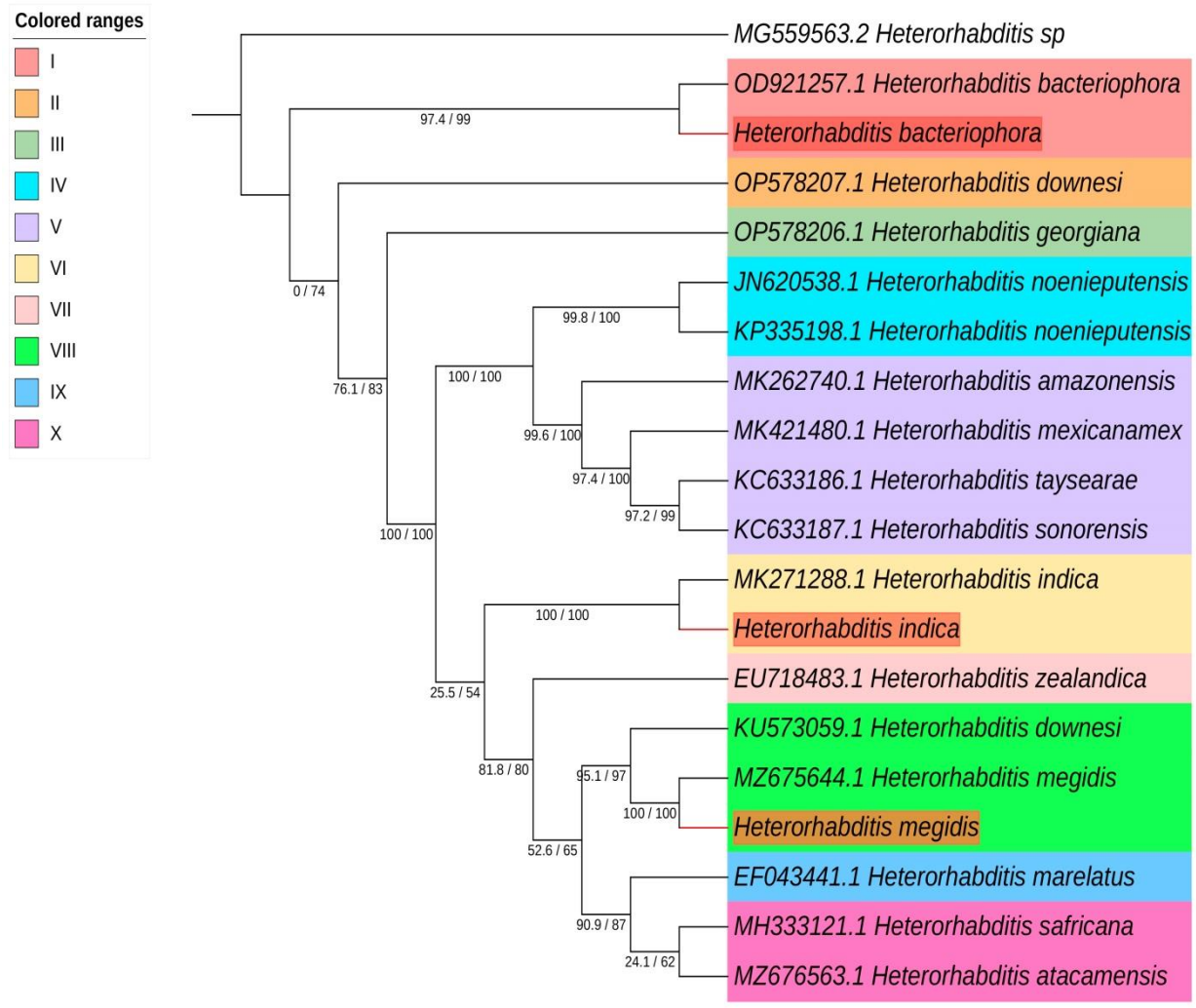

1 – Image. Phylogenetic tree of entomopathogenic nematode species belonging to the steinernematidae family

CONCLUSION

The discovery of a new local entomopathogenic nematode, Heterorhabditis bacteriophora (Poinar, 1976), in Uzbekistan, and the study of its morphobiological characteristics highlight the importance of considering morphometric traits, including those of female and male nematodes, as well as the morphology of infectious larvae, for species identification.

Comparative analysis of mitochondrial DNA COX region-based phylogenetic analysis, ITS sequences from NCBI (GenBank), and morphometric data of the studied samples revealed a match with existing data for Heterorhabditis bacteriophora in the NCBI database.

In conclusion, the samples studied in our research and the ITS sequences obtained from NCBI database formed a single monophyletic group with the representatives of the same species studied by other

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researchers. This confirms the accuracy of our research at the molecular level.

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