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Molecular identification of *Metarhabditis rainai* (Nematoda: Rhabditidae) infecting fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae) on maize in Ghana

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The primary management strategy against the invasive fall armyworm (FAW) (Lepidoptera: Noctuidae) on maize in Ghana involves the application of synthetic insecticides. However, this approach has raised concerns related to human, animal and ecological health, prompting the exploration of alternative, environmentally friendly management strategies. Entomopathogenic nematodes (EPNs) have shown efficacy against FAW and other insect pests. To address challenges associated with morphological identification, this study employed molecular diagnostic tools, specifically PCR sequencing, on EPN samples collected from FAW larvae cadavers across Ghana. The PCR products were subsequently sequenced using the Sanger sequencing approach. A Nucleotide BLAST search identified the EPNs as belonging to the genus Metarhabditis, specifically *Metarhabditis rainai* (formerly named *Rhabditis rainai*). The precise identification of entomopathogenic nematodes through molecular techniques is crucial for the potential utilization of these biocontrol agents against fall armyworm on maize in Ghana and beyond.

Key words: Entomopathogenic nematodes, Detection, Molecular technique, Taxonomy.

INTRODUCTION

The fall armyworm (FAW), *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae), was initially reported in Ghana in 2016 and has since become a significant threat to maize and other economically important crops across the country (Koffi et al., 2020; Asare-Nuamah, 2021). FAW infestation on maize occurs from crop emergence

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until ear formation, potentially leading to more than 34% infestation, defoliation and economic yield reduction (Cruz, 1999). The impact of FAW infestation on maize is particularly alarming for food security in sub-Saharan Africa (SSA), where over 300 million people depend on maize as their staple food (Ekpa et al., 2019). In Ghana, maize is a crucial cereal crop, both cultivated by farmers and consumed widely throughout the country.

The management of FAW infestation on maize in Ghana has predominantly involved extensive synthetic insecticides application to prevent total crop loss. However, this approach has been associated with issues such as human and animal poisoning, as well as surface and groundwater contamination. Consequently, efforts are underway to explore benign management options that minimize synthetic insecticides usage. Utilizing indigenous natural enemies as biocontrol agents emerges as a sustainable management option. Entomopathogenic nematodes (EPNs) have demonstrated lethality against FAW and present promising potential as part of an Integrated Pest Management (IPM) strategy against FAW.

To address challenges related to morphological identification, this study employed molecular diagnostic tools to identify EPN samples collected from FAW larvae cadavers across Ghana. This molecular approach enhances the accurate identification of beneficial nematodes, contributing to the effective utilization of EPNs as biocontrol agents against FAW.

MATERIALS AND METHODS

Soil sampling and FAW larvae collection

Maize crop rhizosphere soils were systematically sampled from FAW-infested maize farms across Ghana, as detailed in Table 1. The sampled areas encompass diverse agroecologies, featuring a mix of both unimodal and bimodal rainfall patterns. On each 0.5- ha FAW-infested maize farm, ten core soil samples were randomly collected from the rhizosphere. These samples were gathered using a soil augur up to a depth of 20 cm and subsequently composited, resulting in a total weight of 0.4 kg. Concurrently, fourth to sixth instar FAW larvae were collected from maize plants infested with FAW on the same farms.

For the purpose of infestation by soil indigenous entomopathogenic nematodes (EPNs) and subsequent recovery of the same, five active FAW larvae were introduced to each composite soil sample (Molina-Ochoa, 2003). To facilitate aeration, the cover of the transparent plastic containers (250 ml) used for the composite soil samples was minimally perforated. To ensure uniform mixing of the FAW larvae and the soil, the plastic containers and their contents were inverted three times. Subsequently, the samples were stored in insulated cool boxes and transported to the laboratory for incubation under conditions of 25°C and 85% relative humidity (Bedding and Akhurst, 1975).

Isolation of EPN infective juveniles from FAW larvae cadavers

Following 72 h of incubation, the cadavers of fall armyworm (FAW) larvae were extracted from the soil for culturing, and the collection of entomopathogenic nematodes (EPN) infective juveniles was carried out using modified white traps (Woodring and Kaya, 1988). To prepare the FAW larvae cadavers for collection, they were rinsed with double-distilled water (ddH_2O) to eliminate adhering soil particles. Subsequently, the cadavers underwent disinfection with 0.1% sodium hypochlorite before being positioned on modified white traps to facilitate the emergence of infective juveniles.

The suspensions containing EPN juveniles in water were harvested daily and concentrated to a final volume of 20 ml after 14 days. The emergence of nematodes ceased after 20 days, at which point the setups were terminated (Bhat et al., 2018, 2019).

Pathogenicity test of entomopathogenic nematodes

To validate the entomopathogenic nature of the isolated nematodes, the completion of Koch's Postulates test was conducted, following the procedures outlined by Campos-Herrera et al. (2019) and Blanco-Pérez et al. (2020). This verification aimed to confirm that the soil-inhabiting native nematodes were responsible for the mortality of the fall armyworm (FAW) larvae applied to the soil, ruling out the involvement of any other opportunistic nematofauna utilizing the cadaver as a resource, especially free-living nematodes (FLNs).

The traditional insect bait method was employed for this purpose. A freshly emerged nematode suspension was sprayed onto FAW larvae and applied to sterilized topsoil. After 72 h (Dillman et al., 2012), the cadavers of the FAW larvae were removed and set up to extract second-generation nematodes (F2) using modified white traps (Bedding and Akhurst, 1975). According to Dillman et al. (2012), the classification of nematodes as entomopathogenic is contingent on their collection in relation to virulence or avirulence.

DNA extraction, polymerase chain reaction (PCR) and sequencing

DNA extraction was carried out from 89 nematode samples using kits provided by Clear®Detections, Wageningen, the Netherlands. The extraction process and subsequent polymerase chain reaction (PCR) were conducted following the manufacturer's instructions, utilizing the 1096F/1912R universal primer (synthesized and supplied by METABION® International AG, Germany) (Table 2). Each PCR amplification reaction volume (10 µL) was individually set up for every sample, containing 50 ng template DNA, 1 × PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTP, 250 nM of each forward and reverse primer, and 0.25 µL Taq polymerase. Nuclease-free water was added to achieve a final volume of 10 µL per reaction. The Gen Amp PCR System (9700 PCR) was utilized with the following cycling conditions: Initial denaturation at 95°C for two minutes, 30 cycles of denaturation at 95°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for one minute, and a final extension for 10 min at 72°C, followed by holding at 4°C.

The amplified fragments were separated through electrophoresis on a 2% agarose gel in a 1x TAE (Tris-acetate-EDTA) buffer at 120 electrical volts for 45 min. The gel was stained with 0.01% ethidium bromide and visualized. Banding patterns were compared between the two amplified products and subsequently sequenced via Sanger sequencing (GENEWIZ®, USA). A Nucleotide BLAST search was then conducted in the GenBank (National Center of Biotechnology Information (Altschul et al., 1990) to identify the taxonomy of the entomopathogenic nematodes (EPNs).

All analyses were performed using the Maximum Composite Likelihood Model in MEGA (11) (Kumar et al., 2019).

RESULTS AND DISCUSSION

All the nematode samples were identified to belong to the

Table 1. Soil and fall armyworm sampling points in Ghana, 2021**.**

Region	District	Coordinates	Rainfall regime
Ashanti	Ejura	N7°22'.958'W1°23.015'	Bimodal
	Ejisu	N6°41'.234'W1°23.674'	
	Asante Akyem Central	N6°36'.881'W1°15.768'	
Brong Ahafo	Nkoranza North	N7°40'.340'W1°44.181'	Bimodal
	Sunyani South	N7°22'.691'W2°12.274'	
	Dormaa Central	N7°15'.940'W2°46.571'	
Western	Prestea Huni-Valley	N5°24'.730'W1°59.796'	Bimodal
	Ahanta West	N4°55'.995'W1°58.648'	
	Shama	N5°02'.505'W1°39.545'	
Central	Abura Asebu Kwamankese	N5°14'.008'W1°11.999'	Bimodal
	Assin South	N5°23'.412'W1°08.965'	
	Assin North	N5°50'.126'W1°19.116'	
Eastern	Kwahu West	N6°28'.065'W1°38.418'	Bimodal
	Akuapem North	N6°01'.215'W0°05.087'	
	Yilo Krobo	N6°03'.786'W0°01.311'	
Greater Accra	Ningo Prampram	N5°46'.104'E0°03.978'	Bimodal
	Ga West	N5°41'.949'W0°17.113'	
	Amasaman	N5°46'.475'W0°19.672'	
Volta	Ketu South	N6°07'.222'E1°08.755'	Bimodal
	Hohoe	N7°11'.467'E0°28.560'	
	Nkwanta South	N8°16'.230'E0°31.486'	
Upper East	Bolgatanga	N10°48'.403'W0°50.273'	Unimodal
	Kasena Nankana	N10°50'.241'W1°00.056'	
	Builsa North	N10°44'.767'W1°16.304'	
Upper West	Lawra	N10°39'.470'W2°53.925'	Unimodal
	Nadowli Kaleo	N10°23'.054'W2°40.796'	
	Wa	N10°05'.101'W2°28.999'	
Northern	Tamale	N9°39'.112'W0°50.532'	Uni/bimodal
	Mamprusi West	N10°24'.246'W0°47.193'	
	Saboba	N9°44'.525'E0°05.234'	

genus *Metarhabditis*. The sequence data was linked to two genes associated with small units of ribosomal RNA, MT012153.1 (16%) and JQ237848.1 (84%), with sequence lengths of 842 and 1410 base pairs, respectively (Table 3). Specifically, four samples from Asakraka (Eastern), Wassa Amenfi (Western), Tarkwa (Western), and Kpong (Eastern regions) exhibited 100% nucleotide sequence similarity, while one sample from Nzema East (Western region) recorded the least similarity at 67%. The generated dendrogram (Figure 1) did not reveal distinct groupings among the samples. All the samples were identified at the species level as *Metarhabditis rainai*, formerly known as *Rhabditis rainai* (Carta and Osbrink, 2005). Figure 2 shows the phylogenetic relationship generated among entomopathogenic nematode populations in Ghana; sequence data tracing to *MT012153.3* gene in red. The samples were classified according to *Nemys* scientific classification details (*Nemys,* 2022):

Kingdom: Animalia*;* Phylum: Nematoda*;* Class: Chromodorea*;* Sub class: Chromadoris*;* Order: Rhabditida; Sub order: Rhabditina; Infra order: Rhabditomorpha; Supa family: Rhabditoidea; Family:

Table 2. Primer information.

Single pair	Sequences (5'-3')	Position	Reference Size	Reference
1096F/	GGTAATTCTGGAGCTAATAC	1076-1095	855 bp	Holterman et al. (2006)
1912R	TTTACGGTCAGAACTAGGG	1913-1931		

Table 3. Export data and sequence similarity information.

Sample location	Region	% Similarity	Gene	Length (bp)
Nzema East	Western	67.0	MT012153.1	842
Asakraka	Eastern	100.0	JQ237848.1	1410
Assin Breku	Central	98.4	JQ237848.1	1410
Nkoransa	Bono East	99.7	JQ237848.1	1410
Tayedo	Central	97.6	JQ237848.1	1410
South Tongu	Volta	95.8	JQ237848.1	1410
Komenda	Central	98.1	JQ237848.1	1410
Attakrom	Bono	99.0	JQ237848.1	1410
Wamanafo	Bono	99.49	JQ237848.1	1410
Ejura	Ashanti	98.64	JQ237848.1	1410
Ejisu	Ashanti	96.00	JQ237848.1	1410
Sunyani	Bono	99.25	JQ237848.1	1410
Dormaa	Bono	96.00	JQ237848.1	1410
Kintampo	Bono East	99.60	JQ237848.1	1410
Adanse Asokwa	Ashanti	97.57	JQ237848.1	1410
Kyeremesu	Bono	98.81	JQ237848.1	1410
Komenda	Central	98.44	JQ237848.1	1410
New Akrade	Eastern	95.00	JQ237848.1	1410
Konongo	Ashanti	95.50	JQ237848.1	1410
Ejisu	Ashanti	94.00	JQ237848.1	1410
Nzema East	Western	95.00	JQ237848.1	1410
Amasaman	Greater Accra	98.00	JQ237848.1	1410
Hohoe	Volta	99.00	JQ237848.1	1410
Atebubu	Bono East	98.00	JQ237848.1	1410
Nkoranza	Bono East	96.00	MT012153.1	842
Tema	Greater Accra	96.00	JQ237848.1	1410
Prestea	Western	96.00	JQ237848.1	1410
Wassa Amenfi	Weatern	100.00	MT012153.1	842
Kpong	Eastern	100.00	MT012153.1	842
Tarkwa	Western	100.00	MT012153.1	842
Kpetoe	Volta	96.00	MT012153.1	842
Ahanta West	Western	92.00	MT012153.1	842
Ho	Volta	96.00	JQ237848.1	1410
Dodowa	Greater Accra	97.00	MT012153.1	842
Elmina	Central	96.00	JQ237848.1	1410
Abura Dunkwa	Central	99.00	JQ237848.1	1410
Larteh	Eastern	93.00	JQ237848.1	1410
Cape coast	Central	99.80	JQ237848.1	1410
Atebubu	Bono East	96.76	JQ237848.1	1410
Tema	Greater Accra	97.00	JQ237848.1	1410
Tema	Greater Accra	98.00	JQ237848.1	1410
Kintampo South	Bono East	93.60	JQ237848.1	1410
Ho	Volta	94.73	MT012153.1	842

Table 3. Contd.

Aboadze	Western	99.00	JQ237848.1	1410
Wassa Amenfi	Western	94.81	JQ237848.1	1410
Cape Coast	Central	95.00	JQ237848.1	1410
Prestea	Western	93.88	JQ237848.1	1410
Nsawam	Eastern	96.27	JQ237848.1	1410
Nzema East	Western	96.32	MT012153.1	842
Yilo Krobo	Eastern	95.00	JQ237848.1	1410
Assin Nduaso	Central	99.00	JQ237848.1	1410
Yilo Krobo	Eastern	96.00	JQ237848.1	1410
Nkoranza	Eastern	93.00	JQ237848.1	1410
Pokuase	G. Accra	96.50	JQ237848.1	1410
Ketu Mite	Volta	96.60	JQ237848.1	1410
Amantin	Bono East	91.00	MT012153.1	842
Odumase Krobo	Eastern	99.70	JQ237848.1	1410
Prestea	Western	96.00	JQ237848.1	1410
Pokuase	Greater Accra	98.11	JQ237848.1	1410
Ninting	Ashanti	98.89	JQ237848.1	1410
Larteh	Eastern	97.80	JQ237848.1	1410
Assin Akrofuom	Central	97.80	JQ237848.1	1410
Adoagyiri	Eastern	92.30	MT012153.1	842
Dangme East	Greater Accra	95.00	JQ237848.1	1410
Dodowa	Greater Accra	96.00	JQ237848.1	1410
Kwahu Tafo	Eastern	90.22	MT012153.1	842
Assin Nsuta	Central	97.60	JQ237848.1	1410
Ketu South	Volta	95.50	JQ237848.1	1410
Mampong	Ashanti	96.89	JQ237848.1	1410
Dzodze	Volta	95.58	JQ237848.1	1410
Wassa Amenfi	Western	98.5	JQ237848.1	1410
Ejisu-Kwaso	Ashanti	96.1	JQ237848.1	1410
Cape Coast	Central	94.51	JQ237848.1	1410
Agbodzome	Volta	94.51	JQ237848.1	1410
Assin Praso	Central	96.51	JQ237848.1	1410

Source: blast.ncbi.nlm.nih.gov.

Rhabditidae; Subfamily: Rhabditinae; Genus: Rhabditis; Species: *R. rainai* (Sudhaus, 2011).

The genus Metarhabditis, as delineated by Tahseen et al. (2004), is classified under the family Rhabditidae (Örley, 1880), with *Metarhabditis andrassyana* being the sole member. In a study by Bhat et al. (2020), *Metarhabditis blumi* (Sudhaus, 1974) and *Metarhabditis amsactae* were identified as sister or associated species of *M. rainai*. Sudhaus (2011) conducted a review of the *Metarhabditis* genus, transferring five species from the genus *Rhabditis* (Dujardin, 1845) into *Metarhabditis* (Tahseen et al., 2004) due to shared morphometric and sequence characteristics with other members of the *Metarhabditis* genus. These transferred species include *Rhabditis adenobia* (Poinar, 1971), *R. blumi* (Sudhaus, 1974), *R. costai* (Martins, 1985), *R. freitasi* (Martins, 1985) and *R. rainai* (Carta and Osbrink, 2005).

The current samples from the study exhibit nucleotide sequence similarities (ranging from 67 to 100%) with *R. rainai,* as reclassified by Sudhaus (2011). *Oscheius amsactae* (Ali et al., 2011), initially placed in the genus Oscheius, was also reclassified into the genus Metarhabditis and renamed *M. amsactae*. *M. amsactae* has been isolated from soils globally, especially in Pakistan and India (Ali et al., 2011). Other nematode species such as *Oscheius chongmingensis, O. carolinensis, and Caenorhabditis briggsae* parasitize insect hosts using the traditional insect baiting method for finding entomopathogenic bactivorous nematodes (Nguyen and Hunt, 2007; Abebe et al., 2010). Abolafia and Peña-Santiago (2017) and Tabassum et al. (2019) also identified new species of Metarhabditis, namely *M. giennensis* and *M. longicaudata* from Spain and Pakistan, respectively. According to Ogier et al. (2023), these

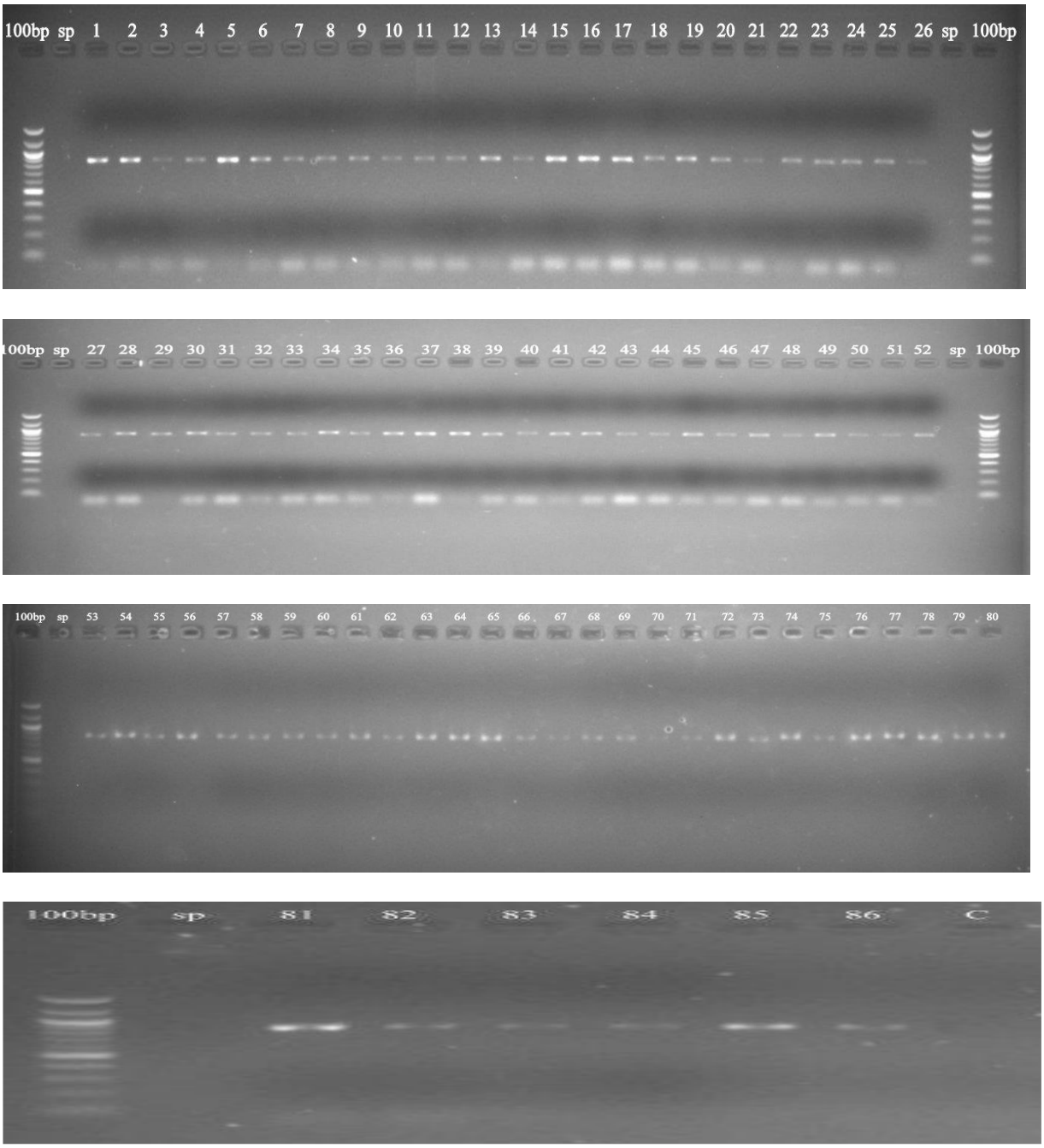

Figure 1. Gel showing EPN DNA amplifications using 1096F/1912R primer pair.

nematodes contribute to the infective success of entomopathogenic nematodes on insect hosts.

The Nemys nematode classification system corroborates that the entomopathogenic nematodes identified in the study inhabit terrestrial habitats and trace their lineage to *Rhabditis Dujardin* (1844). Such nematodes are considered entomopathogenic and hold potential as safe biocontrol agents against insect pests on global food and vegetable crops.

Conclusion

All the samples were identified at the species level as *M. rainai*, formerly known as *R. rainai*, and they belong to the genus *Metarhabditis*. To the best of our knowledge, this study marks the first record of *M. rainai* in Ghana. This finding contributes valuable information that can enhance the exploration of sustainable management efforts against the fall armyworm through biocontrol and

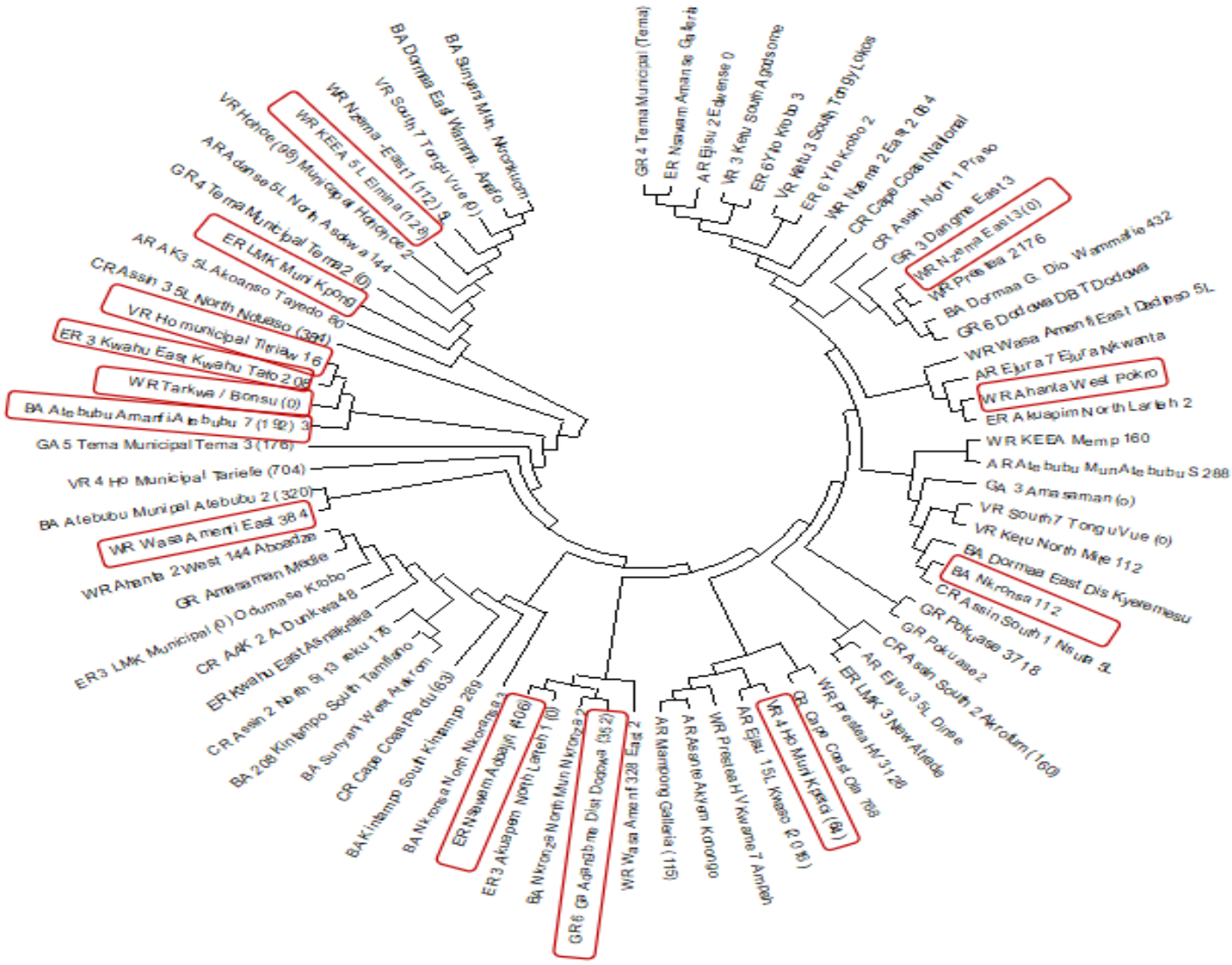

Figure 2. Phylogenetic relationship generated among entomopathogenic nematode populations in Ghana; sequence data tracing to *MT012153.3* gene in red.

Integrated Pest Management (IPM) approaches, ultimately improving maize yield, farmers' income, and livelihoods. The study underscores the promising potential of entomopathogenic nematodes as biocontrol agents against fall armyworm on maize in Ghana, suggesting that further exploration in this direction is warranted.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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