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RESEARCH ARTICLE

Isolation and molecular characterization of an entomopathogenic nematode, *Heterorhabitis* sp. from an arable land in Nigeria

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Abstract

The occurrence of entomopathogenic nematodes (EPNs) in arable soil samples from Nigeria was investigated using Baermann extraction tray and insect-bait (White's trap) techniques. Isolates were tested for infectivity using the larvae of *Galleria mellonella* (greater moth) and *Tenebrio molitor* (mealworm). The study revealed a new species of *Heterorhabditis* (MT371593) in soil samples that were randomly collected from an arable farmland cultivated to cassava TMS-30572 at the Teaching and Research Farm of Landmark University, Nigeria. Amplification of the internal transcribed spacer region (ITS) of the ribosomal DNA produced a nucleotide sequence of 933 base pairs (bp). A BLASTN search of GenBank showed that the sequence of the Nigerian isolate is identical at 99% similarity to *Heterorhabditis* sp. from Thailand. Infectivity test of the isolate showed 100% mortality against *T. molitor* larvae within 48 h of exposure while 80% mortality was recorded on *G. mellonella* only after 1 week of exposure. This is the first account of the *Heterorhabditis* sp. in Nigeria. The varying degrees of infectivity against mealworm and wax moths observed in this study proved that the Nigerian isolate of *Heterorhabditis* sp. could potentially be an attractive option in the management of insect pests of economic crops.

Keywords: biological control, EPNs, *Heterorhabditis* sp., ribosomal DNA, *Tenebrio molitor*

1. Introduction

Entomopathogenic nematodes (EPNs) are obligate soil-inhabiting parasites of many insects. They are ubiquitous in both cultivated and uncultivated soils all over the world. EPNs are widespread in their distribution and have been reported from all the continents of the world except in Antarctica (Campos-Herrera *et al*. 2012; Bhat *et al*. 2020). The potential of EPNs to acts as natural enemies and their ability to suppress the population of insect pests of agricultural importance has made them attractive options as biological control agents in pest management. Their application has formed part of a more integrative approach towards reducing crop losses and frequent damage to crops, including direct injury from pests and damage that comes indirectly from the impact of chemical insecticides as well as other agronomic practices that are aimed at mitigating against insect pests.

More recently, research into the biocontrol potential of EPNs is gaining more attention and has opened up opportunities to explore the rich diversity of nematode species that are beneficial to man and could have commercial value. In Europe and many developed countries of the world, EPNs have been commercialized and have been successfully used for pest management (Lacey and Georgis 2012; Lacey *et al*. 2015; Malan and Ferreira 2017). In countries such as Australia, North America, Europe, Japan, China and Australia commercial applications of EPNs have been successful. Also, many high-value plantations have been protected from the devastating effects of insect pests by the strategic application of EPNs (Ehlers 1996; van Zyl and Malan 2014; Malan and Moore 2016; Gulcu *et al*. 2019; Kapranas *et al*. 2020).

EPNs have potential value as a non-toxic alternative to chemical pesticides, where resistance to insecticides has developed (Ehlers 2001) thereby enabling producers to use an additional biological resource to control pests in an environmentally friendly manner (Platt *et al*. 2020). Biological control using EPNs could be incorporated into existing or emerging IPM strategies (Abd-Elgawad 2019) by developing compatible methods (Shapiro-Ilan *et al*. 2014, 2017; Abd-Elgawad 2017a, b) that are complimentary to chemical nematode management methods (Stevens and Lewis 2017) and synergistic with other agricultural inputs in IPM programmes (Laznik and Trdan 2014, 2017; Malan and Moore 2016; Bajc *et al*. 2017; Gulcu *et al*. 2019; Kapranas *et al*. 2020). When properly applied, EPNs can control a variety of soil-dwelling insect pests (Hiltpold 2015) as well as aboveground herbivorous insects (Shapiro-Ilan *et al*. 2017). However, different factors comprising soil pH, texture, aeration, temperature and atmospheric CO_2 can affect the efficacy of EPNs against many aboveground insect pests (Shapiro-Ilan *et al*. 2012; Hiltpold *et al*. 2020). Lacey and Georgis (2012) gave a detailed account of the successful commercialization and application of EPNs for the control of various insect pests of above and below ground. Reports from South Africa also show that extensive research has been done on the biocontrol potential of EPNs against codling moth, *Cydia pomonella* L. (Lepidoptera: Tortricidae); false codling moth, *Thaumatotibia leucotreta* L. (Lepidoptera: Tortricidae); wax moth *Galleria mellonella* L. (Lepidoptera: Pyralidae) and mealworm *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) (De Waal *et al*. 2011; van Zyl and Malan 2014; Odendaal *et al*. 2016; Malan and Ferreira 2017). Although Galleria larvae is the most commonly used insect bait for EPNs (Woodring and Kaya 1988), there are indications that mass production and commercial application of EPNs have been achieved with mealworm larvae as the insect host (Shapiro-Ilan *et al*. 2012; van Zyl and Malan

2014; Rahoo *et al*. 2019).

Increased awareness on the use of EPNs as effective non-chemical alternatives to insect pest control has resulted in more surveys being conducted across the globe in order to identify and isolate new species that are more virulent and locally adapted to the environmental conditions of the region (Tarasco *et al*. 2015; Yan *et al*. 2016; Kour *et al*. 2020). In Nigeria, studies on the potential use of indigenous EPNs for biological control of insect pests are attracting attention and some species belonging to the families Heterorhabditidae and Steinernematidae have been described from some states within the country. They include: *Heterorhabditis bacteriophora* Poinar (Nematoda: Heterorhabditidae), *H*. *indica* Poinar, Karunakar and David (*Rhabditida: Heterorhabditidae*), an unidentified *Heterorhabditis* sp. and *Steinernema feltiae* Filipjev (Rhabditida: Steinernematidae) (Akyazi *et al*. 2012; Aliyu *et al*. 2015, 2016; Claudius-Cole 2018).

The isolation of indigenous and native species of EPNs is important while searching for potential biocontrol options that have commercial value, antagonistic to indigent insect pests, and adaptable to a range of environmental conditions. Accurate distribution and identification of these indigenous species coupled with a good understanding of their nematode-host interaction capacity are key considerations for their long-term commercial production. This exploit is likely to contribute to the global initiatives at promoting agriculture and sustainably thereby resolving the challenges of poverty and hunger.

This current investigation is therefore aimed at identifying local EPNs that could be incorporated as biocontrol agents into existing Integrated Pest Management (IPM) programs. It also aims to provide baseline information that demonstrates the biocontrol potential of the Nigerian isolate of *Heterorhabditis* sp. against *T*. *molitor*.

2. Materials and methods

2.1. Site description

Samples were randomly collected from different locations of arable farmlands at the Teaching and Research farm of Landmark University, Omu-Aran, Nigeria. The farm is located at 8.1248630°N, 5.0764680°E and 143 m a.s.l. This region is typically a tropical savannah vegetation with an annual rainfall of 1 364 mm and mean temperature of about 25.3°C. The farm was cultivated to cassava (*Manihot esculenta*) variety TMS-30572 for two years after which the land was abandoned to fallow. Soil samples for this study were collected, two years into the fallow period. Conventional tillage was practiced and crops were harvested

after two years after planting.

2.2. Soil sampling and nematode isolation

A total of 30 core soil samples were randomly collected from 1 ha each of a cassava plantation, fallowing cassava and uncultivated farmlands. The samples were collected to a depth of about 20 cm into the soil. The soil samples were then bulked, mixed thoroughly with a hand spade and from them, composite samples were taken for nematode assay. Modified method of Coyne *et al*. (2014) was used for nematode extraction and the set-up was left undisturbed in the laboratory. Several infective stage juveniles (IJs) of the EPNs were observed from the extraction tray after 48 h. Morphological observation for the identification of the nematodes and their morphological features was done under a Leica DM2000 compound microscope.

2.3. Trapping for entomopathogenic nematodes

Entomopathogenic nematodes were isolated in the laboratory from soil samples by trapping with mealworm as the insect bait (Bedding and Akhurst 1975; De Waal *et al*. 2011). Ten mealworm larvae were added to soil samples in separate 500 mL plastic containers. The containers were closed with lids and incubated in a dark room at 25°C. Dead larvae were removed from the soil after a period of 6–7 days and transferred to a modified White's trap (White 1927). This was kept at room temperature until emergence of IJs. The infective juveniles were then harvested after a period of 7 days, maintained by recycling through *T*. *monitor* (Dutky *et al*. 1964) and stored in vented 500 mL culture flasks at 14°C.

2.4. Test for infectivity

Six Petri dishes were lined with filter paper, and *T*. *molitor* larvae were placed in 3 Petri dishes, while five *Galleria mellonella* larvae were placed in the remaining 3 Petri dishes. A total of 1 mL of water, which contains about 800 IJs of the EPNs was added into each Petri dish. Each dish was sealed with parafilm and placed in an incubator at 25°C. The insects were examined after 48 h for mortality by means of dissection (Platt *et al*. 2018).

2.5. Nematode identification

Identification of the nematodes was done using both morphological observations and molecular characterization. Nematodes were dissected from infected *T*. *molitor* cadavers and all life stages of the nematodes (IJs, males, females, and hermaphrodites) were observed under a high power Leica DM2000 Compound Microscope (Leica Microsystems, USA). However, molecular analysis was considered as the primary approach for identifying the species of the nematode. Molecular characterization of the isolates was performed by analysis of the ITS rDNA sequences.

2.6. DNA extraction and molecular identification of nematodes

DNA was extracted from single young female nematodes. Nematodes were picked with sterile needles and placed on the side of 0.2 mL Eppendorf PCR tubes that contained 30 μL lysis buffer (500 mmol L^{-1} MgCl$_2$, 10 mmol L^{-1} DTT, 4.5% Tween 20, 0.1% gelatine and 3 μL proteinase K at 600 μg mL^{-1}). Thereafter, the nematodes were cut into aseptically into many parts with the aid of a sterile insulin needles. The PCR tubes were kept at –80°C for 15 min, and then incubated at 65°C for 1 h and at 95°C for 10 min in a thermocycler.

PCR for the amplification of the ITS regions of the ribosomal DNA was done in a thermocycler using KAPA2G™ Robust Hotstart Ready Mix (KAPA Biosystems, USA) with the primer sets described by Vrain *et al*. (1992) which comprised of a forward primer 18S (TTGATTACGTCCCTGCCCTTT) and reverse primer 26S (TTTCACTCGCCGTTACTAAGG). The cycling condition include; 1 cycle of 94°C for 7 min followed by 35 cycles of 94°C for 60 s, 50°C for 60 s and 72°C for 60 s and a final extension of 72°C for 10 min. The PCR products were separated on 1.5% agarose gel stained with ethidium bromide and visualised under UV light with a transilluminator imaging system.

2.7. Sequencing and phylogenetic analysis

The PCR products were purified using the Nucleo-Fast Purification System (Macherey Nagel, Waltham, Massachusetts, USA). The purified DNA products was sequenced in both directions with the Big Dye Terminator V1.3 Sequencing Kit, followed by the use of electrophoresis on the 3730xl DNA Analyser (Applied Biosystems, USA) at the DNA Sequencing Unit (Central Analytical Facilities, Stellenbosch University, South Africa). Sequence editing and assembly was done using the software for biological sequence alignment editor, Bioedit 7.2 (Hall 1999) and the newly obtained sequence was compared for similarity against other sequences of *Heterhorhabditis* which were obtained from GenBank using BLASTN (Altschul *et al*. 1997). The newly obtained sequence was deposited on the GenBank with accession no. MT371593.

The phylogenetic analysis involved 16 nucleotide sequences of *Heterorhabditis* with *Caenorhabditis elegans* Maupas (Nematoda: Rhabditidae) as outgroup. Sequences

were aligned using Multiple alignment program for amino acid or nucleotide sequences, MAFFT version 7 (Katoh and Standley 2013). Evolutionary analyses were conducted in MEGA X version 10.0.5 (Kumar *et al*. 2018) and the confidence intervals for the various branching patterns in the trees were measured using bootstraps (Felsenstein 1985) with 1 000 replicates. Estimates of the evolutionary divergence between sequences was done using pairwise distance and the number of base differences per sequence are shown in Table 1. Evolutionary analyses were conducted in MEGA X (Kumar *et al*. 2018).

3. Results

A preliminary investigation showed that the *G. mellonema* larvae were not killed after 48 h of exposure to the EPNs. However, 90% mortality was observed after 8–10 days of incubation. IJs were recovered from the cadavers after two weeks of incubation. The mealworm larvae (*T. molitor*) however proved to be a better bait for the *Heterorhabditis* sp. with a 100% mortality rate after 48 h of incubation (Figs. 1 and 2). All life stages of *Heterorhabditis* sp. were observed from the samples obtained from *G. mellonella* and *T. molitor* cadavers. Males were also observed from the second generation of the EPNs.

3.1. Description

Hermaphrodite (First generation hermaphrodite) has a body that is long and robust, takes a C shape when relaxed with heat. The head is slightly rounded and continuous with the body and has 6 distinct protruding pointed lips surrounding the oral aperture. Cheilostome is short with refractile rhabdions. Amphidial pore is obscure. The esophagus is rhabditoid, corpus is cylindrical, metacorpus is undifferentiated and basal bub is pyriform with a distinct valve. Nerve ring surrounds the isthmus posterior to the basal bulb. The vulva is located near the middle of the body and has protruding vulva lips. The tail is tapering to

a pointed terminus (Fig. 3). The 2nd generation female is amphimictic. The genital tract is didelphic and reflexed while the vulva is located close to the middle of the body and has protruding vulva lips. The tail is conoid and tapers to a pointed end. Eggs are oval and arranged in visible rows in the hermaphroditic females (Figs. 4 and 5). Males from the 2nd generation have single testis, reflexed twice. Spicules are paired, separate and slightly curved. Gubernaculum is curved ventrally between the spicules. Bursa is peloderan and adorned by the complement of nine pairs of genital papillae.

The infective juveniles were observed with slightly curved slender bodies that gradually taper at the posterior and ensheathed in the cuticle of the second stage juvenile. The
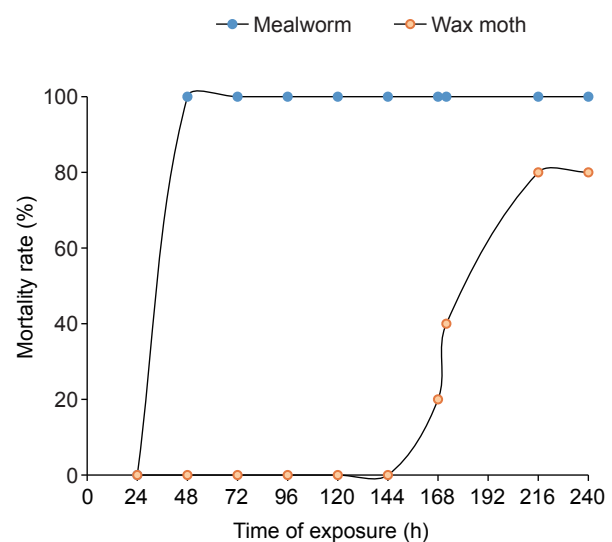

Fig. 2 Mortality rate of *Galleria mellonella* and *Tenebrio molitor* after exposure to *Heterorhabditis* sp.
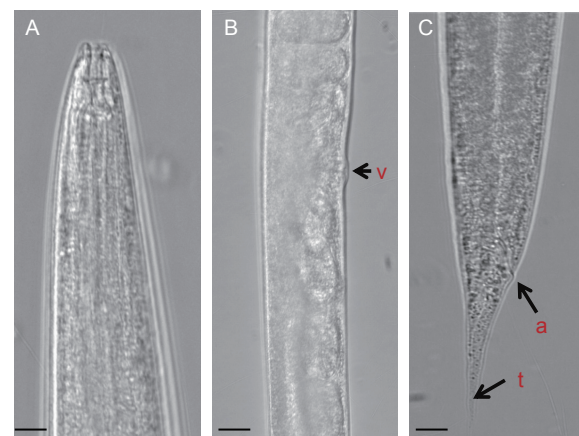

Fig. 3 Light micrographs of *Heterorhabditis* sp. A, head region. B, mid-body region. C, tail region. v=vulva; a=anus; t=tail. Scale bar=50 µm.

Fig. 1 Dead *Tenebrio molitor* larvae after exposure to *Heterorhabditits* sp. in Petri dish.

lip region is continuous and longitudinal ridges run through most of the body length. Mouth and anus are closed. The tail is pointed.

3.2. Phylogenetic analysis

The result of the amplification of the internal transcribed spacer regions (ITS) produced a nucleotide sequence of 933 base pairs (bp) which comprise of the partial 18S, ITS1, 5.8S, ITS2, and partial 28S. A BLASTN search of GenBank revealed that the sequence obtained from the Nigerian isolate was identical at 99% similarity to those of *Heterorhabditis* sp. from Thailand. The pairwise distances of the ITS rDNA regions between *Heterorhabditis* species is shown in (Table 1).

Phylogenetic analysis was based on the maximum parsimony (MP) method. The sequence of the ITS rDNA region confirmed that the Nigerian isolate grouped with other isolates of *Heterorhabditis*, when compared with other species of the genus. The phylogenetic relationships of the Nigerian isolate in comparison with other sequences of closely related *Heterorhabditis* is given in Fig. 6.

4. Discussion

Entomopathogenic nematodes are economically important groups of nematodes reported to have great potential for controlling insect pests of crops (Malan *et al*. 2011; Lacey and Georgis 2012). Reports also attested to their successful use as biological control agents and suppression of insect populations (Shapiro-Ilan *et al*. 2002; Shapiro-Ilan and Gaugler *et al*. 2002; Nguyen *et al*. 2006). An extensive survey for EPNs has been conducted worldwide and research into their biocontrol potential has been reported in all the continents of the world except Antarctica (Popiel and Hominick 1992). In Africa, EPNs have been reported from ten countries including; Egypt, Kenya, Ethiopia, Tanzania, Benin, Morocco, South Africa, Algeria, Cameroon, and Nigeria (Akyazi *et al*. 2012; Bhat *et al*. 2020). EPNs in the family Heterorhabditidae have been described as effective biological control agents of insect pests (Gaugler and Kaya 1990; Stock *et al*. 1996). The family contains one genus, *Heterorhabditis*; with currently more than 21 species described worldwide (Nguyen 2007; Bhat *et al*. 2020)**.**

In Nigeria, research in EPNs is still in its infancy, with only a few species being reported in the country (Akyazi *et al*. 2012; Aliyu *et al*. 2015; Claudius-Cole 2018). In the current investigation, *Heterorhabditis* sp. was identified from soil samples that were obtained from arable land cultivated to cassava. Molecular identification and phylogenetic analysis of this nematode showed that it has not been previously described from Nigeria and the isolate clustered with other species of *Heterorhabditis* that were described from Thailand. Nguyen and Hunt (2007) had linked the occurrence of *Heterorhabiditis* to tropical regions. The climatic conditions of Thailand and Nigeria are similar; with a rainy season which generally runs from mid-May to October and dry season from November to February. This near similar climatic conditions could have contributed to the occurrence of the EPN in these two geographical locations.

The phylogenetic analysis also revealed variability between *Heterorhabditis* sp. observed in this present study and *H. bacteriophora* (Akyazi *et al*. 2012) previously reported from Nigerian soil. The isolates fall within separate clades and the pairwise distance revealed a base pair difference of about 202 nucleotides, thus indicating an appreciable difference and divergence between the two species. In addition, the estimate of the p-distance analysis indicates
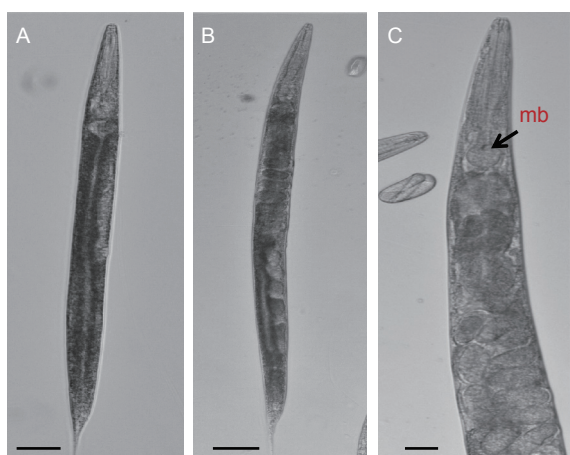

Fig. 4 Lightmicrographs of female *Heterorhabditis* sp. A, young female. B–C, hermaphroditic female. mb=median bulb.
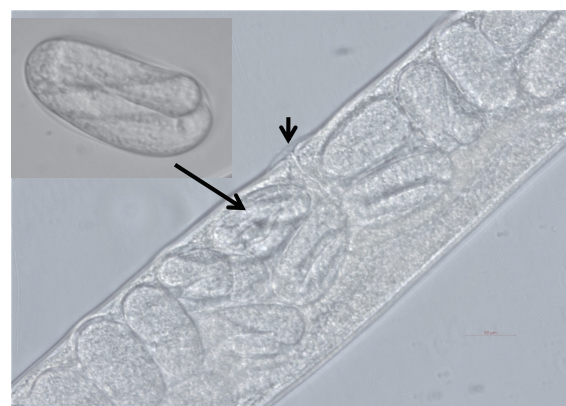

Fig. 5 Lightmicrographs of eggs within and outside an adult female *Heterorhabditis* sp. Vertical arrow indicates the position of the female vulva. The egg enclosing the 1st stage juvenile is also shown (outside and within an adult female).

Table 1 Pairwise distances of the internal transcribed spacer (ITS) regions between *Heterorhabditis* species

ITS regions

#	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
1	MT371593_Heterorhabditis_sp._Nigeria																										
2	MG742141_Heterorhabditis_sp._Thailand	0																									
3	MG742144_Heterorhabditis_sp._Thailand	1	1																								
4	MN635764_Heterorhabditis_sp._India	2	2	3																							
5	MT023075_H._indica_Philipines	0	0	1	2																						
6	FJ751864_Heterorhabditis_sp._SGmg3_India	3	3	4	5	3																					
7	FJ744544_Heterorhabditis_sp._SGgi_India	3	3	4	5	3	6																				
8	HM230723_H._atacamensis_Chile	187	187	187	189	187	188	186																			
10	MH333121_H._safricana_South_Africa	188	188	188	190	188	189	187	6																		
11	KT383776_H._indica_India	189	189	189	191	189	191	188	255	258																	
12	KJ938577_H._megidis_Switzerland	190	190	190	192	190	191	189	23	21	255																
13	MF535520_H._baujardi_South_Africa	196	196	197	198	196	197	195	76	80	261	84															
14	DQ372922_H._floridensis_USA	196	196	197	198	196	197	195	78	82	259	84	3														
15	FJ152545_Heterorhabditis_sp._USA	197	197	197	199	197	197	196	74	78	256	86	33	36													
16	MN028773_H._indica_India	197	197	197	199	197	197	196	74	78	256	86	33	36	0												
17	MK262740_H._amazonensis_Brazil	197	197	198	199	197	198	196	76	80	262	84	2	5	33	33											
18	JN157774_H._indica_Pakistan	197	197	197	199	197	197	196	74	78	256	86	33	36	0	0	33										
19	MH715259_H._indica_Portugal	197	197	197	199	197	197	196	74	78	256	86	34	37	1	1	34	1									
20	KF723814_H._sonorensis_Belgium	198	198	199	200	198	199	197	75	79	260	81	6	7	37	37	6	37	38								
21	MN932152_H._zealandica_South_Africa	198	198	199	200	198	199	197	41	41	263	46	95	96	93	93	95	93	93	94							
22	AY170329_H._indica_China	199	199	199	201	199	199	198	77	81	256	89	37	40	4	4	4	4	5	41	96						
23	KC633186_H._taysearae_Palestine	199	199	200	201	199	200	198	77	81	261	83	10	11	41	41	10	41	42	4	96	45					
24	JX403718_H._bacteriophora_Nigeria	202	202	202	204	202	203	201	52	51	269	62	76	78	72	72	77	72	72	80	77	76	82				
25	HQ225901_H._georgiana_USA	202	202	202	204	202	203	201	54	53	265	64	76	78	71	71	77	71	71	80	79	75	82	4			
26	KU214824_H._indica_Mexico	279	279	280	281	279	281	279	269	267	296	275	272	272	280	280	271	280	280	272	273	281	275	273	272		
27	MK273200_H._indica_India	279	279	280	281	279	281	279	269	267	296	275	272	272	280	280	271	280	280	272	273	281	275	273	272	0	
28	FJ589008_Caenorhabditis_elegans_USA	224	224	225	226	224	224	225	193	191	258	193	199	199	207	207	202	207	207	200	195	208	201	199	198	279	279

that the Nigerian population is not highly divergent from the undescribed species reported from Thailand, and India. We noted that our isolate, which is reported for the first time in Nigeria, is identical to *H. indica* that is described from the Philippines and it clusters with many *H. indica* sequences along with some undescribed species in the phylogenetic tree. This actually puts our isolate in the *H. indica* "sub-group" with *H. indica* as a sister taxon. The outcome is expected, as *H. indica* is frequently encountered in warm tropical climates (Banu *et al*. 2005; Chaerani *et al*. 2018) which the Nigerian climate typifies. This is characterized by relatively high temperature, high humidity and abundant rainfall. We assume that due to the divergent geographical distance among the sites of occurrence of the "indica-subgroup" across the globe, there is a possibility for the isolates of *H. indica* to be "latitudinal clades", or that they might be different species altogether. This phenomenon has been suggested by some authors (Dolgin *et al*. 2008; Dolinski *et al*. 2008). Similarly, it may be attributed to intragenomic rRNA polymorphisms which can be prevalent across wide geographic ranges with slight differences in polymorphism diversity as proposed by Qing (*et al*. 2019). However, more extensive studies will be done on the Nigerian isolate in order to substantiate this claim.

This confirms the fact that there are many uncharacterized and understudied populations of *Heterorhabditis* with possible potentials for biological control still existing in nature. It also

reaffirms the need for more detailed diversity studies of EPNs and nematodes in general for an updated national inventory. This can serve as a baseline reference in research prospecting biotechnological means to harness indigenous species of EPNs and other nematodes for the benefits of humanity (Lacey and Georgis 2012). While very little is currently known about their distribution in the continent, molecular results showed that this is the first account of the isolate in Nigeria and possibly in the West African sub-region.

The biocontrol potential of EPNs had been established for the control of many insect pests including termites *Odontermes obesus* (Isoptera: Termitidae), mealy bugs *Planococcus ficus* (Hemiptera: Pseudococcidae), stem borers *Scirpophaga incertulas* Walker (Pyralidae: Lepidoptera), moths (Lepidoptera, Pyralidae), *Corcyra*

cephalonica Stainton (Lepidoptera; Pyralidae) and weevils *Phlyctinus callosus* (Coleoptera: Curculionidae) (Divya and Sankar 2009; Malan and Ferreria 2017). Its applications have been reported to form part of the integrated management system deployed to tackle the menace of insect pests' attack of pre- and post-harvest crops. Aliyu *et al*. (2015) used Greater wax moth as the bait trap in an investigation that demonstrated the potential bio-insecticidal property of *Steinernema*-bacteria complex. Claudius-Cole (2018) also demonstrated the potential of some EPNs for the control of stem borer, *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae) in Nigeria. In the current study, the infectivity potential of the new species of *Heterorhabditis* sp. was investigated and an interesting result was obtained that validates the isolate as a potential biocontrol of mealworm larva having shown 100% mortality within 48 h of exposure
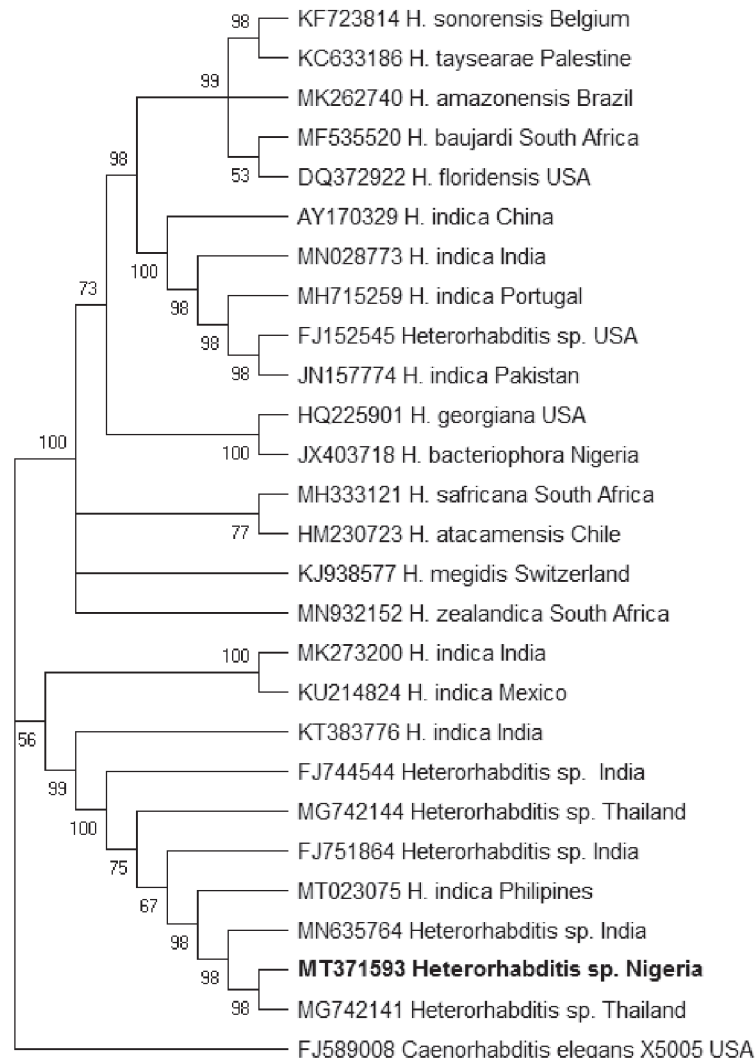

Fig. 6 Phylogenetic relationships among *Heterorhabditis* species, based on analysis of the internal transcribed spacer (ITS) region with maximum parsimony (MP), using *Caenorhabditis elegans* as the outgroup. Newly obtained sequences are indicated by bold letters.

compared to the wax moth. This is in agreement with some research in South Africa where different aspects relating to the efficacy of mealworm as a suitable insect host for commercial production of EPNs have been investigated (van Zyl and Malan 2014, 2015).

According to Popiel and Hominick (1992), EPNs have a preferred host range and are not equally efficient at infecting all insects. In the current investigation, the *Heterorhabditis* sp. showed greater virulence to mealworm larva than wax moth, which is typically common as bait insect in EPN surveys. van Zyl and Malan (2014) posited that the use of *G. mellonella* as bait to obtain EPNs from soil has led to the limited information available on the natural host range of these nematodes. According to Lacey and Georgis (2012), the selection of an EPN for the control of a particular insect pest is determined by factors such as the nematode's host range, host finding or foraging strategy, soil conditions, application methods, tolerance of environmental factors and their effects on survival and efficacy (Shapiro-Ilan *et al*. 2006). This could only suggest that mealworm fall within the host infectivity of the isolated EPN from Nigeria. Native and indigenous EPNs could therefore, provide a rich and valuable resource for biocontrol options because of their adaptability to local environmental conditions. This could also account for their potentiated effectiveness.

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