Acta Scientific AGRICULTURE (ISSN: 2581-365X)

Volume 9 Issue 5 May 2025

Research Article

Phasmarhabditis eobaniae n. sp. (Rhabditidae) a New Snail Parasitic Nematode from Egypt with Comparison with Two Previous Egyptian Species

Karima Mahmoud Azzam*

Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt

***Corresponding Author:** Karima Mahmoud Azzam, Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt.

DOI: 10.31080/ASAG.2025.09.1482

Received: April 14, 2025

Published: April 23, 2025

© All rights are reserved by **Karima Mahmoud Azzam.**

Abstract

Currently, there are known to be 21-23 species of *Phasmarhabditis nematodes* in existence. This research focused on characterizing the newly discovered species, *Phasmarhabditis eobaniae* n. sp., by comparing it morphologically and phylogenetically with previously identified Egyptian species like *Phasmarhabditis tawfiki, Phasmarhabditis eagyptiaca*, and other *Phasmarhabditis* species worldwide.

Nematode-infected and healthy snails were collected from Greater Cairo, Egypt. *P. eobaniae* n. sp.'s 18S ribosomal (18S rRNA) and (ITS) rDNA gene sequences were acquired. The nematodes were photographed, illustrated, and morphological data were measured. The three species of *Phasmarhabditis* from Egypt are compared morphologically and molecularly.

P. eobaniae n. sp. represent the fifth species in Africa and the third new species identified in Egypt, following *P. tawfiki* and *P. eagyptiaca*. Morphological features indicate a close relationship between the new species, *P. eobaniae* n. sp. and existing *Phasmarhabditis* species, particularly *P. tawfiki, Phasmarhabditis neopapillosa*, and *Phasmarhabditis hermaphrodita. P. eobaniae n*. sp. is closely associated with *Phasmarhabditis papillosa* and *Phasmarhabditis apuliae*, according to phylogenetic analysis of the (ITS) rDNA genes. It exhibits genrtic similarty to *P. hermaphrodita* based on SSU rDNA genes

The new nematode species demonstrates a significant capability to control all infected terrestrial snails and slugs, as well as 80 to 90% of aquatic snails and 80% of insect pupae and larvae. Results indicated that this newly identified nematode, *Phasmarhabditis eobaniae* n. sp., may potentially represent a novel addition to the *Phasmarhabditis genus*. It shows promise as a biocontrol agent against gastropod pests.

Keywords: Morphology; phylogeny; Snail Parasitic Nematodes; Genetic Sequencing; Biocontrol Agent

Introduction

The genus *Phasmarhabditis* (Family Rhabditidae) was initially identified as a new genus by Andrassy [1]. In recent years, there has been a surge in the discovery of novel species of *Phasmarhabditis* have been described. *Phasmarhabditis* nematodes have been described in 21 species, including *P. tawfiki* [2] and *P. eagyptiaca* [3]. Table (1) displays these species' geographic distribution arranged from most recently described to older descriptions. They shared morphological traits among these species.

Hooper., *et al*. [4] observed variations in body size of *P. hermaphrodita* reared on slugs and bacterial culture differed in body size, which may affect diagnosis. Growth conditions and host interactions, can significantly influence body size variation, making species differentiation based solely on body size challenging. [5,6]. The source of samples, such as artificial growth conditions, food source, host, or substrate, can have an impact on measurements, and morphometric values are typically very varied [7]. As a result, using body size alone to differentiate between species is challenging.

Recently there has been a great controversy about the nomenclature of the genus *Phasmarhabditis* (Andrassy, 1976). Sudhaus [8] and Tandingan., *et al*. [9] with debates arising regarding a proposed change from *Phasmarhabditis* (Andrassy, 1976) to *Pellioditis* (Dougherty, 1953) and think of *Phasmarhabditis* as a synonym for *Pellioditis.* However, it is possible that *Agfa*, Angiostomatidae, and *Pellioditis* are paraphyletic, as suggested by Tandingan De Ley., *et al*. (2016) [10], Nermuť.,*et al*. (2017) [11], Pieterse., *et al*. (2020) [12], Gorgadze., *et al*. (2022) [13], and Ivanova., *et al*. (2022) [14]. Furthermore, *Phasmarhabditis* and *Pellioditis* species formed two unrelated clades, according to Bayesian and maximum parsimony analyses of all genes. Based on molecular evidence, the synonymy of these two genera can be denied [15,16]. This controversy will be discussed in detail and resolved in the discussion section.

Country	Species of *Phasmarhabditis*	Host	References
Russia	*Phasmarhabditis* (*Pellioditis*) *thermalis* Ivanova and Spiridonov 2023 Two strains (*sindicae* and *thermalis*).	Land snails and slugs	*Ivanova and Spiridonov* (2023) [17]
Egypt	*Phasmarhabditis eagyptiaca* Azzam2023	Land snails	*Azzam* (2023) [3]
Italy	*Phasmarhabditis villasmundi* Ivanova, Clausi, Leone, and Spiridonov	Land gastropods	Ivanova., *et al.* (2022) [14]
Georgia	*Phasmarhabditis thesamica* Gorgadze, Troccoli, Fanelli, Tarasco, and De Luca, 2022	Slugs	Gorgadze *et al.* (2022) [13]
Georgia	*Phasmarhabditis akhaldaba* Ivanova, Gorgadze, Lortkhipanidze and Spiridonov, 2021	Slugs	Ivanova., *et al.* (2021) [18]
Vietnam	*Phasmarhabditis quinamensis* Ivanova and Spiridonov, 2021	Snails and slugs	Ivanova and Spiridonov [19]
Kenya	*Phasmarhabditis kenyaensis* Pieterse, Rowsontiedtmalan, Haukeland and Ross, 2020	Slugs	Pieterse., *et al.* (2020) [12]
China	*Phasmarhabditis zhejiangensis* Zhang and Liu, 2020	Slugs	Zhang and Liu (2020) [20]
Russia	*Phasmarhabditis clausliiae* Ivanova, Geraskina, and Spiridonov, 2020	Snails	Ivanova., *et al.* (2020) [21]
Russia	*Phasmarhabditis circassica* Ivanova, Geraskina, and Spiridonov, 2020	Snails	Ivanova., *et al.* (2020) [21]
South Africa	*Phasmarhabditis safricana* Ross, Pieterse, Malan, and Ivanova 2018	Slugs	Ross., *et al.* (2018) [22]
Vietnam	*Phasmarhabditis meridionalis* Ivanova and Spiridonov, 2017	Snails	Ivanova and Spiridonov (2017) [23]
Czech Republic	*Phasmarhabditis bohemica* Nermuť, Půža, Mekete and Mráček, 2017	Slugs	Nermuť., *et al.* (2017) [11]
USA	*Phasmarhabditis californica* Tandingan De Ley, Holovachov, McDonnell, Bert, Paine and De Ley, 2016	Slugs	Tandingan., *et al.* (2016) [10]
Italy	*Phasmarhabditis apuliae* Nermuť, Půža and Mráček, 2016	Slugs	Nermuť., *et al.* (2016a) [15]
Czech Republic	*Phasmarhabditis bonaquaense* Nermuť, Půža, Mekete and Mráček, 2016	Slugs	Nermuť., *et al.* (2016b) [16]
China	*Phasmarhabditis huizhouensis* Huang, Ye, Ren and Zhao, 2015	Rotting leaves	Huang *et al.* (2015) [24]
Egypt	*Phasmarhabditis tawfiki* Azzam2003	Snails and slugs	Azzam (2003) [2]
UK	*Phasmarhabditis neopapillosa* Andrassy, 1983	Slugs	Hooper., *et al.* (1999) [4]
France	*Phasmarhabditis papillosa* (Schneider, 1866) Andrássy1983	Slugs	Morand (1988) [25]
UK	*Phasmarhabditis hermaphrodita* (Schneider, 1859)	Slugs	Hooper., *et al.* (1999) [4]
Germany		Slugs	Andrássy (1983) [26]

Table 1: Geographical distribution of the *Phasmarhabditis* nematode species that have been described along with their references.

In order to demonstrate that the new species is a novel *Phasmarhabditis* species, this study sought to characterize it morphologically and phylogenetically.

Materials and Methods

In Greater Cairo, Egypt, land snails were collected from fields in 2021–2023. The collected individuals were examined under a microscope in a tiny amount of distilled water after being properly cleaned with tap water and then distilled water to remove any externally attached nematodes or microorganisms. Washing was repeated until all organisms were completely removed if any were discovered by microscopic inspection [3]. The suspicious individuals were placed in plastic pots with a tiny amount of dechlorinated water and covered to keep malacophagous insects out. Every day, a microscopic examination was performed to check for nematodes in the water. Additionally, several snails were dissected to check for nematode infections. Lab-bred snails were used to cultivate the isolated nematodes In order to verify the parasitic nature of the isolated nematodes, they were subsequently cultivated for multiple generations on lab-bred snails. Following this, select offspring were prepared for microscopic analysis by collecting specimens from either liberated nematodes or the deceased bodies of affected snails.

Hot triethanolamine formalin (TAF) was used to kill and fix nematodes in suspension, after which they were mounted in glicerol [3,13]. *P. eobaniae* n. sp. females, males, and infectious young larvae were depicted and captured on camera with a Galaxy A03S and an XSZ-107T microscope. An ocular micrometer lens was used to take the measurements, and a micrometer stage was used to adjust them [3]. In a plastic cage, *Eobania vermiculata* (Müller, 1774) was reared using the same method as explained by Azzam [3].

Bioassays

Bioassays were performed on two groups of 30 individuals from each species, including terrestrial snails (*E. vermiculata* Müller, *Cornu aspersum* Müller, *Theba pisana* Müller, *Monacha obstructa* (Pfeiffer), and *Monacha cartusiana* Müller), terrestrial slugs (*Limax flavus* L., *Lehmania marginata* Müller, and *Deroceras laeve* Müller), and aquatic snails (*Biomphalaria alexandrina* (Ehernberg) and *Bulinus truncatus* Audouin the intermediate hosts of *Schistosoma mansoni* Sambon and *Schistosoma heamatobium* Bilharz in Egypt ,respectively. In addition, several insects, including *Galleria melonella* L., *Spodoptera littoralis* (Boisduval), and *Spodoptera frugiperda* Smith larvae and pupae, were tested in six Petri dishes (9 cm) per group consisting of five individuals /dish for each pest.

The first group was exposed to infection with the isolated nematodes (500 I. J. S. per individual) in distilled water (10 mL for aquatic snails and 2 mL for terrestrial gastropods and insects).). The following Capacity Index (C. I) was calculated for each pest based on the methodology outlined by Azzam [3], as follow: CI = M × R/T This calculation was tailored to align with the parameters of the experiment. Where (M) Mortality rate of pests at the end of two weeks, (R) Percentage emission of the infective stage of nematodes in infected gastropods and (T) Time required in days for nematode larvae to progress to the infective stage following the infection date [3].

Molecular analysis

DNA for *P. eobaniae* n. sp. was isolated from 60 individuals, divided into three repeats of 20 individuals each, and put in an Eppendorf tube with 25 µl of buffers, which comprised 12.5 µl of DreamTaq Green PCR Master Mix (2X), 1 µl of each forward and reverse primer, 2 µl of template DNA ranging from (50 ng to 500 ng), and 8.5 µl of nuclease-free water. The samples were gently vortexed to ensure thorough mixing. Denaturation at 95°C for 5 minutes was followed by 40 cycles of 95°C for 30 seconds, annealing at 57°C for 30 seconds, extension at 72°C for 30 seconds, and a final extension at 72°C for 10 minutes. For PCR, 2 µl of supernatant was utilized. The primers 18S: 5'-TTGATTACGTCCCTGCCCTTT-3' (forward) and 26S: 5'-TTTCACTCGCCGTTACTAAGG-3' (reverse) were used to amplify a fragment of rDNA that contained the internal transcribed spacer regions (ITS1, 5.8S, and ITS2) [3 and 13].The SSU18A (AAAGATTAAGCCATGCATG) and SSU26R (CATTCTTGGCAAATGCTTTCG) primers were used to amplify the other segment that contained small subunits [27]. The Wizard SV Gel and PCR Clean-Up System (Promega) was used to visualize the PCR results in an agarose gel and remove bands for DNA extraction [3]. The purified PCR was sequenced using the BigDye terminator cycle sequencing kit v.3.1

(Applied BioSystems, USA) following the precipitation of the PCR product with ethanol and sodium acetate solution. Sequencing products were resolved on an Applied Biosystems model 3730XE automated DNA sequencing system (Applied BioSystems, USA) at Macrogen, Inc., Seoul, Korea.

The Basic Local Alignment Search Tool (BLAST) (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to look for similar sequences in NCBI GenBank. The ITS and SSU rDNA sequences of the *Phasmarhabditis* genus found in databases from various geographic locations were compared to the acquired sequences. Multalin version 5.4.1 [28], available at http: //multalin.toulouse.inra.fr/, was used to perform the alignments. After performing phylogenetic molecular studies on the aligned 18S sequences, the phylogenetic tree was produced using MEGA11's Neighbor-Joining (NJ) methods [29]. The acquired sequences of *P. eobaniae* n. sp. were put in the (NCBI) GenBank database as OQ351290 for ITS rDNA and PP461475 for SSU rRNA genes and will be made public after publishing this article.

Results

New species classification and description:

Kingdom: Animalia - Phylum: Nematoda - Class: Chromadorea - Order: Rhabditida

Suborder: Rhabditina - Superfamily: Rhabditoidea -

Family: Rhabditidae -

Sub Family: Rhabditinae - Genus: *Phasmarhabditis* - Species: *eobaniae* - Author: Azzam

Table 2 detailed the female, male, and infectious stage measurements in micrometers.

Female

It has an elongated cylindrical body that is 1952 ± 353 µm long and 110 ± 17 µm wide. The width of the lip region is 21 ± 2.5 µm (Table 2). Each of the six unique, separated lips has three pairs of terminal labial papillae. The length of Promesorhabdion is 14 ± 1.9 µm. Tubular stoma 24 ± 4. The pharynx is 312 ± 40.3 µm long, with a corpus of 149 ± 22.4 µm, an isthmus of 79 ± 9.3 µm (Table 2), and the nerve ring encircling its anterior portion, a basal bulb of 65 ± 9.5 with a valve (Figure 1a and Figure 2a). The excretory orifice is located close to the basal bulb's anterior region (Figure 1a). After the base of the basal bulb is the cardiac region (Figure 1a). The vulva is located nearly in the middle of the body length (52.7 ± 3.7 %), and in mature females, the paired ovaries reflected inversion to the vulva (Figure 1, b). The oocytes fill the majority of the body cavity, where the embryonated ones are located near the vulva. The vagina is muscular and straight, and the uterus is capacious and involved in some eggs (Figure 1b). The tail is 161 ± 26.7 µm long (Table 2), with a conoid tapering to a filiform terminus (Figure 1 c and d and Figure 2 b). A short phasmid is located on each side of the tail near the terminus. Anus narrow, curved anteriorly, transverse slit, and rectum somewhat inflated (Figure 1d).

Male

It is 1445 ± 254 µm in length and 84 ± 10 µm in width. Lip region 17 ± 1.8 in wide. Promesorhabdion 11 ± 1.1 µm. Stoma 18 ± 2 µm in length (Table 2).The pharyngeal region is 230 ± 29.7 µm in length and is distinctly divided into the corpus 111 ± 16.1, isthmus 60 ± 8.3 (Table 2), and valvular basal bulb 49 ± 6.8 (Figure 1 e).Single testis reflection on the right side of the intestine. Tail sharply pointed, conical, shorter than female, about 50 ± 7.8 µm long and enveloped by an open peloderan bursa, bearing nine prominent radial papillae and phasmid on each side. The GP formula is 2+1+2+1+3 (Figure 1f and g, Figure 2c and d). GP 1-2, 4-5 closed together, also 7-9 close together and near the phasmid. GP 4, 7, 8 and 9 not reaching the bursa margin, GP1-5 pre-cloacal, GP 6 ad cloacal, and GP 7-9 post-cloacal. (Figure 1g, Figure 2d).

Infective Juvenile Stage: Body 1131 ± 266 µm in length and 59 ± 10.4 µm in width (Table 2), typically having second-stage juvenile cuticles (Figure 1h and 2e) and being shorter and more slender than females and males. The lip region is 12 ± 2.2 µm wide (Table 2), in line with the body, and is made up of six different lip sectors, each of which has a papilla. The mouth aperture is closed (Figure 1h and 2f). The length of Promesorhabdion is 9 ± 1.9 µm. The stoma is long and narrow, measuring 16 ± 3.4 µm. The pharyngeal area, which is 180 ± 34.1 µm long, is clearly divided into a short valvular basal bulb (38 ± 7.7), a narrow isthmus (51 ± 10.2 µm) surrounded by a nerve ring in the middle, and a corpus (91 ± 20 µm) with a slight metacorpal extension (Figure 1h).Compared to both males and females, the tail is longer, measuring 171 ± 40 µm in length.

- **Eggs:** The fully grown eggs were 42 ± 6.2 µm wide and 75 ± 10 µm long (Table 2).
- **Type host:** *Eobania vermiculata* (Müller, 1774)
- **Type locality:** Giza, Greater Cairo, Egypt. Latitude 30°, 1'35.6556, Longitude 31 °.208260
- **Site of infection:** Ornamental plants.

- **Site in host:** Head foot region, ovotestis, and hepatopancreas.
- **Rate of infection:** 178 of 948 (18.78%)
- **Type material:** Specimens of female, male, and infective dauer stages were deposited in the collection of animals of the Department of Harmful Animals, Plant Protection Research Institute, Egypt, No KMAN3.
- **Host material:** *Eobania vermiculata* snails deposited in the collections of animals of the Department of Harmful Animals, Plant Protection Research Institute Egypt, No KMAEV2.
- **Etymology:** The specific epithet (*eobaniae*) refers to the type host, *Eobania.*
- **GenBank accession numbers:** OQ351290 for ITS and PP461475 for SSU, ZooBank LSID is urn:lsid:zoobank.org:pub:540AC897-F2B3-45C1-9762-2E2F9304BC7F

Character	Female	Male	I. D. J	Egg
Body length	1952 ± 353 (1320-2469)	1445 ± 254 (1016-1782)	1131 ± 266 (739-1584)	75 ± 10 (54-92)
Body diameter	110 ± 17 (85-158)	84 ± 10 (64-102)	59 ± 10.4 (40-75)	42 ± 6.2 (29-53)
a	17.7 ± 1.9 (14.3-20.2)	17.2 ± 2.1 (12.8-20)	19 ± 1.8 (15.4-21.1)	-
b	6.9 ± .30 (6.5-7.5)	6.5 ± 0.35 (5.9-7.14)	6.4 ± .3(5.8-7.1)	-
c	12.1 ± 0.6(11.1- 13.4)	28.8 ± 2.6(25.7- 33.8)	6.6 ± 0.3(6.4-7.6)	-
ć	2.5 ± 0.2(2.4-3)	1.1 ± 0.1(1-1.3)	4.7 ± 0.6(4- 6.9)	-
d	0.89 ± 0.03 (0.85-0.96)	0. 85 ± 0.03 (0. 8-0.9)	0.8 ± 0.04 (0.7-0.9)	-
e	1.58 ± 0.2(1.3- 2.2)	3.8 ± 0.5(3-4.9)	0.9 ± 0.1(0.7-0.9)	-
F	0.7 ± 0.07(0. 6-0.8)	1.7 ± 0.2(1.4- 2)	0.4 ± 0.04(0.3- 0.4)	-
V	52.7 ± 3.7(48- 64.6)	-	-	-
G1	33.9 ± 2.6(26.4-37)	-	-	-
G2	32.1 ± 2.3(25.1-34.6)	-	-	-
Lip region width	21 ± 2.5(17 - 27)	17 ± 1.8(13 -20)	12 ± 2.2(8-16)	-
Stoma length	24 ± 4(19-34)	18 ± 2(14-23)	16 ± 3.4(11-22)	-
Stoma width	7 ± 1(5-9)	5 ± 0.6(4-6)	4. 5 ± 0.9(3-6)	-
Promesorhabdion length	14 ± 1.9(11- 18)	11 ± 1.1(8- 13)	9 ± 1.9(6- 13)	-
Pharynx length	312 ± 40.3(224-365)	230 ± 29.7(173- 273)	180 ± 34.1(126-230)	-
Corpus length	149 ± 22.4(106-180)	111 ± 16.1(81-134)	91 ± 20(62-130)	-
Isthmus length	79 ± 9.3(59-97)	60 ± 8.3(45-79)	51 ± 10.2(37- 71)	-
Basal bulb length	65 ± 9.5(46-79)	49 ± 6.8(36- 60)	38 ± 7.7(26-51)	-
Distance from anterior end to nerve ring	205 ± 34.7(145-261)	153 ± 26(112-195)	121 ± 29(83-176)	-
Distance from anterior end to excretory pore	251 ± 42(174-316)	190 ± 30(132- 228)	145 ± 34(100- 201)	-
Distance from anterior end to Pharynx base	281 ± 42.5(199-337)	223 ± 40(154-280)	178 ± 42.5(115-248)	-
Tail length	161 ± 26.7(111- 222)	50 ± 7.8(37-64)	171 ± 40(115-238)	-
Anal body diameter	64 ± 8.4(46-79)	46 ± 6.9(32-55)	37 ± 7(27-49)	-
Spicule length	-	67 ± 8.1(55-86)	-	-
Gubernaculµm length	-	34 ± 3.9(27-43)	-	-

Table 2: Measurements in µm of 20 individuals from each of females, males and infective dauer larvae of *Phasmarhabditis eobaniae* n. sp. expressed as mean ± SD and (range).

a: Body length/Body width. , b: Body length/ Distance from anterior end to pharynx base. c: Body length/Tail length. ć: Tail length/Anal body diameter d: Distance from anterior end to excretory pore/Distance from anterior end to pharynx base. e: Distance from anterior end to excretory pore/Tail length. , f : Greatest width/Tail length. G1: Distance from vulva to anterior flexure of gonad/Body length x 100. G2: Distance from vulva to posterior flexure of gonad/Body length x 100. V: Anterior end to valve or middle of gonad/Body length

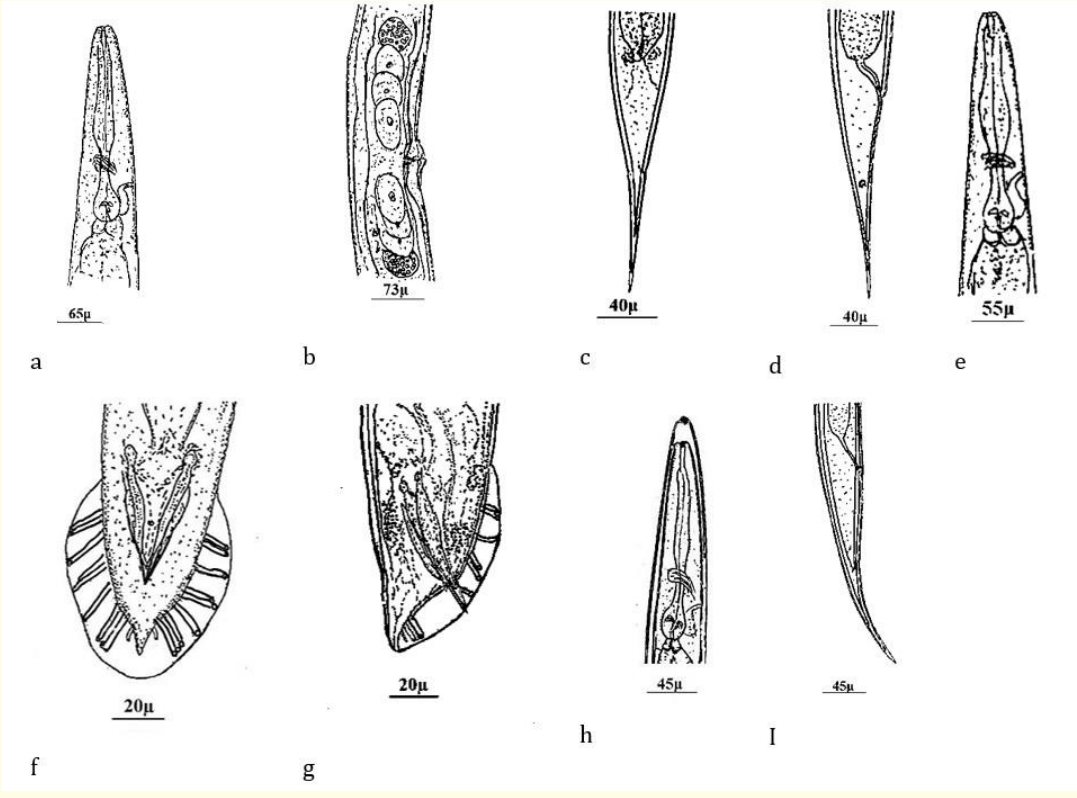

Figure 1: a-i) Line drawing of *Phasmarhabditis eobaniae* n. sp. (Nematoda: Rhabditidae) from the snail *Eobania vermiculata* (Müller) from Great Cairo, Egypt, Africa.

a: Female anterior end, b: Velva region, c: Ventral view of female tail, d: Lateral view of female tail, e: Male anterior end f: Ventral view of male tail g: Lateral view of male tail, h: Infective dauer stage anterior end i: Infective dauer stage posterior.

Relationships and diagnosis: There are currently 21-23 species recognized within the genus of *Phasmarhabditis* Andrássy, 1976. *Phasmarhabditis eobaniae* n. sp. shares many features with those species, i.e., a head with six distinict lips arranged in three pairs, a narrow stoma, a puffed pharyngeal corpus, a fan-like bursa with nine pairs of genital papillae plus a phasmid, a male has a single testis, slightly curved spicules, and a female with paired ovaries and a vulva positioned centrally along the body.

Some of the morphometric characteristics of *P. eobaniae* n. sp. differ from those of other known species. The presence of males, which is absent from *P. hermaphrodita* and *P. californica*, sets it apart from the latter two species. Males are present but uncommon, similar to *P. tawfiki*, but differ in GP formula: 2+1+2+1+3 in *P. eobaniae* n. sp. *vs.* 2 + 1 + 4+ 2 in *P. tawfiki.* It also differs in GP formula with all previously described species. 2+1+2+1+3 in *P. eobaniae* n. sp. *vs.* 1 + 1 +3 + 1 + 3 in *P. eagyptiaca,* characterized by a tail adorned with a spike that extends beyond the bursa's edge, and a domed end of female. *vs.* 1+ 1+ 1+ 2/1+ 3 in *P. thermalis*, *P. safricana*, *P. quinamensis, P. circassica, P. clausliiae*, *P. villasmundi, P. meridionalis, P. bonaquaense, P. neopapillosa,* 1 + 1 + 1 + 2 + 1 + 3 in, *P. bohemica*, *P. zhejiangensis*, *P. huizhouensis* and *P. apuliae* 1 +1 + 1/2 +1 + 3 in *P. thesamica.* 1 + 2 + 2/1 + 3 in *P. akhaldaba,* 1 + 1 + 1 + 2+1 + 1 + 2 in *P. papillosa,* and 1 + 1 + 1 + 1 + 1 + 1 + 1 + 3 in *P. kenyaensis.*

Comparative between the new species and previously recorded Egyptian species

Table 3 shows a comparative between the adult female, male, and infectious stages of the newly identified species *P. eobaniae* and those of the two Egyptian taxa, *P. eagyptiaca* and *P. tawfiki*. As seen in this table and Figure 2 b, h, i, m, n, and *o*. *P. eagyptiaca* females

and males were the longest, while *P. tawfiki* was the shortest. While the females of *P. eobaniae* and *P. tawfiki* exhibited conical tails, *P. eagyptiaca* exhibited copula tails *(*Figure 2, j, k and l*). P. eobaniae* had the longest tail, while *P. eagyptiaca* had the shortest one *(Table 3). Male* GP formula 2+1+2+1+3 in *P. eobaniae* n. sp. (Figure 1 F and g, Figure 2 c and d) *vs.* 1 + 1 + 3 + 1 + 3 in *P. eagyptiaca* and *vs.* 2+1+4+2 in *P. tawfiki* (Figure 2 p and q). In *P. eagyptiaca*, the male tail tip ends with a spike exceeding the bursa margin (but not in *P. eobaniae* and *P. tawfiki* (Figure 2d, p and q). in *P. tawfiki,* GP 1-2 pre-cloacal,GP3 ad cloacal, and GP 4-9 post-cloacal while in *eobaniae* GP1-5 pre-cloacal, GP 6 ad cloacal, and GP 7-9 post-cloacal (Figure 2 d and q).

Character	Female			Male			I. D. J		
	P. eobaniae	***P. eagyptiaca***	***P. tawfiki***	***P. eobaniae***	***P. eagyptiaca***	***P. tawfiki***	***P. eobaniae***	***P. eagyptiaca***	***P. tawfiki***
Body length	1952 ± 353 (1320 -2469)	2007 ± 384 (1430-2656)	1715.9 ± 346.9 (1150-2370)	1445 ± 254 (1016 -1782)	1647 ± 327.3 (1152 - 2158)	1337.5 ± 159.2 (980-1535)	1131 ± 266 (739 -1584)	1112 ± 109.6 (922- 1322)	965.7 ± 109.4 (750-1140)
Body diameter	110 ± 17 (85 -158)	112 ± 15 (89-142)	95.55 ± 13.2 (70-110)	84 ± 10 (64- 102)	93 ± 12.7 (73- 115)	80.9 ± 9.1 (65-100)	59 ± 10.4 (40- 75)	54 ± 6.8 (44-67)	40.3 ± 3.8 (30-45)
a	17.7 ± 1.9 (14.3 - 20.2)	16.8 ± 2.5 (12.8- 20.1)	17.3 ± 2.5 (12-20)	17.2 ± 2.1 (12.8 - 20)	19 ± 1.9 (14-22)	17.9 ± 1.67 (14.8-19.9)	19 ± 1.8 (15.4- 21.1)	22.2 ± 2.3 (19.1- 26.9)	23.9 ± 2.3 (18.8-27.5)
c	12.1 ± 0.6 (11.1 - 13.4)	22.7 ± 3.9 (16- 27.6)	11.1 ± 3.3 (5.9-15.6)	28.8 ± 2.6 (25. 7- 33.8)	26.5 ± 3.9 (17.4- 31.1)	27.5-4.6 (18.2-34. 9)	6.6 ± 0.3 (6.4-7.6)	8.62 ± 1.42 (6.47- 11.8)	7 ± 1.3 (5-8.8)
d	0.89 ± 0.03 (0.85-0.96)	0.8 ± .1 (0.7-1)	0.8 ± 0.1 (0.7-1)	0. 85 ± 0.03 (0.8-0. 9)	0.7 ± .1 (0.6- 1)	6.8 ± 0.1 (0. 7-0.9)	0.8 ± 0.04 (0.7-0.9)	0.8 ± 0.1 (0.6- 1)	0.9 ± 0.1 (0.7-1)
e	1.58 ± 0.2 (1.3- 2.2)	3.2 ± 0.6 (2.2-4.3)	1.8 ± 0.3 (1.3-2.4)	3.8 ± 0.5 (3-4.9)	3.7 ± 0.8 (2.8- 6.3)	3.9 ± 0.7 (2.6-5.1)	0.9 ± 0.1 (0.7-0.9)	1.30 ± 0.2 (1- 1.6)	1.3 ± 0.2 (1-1.6)
f	0.7 ± 0.07 (0. 6-0.8)	1.3 ± 0.1 (1- 1.5)	0.8 ± 0.2 (0.6-1.2)	1.7 ± 0.2 (1.4- 2)	1.5 ± 0.3 (1.1-2.2)	1.6 ± 0.2 (1.3-1.9)	0.4 ± 0.04 (0.3-0.4)	0.4 ± 0.1 (0.3-0.6)	0.3 ± 0.06 (0.2-0.4)
G1	33.9 ± 2.6 (26.4-37))	34.5 ± 5.3 (25.2-41.5)	31.5 ± 7.3 (21-46)	-	-	-	-	-	-
G2	32.1 ± 2.3 (25.1-34.6)	32.2 ± 5.2 (23.2-39.8)	30.1 ± 7. 5 (21-44)	-	-	-	-	-	-
Tail length	161 ± 26.7 (111-222)	88 ± 7 (71-97)	127.95 ± 22.7 (85-140)	50 ± 7.8 (37- 64)	63 ± 10 (47-86)	49.1 ± 3.8 (44-54)	171 ± 40 (115-238)	134 ± 26.6 (100- 172)	144.2 ± 29.3 (105- 180)
Spicule length	-	-	-	67 ± 8.1 (55-86)	74 ± 13.5 (52- 93)	62.9 ± 8.2 (54-75)	-	-	-
Gubernaculum length	-	-	-	34 ± 3.9 (27-43)	35 ± 5.5 (27- 44)	35.9 ± 3.24 (33-40)	-	-	-

Table 3: Comparative between the three Egyptian *Phasmarhabditis* species.

Bioassay

The new nematode species exhibited the ability to eradicate all infected terrestrial snails and slugs, as well as 80 to 90% of aquatic snails and 80% of insect pupae and larvae, with the exception of *G. molenella*, perished entirely .These data indicate that terrestrial snails and slugs are more favorable and suitable hosts for this nematode compared aquatic snails, insect larvae, and pupae; thus, this nematode could be used as a biocontrol agent against those pests (Table 4).

The highest value of capacity index 984.25 reported for *E. vermiculata,* the type natural host of the parasitic nematode followed by *C. aspersum* then *M. obstructa* and *M. cartusiana.* Then slugs followed by aquatic snails and lastly insect larvae and pupae except *G. melonella* larvae which showed a higher C.I (715.49) due to its high susceptibility to parasites.

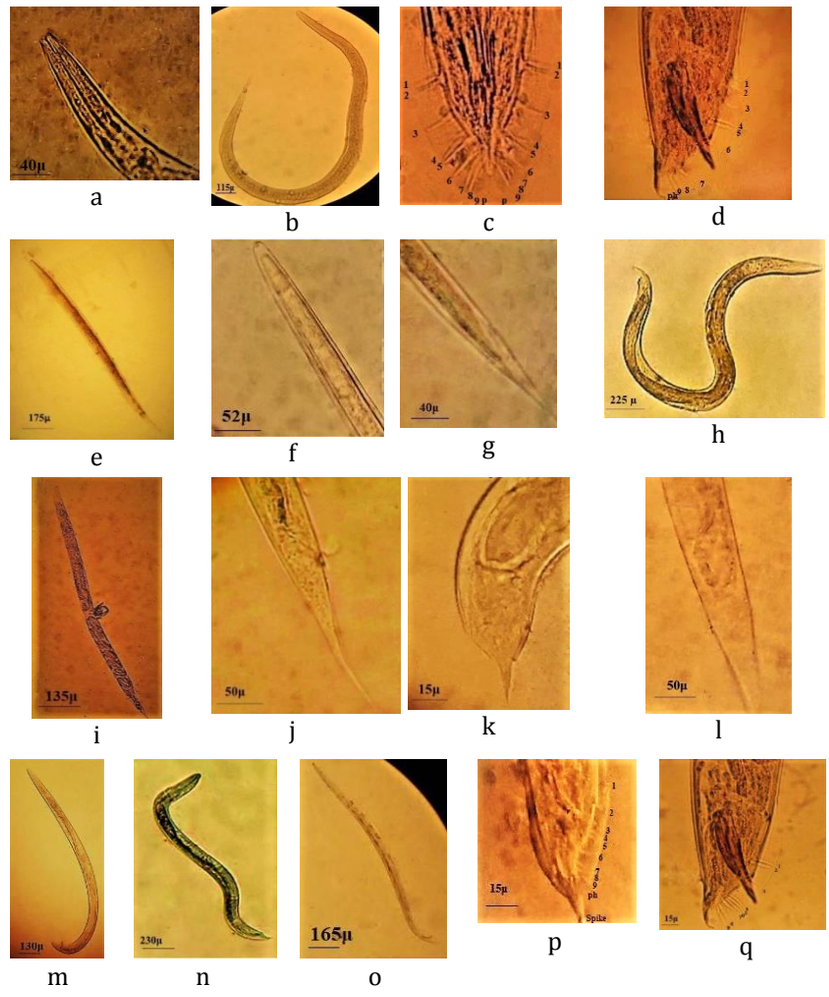

Figure 2: (A-q). Microphotographs of *Phasmarhabditis eobaniae* n. sp., *P. eagyptiaca,* and *P. tawfiki* (Rhabditidae).

a: Female anterior end of *P. eobaniae* n. sp. b: Female body of *P. eobaniae* n. sp. c: Ventral view of male tail of *P. eobaniae* n. sp. d: Lateral view of male tail of *P. eobaniae* n. sp. e: Infective dauer stage of *P. eobaniae* n. sp. f: Infective dauer stage anterior end of *P. eobaniae* n. sp. g: Infective dauer stage tail. h: Female body of *P. eagyptiaca.* i: Female body of *P. tawfik*i. j: Female tail of *P. eobaniae* n. sp. k: Female tail of *P. eagyptiaca.* l: Female tail of *P. tawfiki.* m: Male body of *P. eobaniae* n. sp. n: male body of *P. eagyptiaca* o: Male body of *P. tawfiki.* p: Lateral view of male tail of *P. eagyptiaca.* q: Lateral view of male tail of *P. tawfiki*

Molecular and phylogenetic analysis

The ITS rDNA PCR result of 60 *P. eobaniae* n. sp. individuals yielded 773 pb. with a high bootstrap value of 99.87%, the sequence of *P. eobaniae* n. sp. is consistent with *P. papillosa* (KX267675) from South Africa, *P. papillosa* (MT819980) from Slovenia, and *P. papillosa* (KM510205) from the USA, with a 99.49% compatibility rate. Furthermore, the respective percentages of *P. apuliae* (KX017488) and *P. apuliae* (KX017486) from Italy were 96.64% and 96.25%. The Czech Republic's *P. bonaquaense* (KX017491) achieved a 95.88 percent success rate. *P. pelhami* (OR059186) from North America had 91.56 percent, but *P. californica* (KM510204) had 94.19 per- cent and *P. californica* (KM510203) had 94.08 percent. Further more, *P. eobaniae* n. sp aligned with *P. bohemica* (KX017489) and (KX017490) from the Czech Republic at 88.29%, as well as with *P. pellio* (OR059187) from the USA at 88.11% (Figure 3).

It also coincided in 85.03% with *P. clausliiae* (MK500249) and 84.12% with *P. circassica* (MK500250) from Russia. Additionally, 83.61% with *P. meridionalis* (MG543920) and 83.39% with *P. quinamensis* (MW843494) from Vietnam. However, it coincided in 82.47% with *P. zhejiangensis* from China and 81.55% with *P. hermaphrodita* (KM510201) from North America and 81.37% with *P. kenyaensis* from Kenya, 80.78% with *P. neopapillosa* (OP626247) and (OP626246) from England.

Group	Species	Mortality rate	Ability of died individuals to recovering nematodes	Rate % of individuals recovered nematodes	Infectivity of recovered nematodes	Capacity index	Rank
Terrestrial Snails	*E. vermiculata*	100%	+	100	+	984.252	1
	C. aspersum	100%	+	100	+	906.62	2
	T. pisana	100%	+	70	+	726.89	4
	M. obstracta	100%	+	70	+	739.18	3
	M. cartusiana	100%	+	70	+	739.18	3
Terrestrial slugs	*L. flavus*	100%	+	100	+	572.41	7
	L. marginata	100%	+	90	+	548.45	8
	D. laeve	100%	+	80	+	636.94	6
Aquatic snails	*B. alexandrina*	80%	+	70.83	+	448.22	10
	B. truncatus	80%	+	70.83	+	444.68	11
	P. acuta	90%	+	66.66	+	509.72	9
Insect larvae	*G. melonella*	90%	+	88.88	+	715.49	5
	S. litoralis	80%	+	50	+	428.27	13
	*S. frugiperda**	80%	+	50	+	430.11	12
Insect pupae	*G. melonella*	90%	+	85.18	+	397.83	14
	S. litoralis	80%	+	62.5	+	306.37	15
	*S. frugiperda**	80%	+	62.5	+	299.04	16

Table 4: Results of bioassays assessing the susceptibility of different invertebrate to infection by *Phasmarhabditis eobaniae* under laboratory conditions of 23 ± 2 C° and 65 ± 5% R.H.

*Ten individuals only from field were used.

A well-supported monophyletic subclade was formed by *P. eobaniae* n. sp. appearing in the tree that is closely related to a group of *P. papillosa* (KM510205) from the USA, *P. papillosa* (KX266775) from South Africa, and *P. papillosa* (MT 819980) from Slovenia (Figure 3). This specific subclade is closely related to another subclade dominated by Italian *P. apuliae* (KX017486, KX017487, and KX017488) and *P. bonaquaense* (KX017491) from the Czech Republic, these two subclades together constitute a monophyletic clade. (Figure 3).

A high bootstrap value of 94 .97% for *P. eobaniae* n. sp. SSU (750 bp) demonstrated compatibility with *P. hermaphrodita* (FJ516755 and with all of the UK *P. hermaphrodita* (DQ639980) 91.44% and (DQ639981) 91.31%. In addition to (JQ965811)91.29% from New Zealand (slugs), KM510206)91.58% from the USA (slugs) and *P. tawfiki* (OQ975293) 91.31% from Egypt (snails and slugs) (Figure 4).

It shows a 90.41% compatibility with *P. bonaquaense* (KX017480) from the Czech Republic (slug), and 90.64% with *P. californica* (KM510210) and *P. papillosa* (KM510211) from the USA (slugs) and *P. apuliae* (KX017477) from Italy (slugs). Also it coincident 90.37% with *P. papillosa* (KX267673) from South Africa (slugs) ,90.5% with *P. apuliae* (KX017476) from Italy (slugs),

and 90.7 % with *P. papillosa* (MT797805) from South Africa (slugs) and *P. thermalis* (OR373881) and (OR373882) from Russia (snails and slugs).*P. kenyaensis* (MN626643) from Kenya (slugs) are coincident 90.41 %. Additionally, *P.eobaniae* n. sp. is compatible with *P. huizhouensis* (KP017252) from China (91.33%), *P. thesamica* (OU753546) from Russia (90.4%) ,and *P. bohemica* (KX017478)90.68% and (KX017479) 90.18%from the Czech Republic (slug).Also 90.65% with *P. safricana* from South Africa (slugs)and 90.91 % with *P. ellio.* Finally, 90.51 % with *P. pelhami* (EU196008) and (OR059186) from the USA earthworm.

NJ analyses show a tight relationship with *P. hermaphrodita* (UK/Europe) which linked to two clades (Figure 4). The first clade comprises four species of *P. hermaphrodita* from Europe, including four from the United Kingdom and one from New Zealand, as well as *P. tawfiki* from Egypt/Africa. The second clade was divided to two sub clades, the first one divided also to two sub-subclades, one of which encompasses *P. pellio* and *P.bohemica* while the other includes *P.thermalis* and *P. kenyaensis.* The second sub clade includes 12 species i.e. *P. papillosa, P. apuliae, P. huizhouensis*, *P. bonaquaense*, *P. safricana, P. califórnica* and *P. pelhami.*

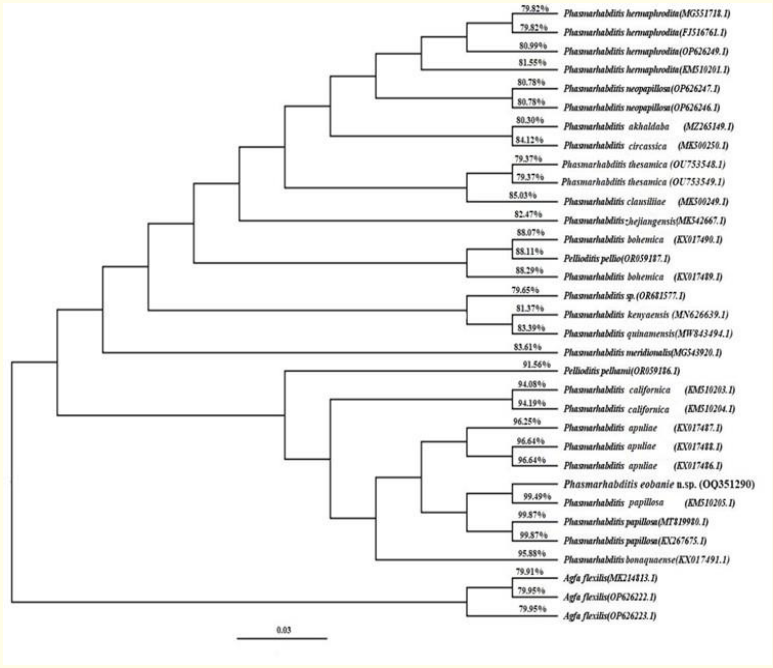

Figure 3: Phylogenetic relationships of *Phasmarhabditis eobaniae* n. sp. (Nematoda: Rhabditidae) from the snail *Eobania vermiculata* (Müller) from Great Cairo, Egypt, Africa, and other *Phasmarhabditis species*, as inferred from (NJ) analysis of 773 bp (ITS) rDNA. Bootstrap values are indicated next to the relevant nodes (as a percentage) *Agfa flexillis* used as an out group.

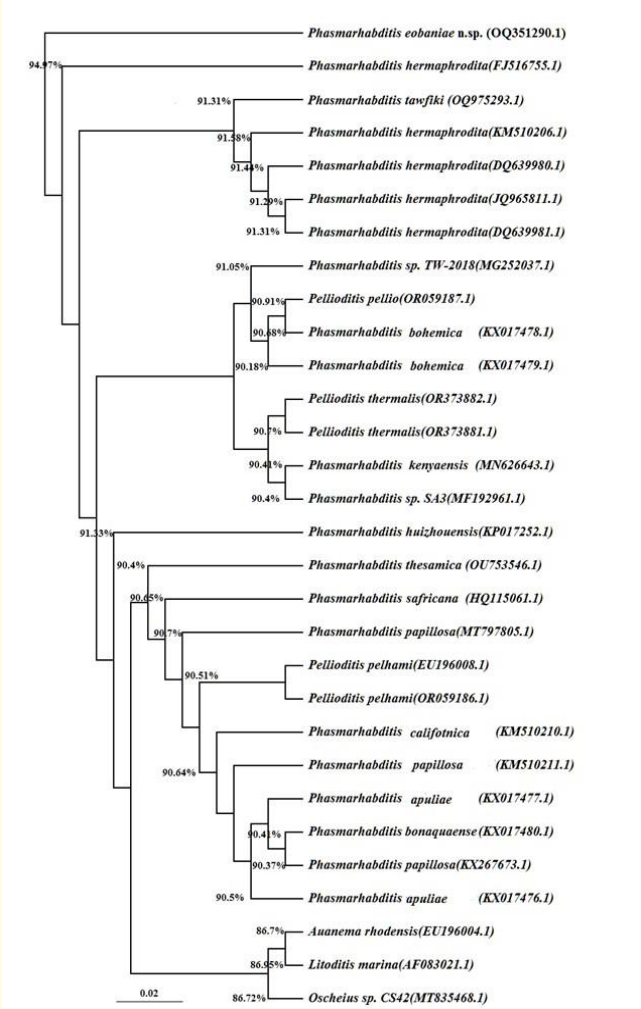

Figure 4: Phylogenetic relationships of *Phasmarhabditis eobaniae* n. sp. (Nematoda: Rhabditidae) from the snail *Eobania vermiculata* (Müller) from Great Cairo, Egypt, Africa, and other *Phasmarhabditis species*, as inferred from (NJ) analysis of 750 bp (SSU) rDNA (18S) genes. Bootstrap values are indicated next to the relevant nodes (as a percentage).

Auanema rhodensis, Litoditis marina and *Oscheius* sp.were used as an out group.

Discussion

The recent publication of sequence data for the species *Pelliodi-tis pellio*, which indicates that it is a member of the same clade as *Phasmarhabditis*, provided Sudhaus (1923) [8] with an explanation for the retreat the name of *Phasmarhabditis* to *Pellioditis*. While in his opnion that *P. tawfiki* is not a legitimately recognized species of *Phasmarhabditis*,although it is also belongs to the same clade as *P. hermaphrodita*, which is sister to the clade of *P. neopapillosa* as seen from the tree of Azzam (2023) [3] and Ivanova and Spiridonov (2023) [17] and isolated only from terrestrial snails and slugs and showed capability to kill all snails and slugs exposed to its infection. Tandingan De Ley., *et al*. (2023) [9] determined that *Phasmarhabditis* is a junior synonym of *Pellioditis,* considering *Pellioditis pellio* is the type species of *Pellioditis.* Additionally, they mentioned that in cases where the genus name (*Pellioditis)* holds seniority, it

supersedes the junior synonym once a type species is moved to a different genus (*Phasmarhabditis*). Consequently, the junior synonym ought to be phased out. It is possible that *Agfa*, Angiostomatidae, and *Pellioditis* are paraphyletic, as suggested by Nermut'., *et al*. (2017) [11], Tandingan De Ley., *et al*. (2016) [10], Pieterse., *et al*. (2020) [12], Gorgadze., *et al*. (2022) [13], and Ivanova., *et al*. (2022) [14]. Furthermore, *Phasmarhabditis* and *Pellioditis* species formed two unrelated clades, according to Bayesian and maximum parsimony analyses of all genes. Based on molecular evidence, the synonymy of these two genera can be rejected [9,15,16].

In Sudhaus's opinion [8], the phylogenetic position of *Angiostoma* has not yet been demonstrated with any degree of certainty, particularly since only members of the gastropods are bound to the *Limacis* group, whereas *Pellioditis* is necromenic for terrestrial gastropods and earthworms. It is noteworthy that *Pellioditis pellio* and *P. pelhami* were isolated from earthworms solely and infected gastropods only experimentally rather than in nature [9]. However, several species of *Angiostoma* have been isolated from terrestrial snails and slugs, i.e., *Angiostoma amilacis*, which parasitizes milacid and agriolimacid slugs, and closely related to *A. asamati* Spiridonov 1985, which was recovered from a slug [30]. *A. spiridonovi* from *Limax flavus* [31] and *A. kimmeriensis* from terrestrial mollusks [32]. In a zonitid snail, *A. zonitidis* was discovered [33]. Additionally, land snail parasite *A. glandicola* [34] *A. namekuji* from slugs in Japan [35], *A. norvegicum*, *A. dentiferum* (Mengert, 1953), and *A. limacis* (Dujardin, 1845) from arionid slugs [36]. *Angiostoma gandavense*, *Agfa flexilis*, and *Angiostoma margaretae* from UK slugs [37], *A. gandavense* and *A. dentiferum* from Belgium's *Arion hortensis* slug [38]. Even so, Sudhaus (1923) [8] thought that this genus had tenuous connection with gastropods, and suggesting that *Pellioditis* functioned as a necromenic for gastropods.

Based on Hodda's research [39], *Pellioditis* and *Phasmarhabditis* are distinct genera. As the reasoning behind the name alteration is deemed illogical, *Phasmarhabditis* stands as the valid name for the genus and should not be reverted to *Pellioditis*.

From the above-mentioned it is evident that *Phasmarhabditis* (Andrassy, 1976) and *Pellioditis* (Dougherty, 1955) are two different genera [39]. Consequently, *Pellioditis* may fall within the *Phasmarhabditis* clade, which is paraphyletic like *Angiostoma* and *Agfa*, [10-14]. Consequently, *Pellioditis pellio* and *P. pelhami* might be members of the genus *Pellioditis* (Dougherty, 1955).Thus, *P. eobaniae* n. sp. is the 22nd species in the globe, the fifth species in Africa, and the third species documented and described from Egypt. The morphometric characteristics, female tail appearance, male genital papillae formula, presence or lack of males, and molecular phylogenetic analysis are currently used to distinguish registred species in the genus *Phasmarhabditis*. Generally, some factors, i.e., artificial growth media, food source, host [7], and size of the host [6], have a major impact on morphological measurements. This indicates that it is challenging to distinguish between species based solely on body size.

The idea that *P. eobaniae* n. sp. is a new species of *Phasmarhabditis* is supported by the fact that its bursal papillae formula is different from that of any other species that has been breviously identified. Strong bootstrap support values of *P. papillosa* (99.49%, 99.87%), and *P. apuliae* (96.25–96.64%) indicate that *P. eobaniae* n. sp. ITS is most closely linked to the *P. papillosa* group, based on the phylogenetic connection. *P. eobaniae* n. sp. was positioned by SSU close to *P. hermaphrodita*, with bootstrap support values ranging from 90.31% to 94.97%. These show that the nematode belongs to the genus *Phasmarhabditis*. However, due to clear morphological variations from these species, it is not the same species as previously found.

Therefore, it is possible to confirm that it is a novel species of *Pasmarhabditis*.

The new nematode species could kill all afflicted terrestrial snails and slugs. *P. eagyptiaca* killed 100% of terrestrial snails and slugs and 70% of aquatic snails, according to Azzam [3]. Hundred percent of the *D. reticulatum* slugs perished after 12 days of *P. safri-*

cana infection, according to Ross., *et al*. [22]. Nermut., *et al*. [15] did not report mortality in the slug *Milax sowerbyi* (Ferussac, 1823) (Gastropoda, Stylommatophora, Milacidae) and the citrus snails *Theba* sp., (Gastropoda, Stylommatophora, Helicidae). The reason might be attributed to the substantial impact of bacteria on pathogenicity, similar to *P. hermaphrodita*.

Conclusion

With high bootstrap support values, the phylogenetic tree shows a tight relation between *P. eobaniae* n. sp. and other species in the genus *Phasmarhabditis*; yet, it differs morphologically from all described species in basic taxonomic features. This indicates that it is not the same species that was previously registered. Consequently, it could be verified that *P. eobaniae* is a new species of the *Phasmarhabditis* genus.

The laboratory bioassay conducted on *P. eobaniae* n. sp. indicated that it is capable of serving as biological control for terrestrial snails and slugs and partialy some aquatic snails and insects.

It's important to continue exploring the ecological implications and potential applications of *P. eobaniae* n. sp. in pest management strategies, considering its demonstrated effectiveness against various snails, slugs, and potentially aquatic organisms. Further research and field studies can provide valuable insights into harnessing the biological control capabilities of this newly identified species for sustainable pest control practices.

Declarations

Conflict of interest

The authoress has no competing interests to declare that are relevant to the content of this article.

The authoress has no financial or proprietary interests in any material discussed in this article.

Ethics approval and consent to participate

No approval of research ethics committees was required to accomplish the goals of this study because experimental work was conducted with an unregulated invertebrate species.

Consent for publication

Not applicable

Author contributions KA

All work.

The authoress read and agreed with the content of manuscript to submission.

The authoress confirms that the content of the manuscript was not published elsewhere.

Funding: No funding was received for conducting this study.

Bibliography

1.Andrassy I. "Evolution as a basis for the systematization of nematodes". London, UK, Pitman Publishing 286 (1976).

2.Azzam KM. "Description of nematode *Phasmarhabditis tawfiki* n. sp. isolated from Egyptian terrestrial snails and slugs". *Journal of the Egyptian German Society of Zoology* 42D (2003): 79-87.

3.Azzam KM. "*Phasmarhabditis eagyptiaca* n. sp. (Nematoda: Rhabditidae) isolated from Egyptian terrestrial snails and its role as control bio agent to gastropods". *Acta Parasitologica* 68.3 (2023): 637-650.

4.Hooper DJ., *et al*. "Some observations on the morphology and protein profiles of the slug parasitic nematodes *Phasmarhab- ditis hermaphrodita* and *P. neopapillosa* (Nematoda: Rhabditi- dae)". *Nematology* 1 (1999): 173-182.

5.Nermut' J., *et al*. "The effect of different growing substrates on the development and quality of *Phasmarhabditis hermaphro- dita* (Nematoda: Rhabditidae)". *Biocontrol Science and Tech- nology* 24 (2014): 1026-1038.

6.Azzam KM and El-Abd N. "First record of *Phasmarhabditis* sp. from eggs of *Eobania vermiculata* (Müller) snails in Egypt and their response to host size". *Egypt. Journal of Biological Pests Control* 31.48 (2021): 722-728.

7.Tandingan De Ley I., *et al*. "*Phasmarhabditis:* The slug and snail parasitic nematodes in North America". ©CAB International (2017): 560-578.

8.Sudhaus W. "An update of the catalogue of paraphyletic 'Rhabditidae'(Nematoda) after eleven years". *Soil Organisms*

95.1 (2023): 95-116.

9. Tandingan De Ley I., *et al*. "*Pellioditis pelhami* n. sp. (Nematoda: Rhabditidae) and *Pellioditis pellio* (Schneider, 1866), earthworm associates from different subclades within *Pellioditis* (syn. *Phasmarhabditis* Andrássy, 1976)". *PLOS ONE* 18.9 (2023): e0288196.

10. Tandingan De Ley IT., *et al*. "Description of *Phasmarhabditis californica* n. sp. and first report of *P. papillosa* (Nematoda: Rhabditidae) from invasive slugs in the USA". *Nematology* 18 (2016): 175-193.

11. Nermut' J., *et al*. "*Phasmarhabditis bohemica* n. sp. (Nematoda: Rhabditidae), a slug-parasitic nematode from the Czech Re- public". *Nematology* 19 (2017): 93-107.

12. Pieterse A., *et al*. "*Phasmarhabditis kenyaensis* n. sp. (Nemato- da: Rhabditidae) from the slug, *Polytoxon robustum*, in Kenya". *Nematology* 23 (2020): 229-245.

13. Gorgadze O., *et al*. "*Phasmarhabditis thesamica* n. sp. (Nema- toda: Rhabditidae), a new slug nematode from southern slope of Caucasus, Georgia". *Nematology*.24.6 (2022): 617-629.

14. Ivanova ES., *et al*. "*Phasmarhabditis villasmundi* sp. n. infecting land gastropods in the Nature Reserve 'Speleological Complex Villasmundo - S. Alfio' in Syracuse Province, Sicily". *Nematol- ogy* 25.2 (2022): 169-180.

15. Nermut' J., *et al*. "*Phasmarhabditis apuliae* n. sp. (Nematoda: Rhabditidae), a new rhabditid nematode from milacid slugs". *Nematology* 18 (2016a): 1095-1112.

16. Nermut' J., *et al*. "*Phasmarhabditis bonaquaense* n. sp. (Nema- toda: Rhabditidae), a new slug-parasitic nematode from the Czech Republic". *Zootaxa* 4179 (2016b): 530-546.

17. Ivanova ES and Spiridonov SE. "Synopsis of gastropod-asso- ciated nematodes of Ciscaucasia (Russian Federation) with the description of a new species of *Pellioditis* Dougherty, 1953 (syn. *Phasmarhabditis* Andrássy, 1976)". *Journal of Helmin- thology* 97.e90 (2023): 1-20.

18. Ivanova ES., *et al*. "*Phasmarhabditis akhaldaba* sp. n. associ- ated with a slug *Deroceras reticulatum* in Lesser Caucasus mountains in Republic of Georgia". *Russian Journal of Nematol- ogy* 29.1 (2021): 75-88.

19. Ivanova ES and Spiridonov SE. "*Phasmarhabditis quinamensis* sp. n. (Nematoda: Rhabditidae) from tropical terrestrial gas- tropods in southern Vietnam". *Nematology* 33.1 (2021): 1-15.

20. Zhang CN and Liu QZ. "*Phasmarhabditis zhejiangensis* sp. nov. (Nematoda: Rhabditidae), a new rhabditid nematode from Zhejiang, China". *PLOS ONE* 15.11 (2020): e0241413.

21. Ivanova ES., *et al*. "Two new species of *Phasmarhabditis* An- drássy, 1976 (Nematoda: Rhabditidae) associated with land snails in Northwest Caucasus, Russian Federation: description and molecular affiliation". *Nematology* 22 (2020): 179-197.

22. Ross JL., *et al*. "*Phasmarhabditis safricana n*. sp. (Nematoda: Rhabditidae), a parasite of the slug *Deroceras reticulatum* from SouthAfrica". *Zootaxa* 4420 (2018): 391-404.

23. Ivanova ES and Spiridonov SE. "*Phasmarhabditis meridionalis* sp. n. (Nematoda: Rhabditidae) from a land snail *Quantula stri- ata* (Gastropoda: Dyakiidae) from southern Vietnam". *Russian Journal of Nematology* 25 (2017): 129-140.

24. Huang RE., *et al*. "Morphological and molecular characteriza- tion of *Phasmarhabditis huizhouensis* sp. nov. (Nematoda: Rhabditidae), a new rhabditid nematode from south China". *PLOS ONE* 10.12 (2015): e0144386.

25. Morand S. "Contribution a l` etude d`un systeme hotes-para- sites: Nematodes associes a quelques mollusques terrestres". These de Doctorat, Universite de Rennes. I. France (1988): 335.

26. Andrássy I. "A taxonomic review of the suborder Rhabditina (Nematoda: Secernentia)". Paris, France, ORSTOM (1983): 242.

27. Wang A., *et al*. "Molecular Phylogeny Inferred from 18S rRNA Gene Sequences of Nematodes Associated with *Cernuella vir- gata*, a Pest Snail in Australia". *Journal of Phylogenetics and Evolutionary Biology* 3 (2015): 148.

28. Corpet F. "Multiple sequence alignment with hierarchical clus- tering". *Nucleic Acid Research* 16.22 (1988): 10881-10890.

29. Tamura K., *et al*. "Molecular Evolutionary Genetics Analysis Mega 11Version 11.0.13". *Molecular Biology and Evolution* 38 (2021): 3022-3027.

30. Spiridonov SE. "*Angiostoma asamati* n. sp. (Angiostomatidae: Rhabditida)-new species of nematodes from slugs (Mollus- ca)". *Helminthologia* 22.25 (1985): 3-261.

31. Morand S. "*Angiostoma spiridonovi* n. sp. (Nematoda:Angiostomatidae) from *Limax flavus* (Gastropoda: Limacidae)". *Journal of the Helminthological Society of Wash- ington* 59 (1992): 212-217.

32. Korol EN and Spiridonov SE. "*Angiostoma kimmeriensis* sp. n. and *Agfa tauricus* sp. n. - parasitic Rhabditida (Nematoda) from Crimean terrestrial molluscs". *Helminthologia* 28 (1991): 179-182.

33. Ivanova ES and Wilson MJ. "Two new species of *Angiostoma* Dujardin, 1845 (Nematoda: Angiostomatidae) from British terrestrial molluscs". *Systematic Parasitology* 74 (2009): 113-124.

34. Ivanova ES and Spiridonov SE. "*Angiostoma glandicola* sp. n. (Nematoda: Angiostomatidae): a parasite in the land snail *Megaustenia* sp. from the Cat Tien Forest, *Vietnam". Journal of Helminthology* 84.3 (2010): 297-304.

35. Waki T., *et al*. "*Angiostoma namekuji* n. sp. (Nematoda: Angios- tomatidae) from terrestrial slugs on Oshiba Island in the Seto Inland Sea, Japan". *Systematic Parasitology* 95 (2018): 913-920.

36. Ross JL., *et al*. "*Angiostoma norvegicum* n. sp. (Nematoda: Angi- ostomatidae) a parasite of arionid slugs in Norway". *Systematic Parasitology*. 94 (2017): 51-63.

37. Andrus PS., *et al*. "Nematodes and trematodes associated with terrestrial gastropods in Nottingham,England". *Journal of Hel- minthology* 96.e81 (2022): 1-13.

38. Cortes MA., *et al*. "Nematodes associated with terrestrial gas- tropod molluscs in Belgium and additional characterisation of *Pellioditis californica* and *P. hermaphrodita". Journal of Helmin- thology* 98.e27 (2024): 1-14.

39. Hodda M. "Phylum Nematoda: a classification, catalogue and index of valid genera, with a census of valid species". *Zootaxa* 5114.1 (2022): 1-289.