

Nematode biodiversity in south-western Nigerian watermelon cropping systems, with reference to *Meloidogyne* and its management

TT Bello

 **orcid.org 0000-0002-9657-0504**

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Promoter:

Prof H Fourie

Co-promoter:

Prof DL Coyne

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27216276



Private Bag X6001, Potchefstroom
2520, South Africa

Tel: 016 910-3111

Fax: 016 910-3116

Web: <http://www.nwu.ac.za>

Unit for Environmental Sciences and
Management

Sub-program: Integrated Pest Management

Tel: 018 299 2376

E-pos: johnnie.vandenbergh@nwu.ac.za

2 August 2019

To whom it may concern

The following student is conducting research in Environmental Sciences with zero risk.

Name	Mr Tesleem Bello
Student nr	27216276
Degree	PhD Environmental Sciences
Title	Nematode biodiversity in south-western Nigerian watermelon cropping systems, with reference to <i>Meloidogyne</i> and its management
Supervisors	Prof Hendrika Fourie (NWU) Dr Danny Coyne (IITA, Nigeria)

Prof Johnnie van den Berg: Chair of the Scientific Committee, Integrated Pest Management (IPM) sub-programme.

Prof Nico Smit:

Director, Unit for Environmental Sciences and Management

‘I dont know where the limits are, but I would like to go there’

Eliud Kipchog

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ABSTRACT

Watermelon is increasingly produced and consumed in Nigeria and sub-Saharan Africa (SSA). However, limited information exists regarding the nematode fauna associated with the crop. Therefore, the overall aim of this study was to determine the nematode assemblages associated with watermelon, to investigate the reproduction potential of populations of the predominant plant-parasitic nematodes identified and to assess the host status of commercially available cultivars in south-west Nigeria to the predominant nematode pest species. Of the 30 free-living nematode genera identified from soil samples, *Cephalobus*, followed by *Rhabditis*, *Aphelenchus* and *Aporcelaimus*, were predominant. Variation in nematode community structures across the 50 fields was apparent for mean maturity indices, metabolic footprints, feeding-type composition and coloniser-persister (c-p) structure. Faunal analyses characterised 52% of the fields as having stable and enriched soil food webs, which is beneficial for crop production. A new species, *Aporcelaimellus nigeriensis* sp. n., was furthermore identified and described from this study. Of the 12 plant-parasitic nematode species identified, *Meloidogyne* spp. were predominant, followed by *Helicotylenchus dihystra*, *Pratylenchus zae* and *Scutellonema bradys*. Applying morphological and molecular techniques, four *Meloidogyne* spp. were identified from the sampling sites. *Meloidogyne enterolobii* was the most prevalent, followed by *M. incognita*, *M. javanica* and *M. arenaria*. *Meloidogyne arenaria* is reported for the first time from south-west Nigerian cropping systems. Significant associations were observed between the frequency of occurrence of the predominant nematode pest genera/species and soil properties as well as rainfall. The reproduction potential of 25 *Meloidogyne* spp. populations (containing single-species) and/or communities (containing mixed-species) obtained from watermelon fields were determined under glasshouse conditions, while the host response of six commercially available watermelon cultivars to the three predominant root-knot nematode species (*M. incognita*, *M. javanica* and *M. enterolobii*) were also done. For both studies an initial and repeat experiments were conducted over 56 days. For the reproduction potential experiments, $\pm 5\,000$ eggs and second-stage juveniles (J2) of each of the 25 *Meloidogyne* populations and/or communities were inoculated on roots of two-leaf stage seedlings of the root-knot nematode susceptible tomato (*Lycopersicon esculentum* Mill.) cultivar Tropimech. For the host status experiments, roots of six commercially available watermelon cultivars were inoculated with

±5 000 eggs and J2 of *in-vivo* reared, single-species populations of *M. incognita*, *M. javanica* and *M. enterolobii*. The reproduction potential of the *Meloidogyne* spp. communities and the host response of the cultivars were assessed based on the i) number of egg masses, ii) final nematode population (Pf) and iii) reproduction factor (Rf) per root system. No significant interaction existed between the initial and repeat experiments of the reproduction potential experiments, while a significant interaction was apparent between the two host status experiments. However, for the reproduction potential experiments higher Pf and Rf values were recorded for most of the cultivars for the initial compared to the repeat experiment. The highest Rf was obtained for a mixed community of *M. enterolobii* and *M. javanica* (L 15), while the lowest Rf was ascribed to a mixed species community (L16) containing *M. arenaria* and *M. enterolobii*. Host status assessments of cultivars showed that all cultivars evaluated were susceptible (Rf >1) to the three species of *Meloidogyne*, although substantial variation among the cultivars' host responses to the three *Meloidogyne* spp. existed. For example, cultivar Koloss F1 supported the lowest population densities for *M. enterolobii* (Pf = 40 002; Rf = 6.1); Sugar Dragon for *M. javanica* (Pf = 12 947; Rf = 2.6); and *M. incognita* (Pf = 10 670; Rf = 2.1); the highest population densities were maintained in roots of cultivar Charleston Gray for *M. enterolobii* (Pf = 73 522 ; Rf = 14.7); Erato F1 for *M. javanica* (Pf = 47 684 ; Rf = 9.5); and Charleston Gray for *M. incognita* (Pf = 63 395; Rf = 12.7). All Pf and Rf values recorded across the treatments were significantly lower than those of the susceptible tomato standard check. This study provides novel information regarding i) the free-living and ii) plant-parasitic nematodes associated with watermelon from SSA; iii) a new *Aporcelaimellus* sp. report; and baseline information on iv) the reproduction potential of *Meloidogyne* spp. populations and communities occurring in south-west Nigeria; as well as the v) the host status of commercially available watermelon cultivars grown across south-west Nigerian agro-ecological systems to single-species *Meloidogyne* populations. The data generated from this study hence represent valuable and useful information to watermelon growers and can contribute towards sustainable cultivation of the crop in Nigeria.

Keywords: bio-indicators, cultivars, host status, molecular techniques, morphology, nematodes, reproduction potential.

PREFACE

This thesis is written in line with article format style prescribed by North-West University. Thus, the articles are in the publishable format, while the manuscript (Chapter 2, which has already been published) and other chapters (Chapters 3 and 4, which have been submitted for publication to the journal *Nematology*) are written according to the authors instructions of the internationally accredited journal *Nematology*. Chapter 5 has also been prepared for the submission in the latter journal. As required by North-West University, contributions of authors for each article/ chapter as well as their accent for use as part of the thesis are provided in Table A.

This thesis contains the following chapters:

Chapter 1 – Introduction and literature review: **European Journal of Plant Pathology (Springer) (only for referencing style)**

Chapter 2 – Article 1 (Published): **Nematology (Brill)**

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Chapter 4 – Article 3 (Submitted): **Nematology (Brill)**

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Chapters 1 and 6 were prepared according to the springer format of which an excerpt is available in Appendix A. The submitted (Chapter 3: Article 2, Chapter 4: Article 3 and Chapter 5: Article 4) as well as the unpublished (Chapter 6: Article 5) were prepared according to the instructions to authors of the journal *Nematology* (instructions for authors is available in Appendix B). Finally, the printed version of Article 2 as well as proofs of submission of articles 3 and 4 are provided in Appendices C and D, respectively.

Access links to raw data of Chapter 2; Article 1, Chapter 3, Article 2, Chapter 4: Article 3 and Chapter 5: Article 4 are available in Appendices E, F, G and H respectively.

Table A. Contribution of a Authors and consent of use as part of this thesis

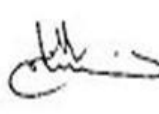
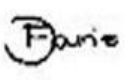
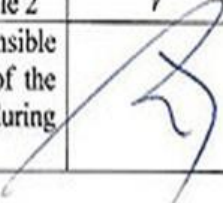
Author	Article	Contribution	Accent
T.T. Bello	Article 1 – 4	Principal investigator: Responsible for conducting the study design, sampling, data analyses as well as interpretation. Also, the first author responsible for writing of articles and thesis.	
H. Fourie	Article 1 – 4	Promoter: Supervised the study design, and monitored the progress. Also provided intellectual input during the practical work and writing of articles and thesis.	
D. Coyne	Article 1– 4	Co- promoter: Provided intellectual input during the practical work and writing of articles and thesis.	
M. Rashidifard	Articles 2 and 3	Provided intellectual input on molecular analyses and gave guidance in writing the article. Also, the first author of Article 2	
R. Pena Santiago	Article 2	Provided technical input and responsible for the morphological description of the new species of nematode identified during the study.	

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 General introduction

Nematodes are abundant in agricultural soils worldwide, with plant-parasitic nematodes causing significant yield losses to agricultural crops (Jones *et al.*, 2013). Conversely, beneficial nematodes (from here on referred to as free-living nematodes for the purpose of this thesis) serve as bioindicators of soil conditions where they play important roles in food webs (Neher, 2001; Ferris, 2010).

Although watermelon (*Citrullus lanatus*) (Thunb) Matsum & Nakai is regarded as an exotic vegetable crop in Nigeria, its production in the country dates back to the early 1980s when it was limited to the drier northern and middle belt regions (Iheke, 2009). Awareness about the nutrition and health benefits of the crop increased, resulting in expansion of watermelon production in the southern parts of the country where it became a lucrative enterprise with demand generally exceeding supply of the crop produce (Adeoye *et al.*, 2011; Okunlola *et al.*, 2011). Watermelon production worldwide is hampered by several plant-parasitic nematode genera of which *Meloidogyne* spp. are considered as the most damaging and destructive (Davis, 2007; Ngele and Kalu, 2015). No study has, however, investigated the association of plant-parasitic nematodes with watermelon crops in south-west Nigerian cropping systems, except for three studies (Mary *et al.*, 2013; Eche *et al.*, 2015; Alabi *et al.*, 2017) that focused on nematodes, but only for some areas of Nigeria and for a few crops. Therefore, an urgent need for nematode research on watermelon exists since production of the crop is expected to be sustained within south-west Nigeria. Furthermore, strategies to manage economically important nematode pests of watermelon should also be investigated once the predominant genera have been identified. This will enable producers to grow the crop sustainably in mixed cropping systems in the country.

The overall aim of this study was to determine the nematode assemblages associated with watermelon, to investigate the reproduction potential of populations (representative of single species) and/or communities (representative of mixed species) of the predominant plant-

parasitic nematodes identified and to assess the host status of commercially available cultivars in south-west Nigeria to the predominant nematode pest species. The specific objectives were:

- to record the abundance and diversity of plant-parasitic and free-living nematode assemblages obtained from watermelon production areas by means of a survey.
- to identify any new species of the free-living nematode genera found as well to identify the species of the predominant plant-parasitic nematode genus found,
- to determine the reproduction potential of populations and/or communities of the predominant genus identified and
- to screen watermelon cultivars that are commercially available in Nigeria against the most abundant species of the predominant plant-parasitic nematode genus recorded.

The thesis commences by introducing the reader, by means of a concise overview, to various aspects about watermelon: e.g. its history, classification, anatomy, agronomy and production (globally and in Nigeria). Aspects about nematodes, with reference to nematode classification, biology, morphology, feeding habits (trophic groups) and an overview of nematode pests associated with watermelon are briefly elaborated on next. In this section, the focus is particularly on the genus *Meloidogyne*, with the symptoms and damage they cause to watermelon crops being discussed and illustrated. Ultimately, the thesis is concluded with a discussion about nematode management strategies; the emphasis being on host plant resistance (representing one of the objectives of the current study). A discussion about the role of free-living nematodes, that are increasingly used as bio-indicators of soil quality, are then referred to with emphasis on their role in the soil-food web, where these organisms occupy several trophic levels. Techniques used to accurately identify nematode species, free-living and plant-parasitic, are then discussed with focus being placed on the most important morphological and molecular aspects currently used. In this part special reference are given to *Aporcelaimellus* (a new species identified as a result of this study) and *Meloidogyne* (identified as the predominant nematode pest genera of watermelon during this study). This introductory chapter serves as a background for the technical chapters that follow, of which a short overview is given below.

The technical part of the study dealt with several individual objectives of which the first entailed recording the abundance and diversity of free-living and plant-parasitic nematode

assemblages from 50 fields across southwest Nigeria where watermelon was grown during the 2016/17 growing season. This represented the first study of its kind since no extensive watermelon-nematode association study have been done before for the crop in this country, but moreover, on the African continent. The second and third objectives respectively dealt with identification of a new free-living nematode species of the genus *Aporcelaimellus* and species of the predominant plant-parasitic genus *Meloidogyne* using morphological and molecular techniques. The fourth objective entailed determining the reproduction potential of *Meloidogyne* spp. populations and/or communities identified from the 25 sampling sites, followed by the fifth objective that focused on assessing the host status of six commercially available watermelon cultivars to the three most abundant *Meloidogyne* spp. identified; *Meloidogyne enterolobii* (Yang and Eisenback, 1983), *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1949, and *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949. The thesis is concluded with a conclusions chapter, indicating the way forward in terms of research on this topic.

1.2 Literature review

1.2.1 Watermelon

1.2.1.1 History and botanical classification

Watermelon (*Citrullus lanatus*) (Thunb) Matsum & Nakai, a member of the family Cucurbitaceae, is monoecious and bears fruit annually where it is cultivated in warm climates of the world (Figure. 1.1). The family includes squash and pumpkins (*Cucurbita pepo* L. var. *ovifera* (L., Harz) cucumbers (*Cucumis sativus* L.), muskmelons (*Cucumis Melo* L.) and gourds (*Curcubita lagenaria* L.) (Ferriol and Picó, 2007). During the 19th century Alphonse de Candolle traced the origin of watermelon to indigenous tropical Africa (De Candolle, 1882). Recent findings based on chloroplast deoxyribonucleic acid (DNA) investigations have revealed that the cultivated and wild watermelon (*Citrullus colocynthis* L. Schrad) diverged independently from a common ancestor, probably *Citrullus ecirrhosus* (Cogn) that is commonly known as ‘Namib Tsamma’ which originated from Namibia (Dane *et al.*, 2006).

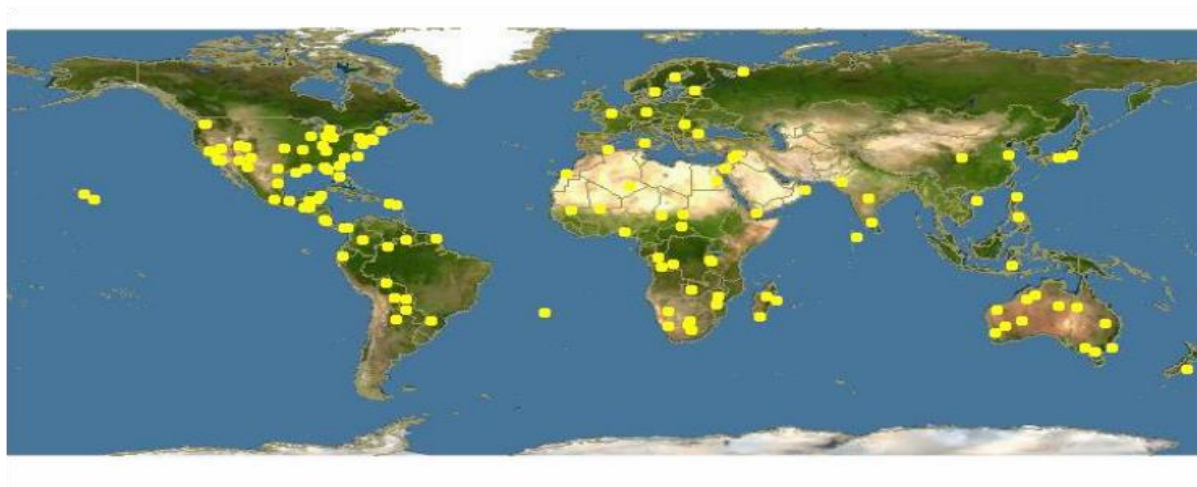


Fig 1.1. World map showing watermelon producing areas. (Source: [www.edibleplantsin vietnam.com](http://www.edibleplantsinvietnam.com)).

Watermelon is classified as follows (<http://plants.usda.gov/java/profile?symbol=CILAL>):

Phylum: Embryophyta

Class: Dicotyledoneae

Order: Cucurbitaceae

Genus: *Citrullus*

Species: *lanatus*

1.2.1.2 Anatomy and morphology

Detailed descriptions of the anatomy and morphology of watermelon can be found in Maynard *et al.* (2012) and Mercy *et al.* (2013). The anatomy of *C. lanatus* var. *lanatus* has been described in detail by Barber (1909). The fruit represents a fleshy berry of which the tissues are derived from the pericarp and the flesh separated along the placental bundles with no true cavity being formed (Maynard *et al.*, 2012; Mercy *et al.*, 2013). The rind is divided into four layers of tissue viz: epicarp (first differentiated layer), hypodermis, outer-mesocarp and the middle-mesocarp. The outermesocarp represents the most distinct tissue in the rind, consisting of tightly packed bands of brachysclereids which has locules (in which the seeds are formed) (Maynard *et al.*, 2012; Mercy *et al.*, 2013).

Watermelon are trailing herbaceous plants possessing annual vines and woody rootstocks. Monoecious yellow flowers (representing both male and female) grow on the same plant with male flowers having longer peduncles than female flowers, which enlarged at the base that contains the ovary (Mercy *et al.*, 2013). The fruit shapes are oval to round (usually between 25 to 85 cm in length). The rind has as mid- to dark green colour (usually mottled or striped) while the flesh (containing numerous seeds) colour can range from red or pink (most commonly) to orange, yellow, green and sometimes white (Maynard *et al.*, 2012).



Figure 1.2. Watermelon plants with runners along the ground and fruits (Source: Keywordsuggest.org).

1.2.1.3 Production and importance of watermelon worldwide and in Nigeria

Watermelon produces fruits with edible rinds which are sometimes used as a vegetable. The rinds are stir-fried, stewed or more often pickled (Wehner, 2008). Watermelon juice can also be made into wine. Watermelon fruits have been associated with mildly diuretic properties and contain large amounts of beta carotene (Edwards *et al.*, 2003; Jaskani *et al.*, 2005). The red fleshy fruit is furthermore a significant source of lycopene ($\pm 4\ 100\ \mu\text{g}/100\ \text{g}$), which is associated with cancer risk reduction. These high lycopene concentrations demonstrate the health benefits of watermelon which is substantially higher than those in other fruits, e.g. $\pm 3\ 100\ \mu\text{g}/100\ \text{g}$ in raw tomato (*Solanum lycopersicon* L.); $\pm 3\ 362\ \mu\text{g}/100\ \text{g}$ in pink grapefruit (*Citrus paradisi* Macf.), but less than the $\pm 5\ 400\ \mu\text{g}/100\ \text{g}$ in raw guava (*Psidium guajava* L.) (Choudhary *et al.*, 2009).

World production of watermelon in 2017 was estimated at 118.4 million tons, with 67% of it produced in China. The other major watermelon producing countries are Turkey (3.9%), Iran (2.2%), Brazil (1.9%), Egypt (1.8%) and USA (1.4%). Total production from Africa stands at 6.2 million tons (FAOSTAT, 2017).

Watermelon is an important vegetable crop that is mainly used grown for its fruit but also for processed products (Akintoye *et al.*, 2009). In Nigeria, watermelon is regarded as an exotic vegetable that generates a higher profit and provides more employment and income opportunities to farmers than other available indigenous vegetable crops (Ajewole and Folayan, 2008). Cultivation of watermelon in Nigeria was originally confined to the drier savannah regions in the north but is now gradually increasing towards the south-western part of the country. This progressive increase in its production across the country is stimulated by increased public demand and was brought about by enhanced consumer awareness of the health and dietary benefits of fresh vegetable consumption (Iheke, 2009). According to a report by Adeoye *et al.* (2007), watermelon was rated as the most preferred among five other exotic vegetables examined in south-western states of the country. In this part of Nigeria, an average yield of 38.7 t/ha was recorded for watermelon production (Okunlola *et al.*, 2011), indicating that farmers' yields are lower than the global average of 118.4 million tons for 2017 (FAOSTAT, 2017). These low watermelon yields recorded for Nigeria have been attributed to a decline in the unit output from various agricultural inputs such as capital, land, labour and

management practices (Ajewole, 2015). Other constraints identified include soil-fertility decline, soil-borne diseases, insects and nematode pests (Ajewole, 2015). Since this study addressed the nematode assemblages (free-living and plant-parasitic) associated with watermelon in southwest Nigeria, the next phase of this chapter will focus on the basic classification and morphology of nematodes

1.2.2 Nematodes

1.2.2.1 Classification, basic biology and morphology of nematodes

Nematodes belong to the Phylum Nematoda. The phylum consists of two classes, namely Chromadorea (containing the Order Rhabditida) and the Class Enoplea (containing the orders Dorylaimida and Triplonchida) (Decraemer and Hunt, 2013) (Figure 1.3). Nematodes have been described as the most abundant and numerous multicellular organisms on earth, which inhabit various habitats in soil, water and several other substrates (Decraemer and Hunt, 2013). Although many of them are parasites of animals, humans and insects, some of them are economically important pests of plants while the majority represents free-living nematodes. About 10 681 terrestrial free-living nematode species have been described by the turn of the century (Hugot *et al.*, 2001) and about 4 100 species of plant-parasitic nematodes species by 2013 (Decraemer and Hunt, 2013).

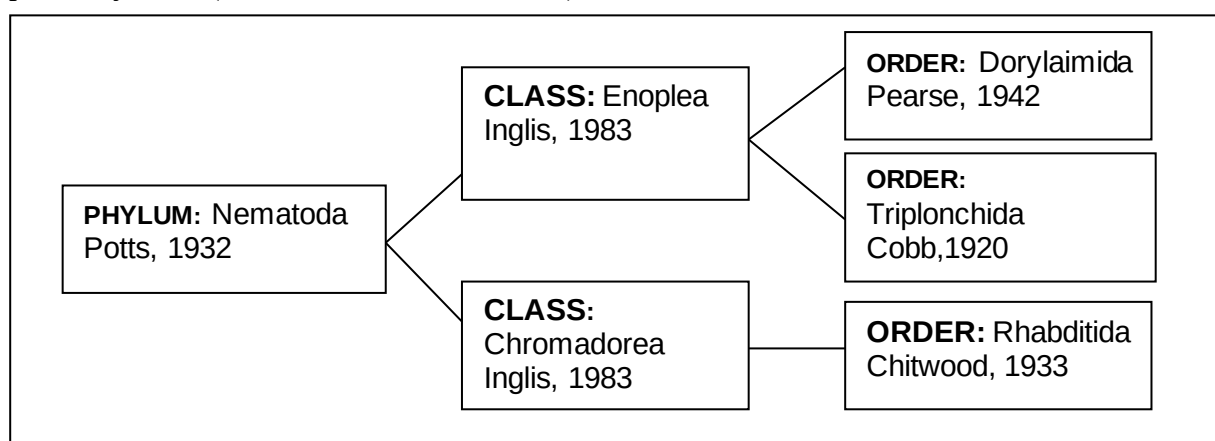


Figure 1.3. Taxonomic classification of plant-parasitic nematodes to order level (Decraemer and Hunt, 2013).

The anatomy and morphology of nematodes are described by Decraemer and Hunt (2013) and Hunt *et al.* (2018). In short, nematodes are microscopic, bilateral, symmetrical organisms of which most life stages are vermiform. In some genera, for example *Meloidogyne*, the female however loses the vermiform shape and becomes obese and globose in form; differing from

the other vermiform life-stages such as infective juveniles and males. This phenomenon is known as sexual dimorphism.

The nematode body is enclosed in a cuticle which is usually transversely annulated. The central cavity of the nematode body is a pseudocoelum which acts as a hydrostatic skeleton. The digestive system generally consists of the mouth region, oesophagus, intestine and rectum, while the reproductive systems in both sexes are tubular in structure. The female genital system may consist of two branches (didelphic) or can be reduced to a single branch (monodelphic). Each branch consists of four major parts: ovary, oviduct, uterus and the vagina which opens to the exterior via the vulva. There may also be a specialized structure for storing sperm (spermatheca) in the female's body. The male reproductive system is less variable with a single genital tube that consists of seminal vesicle and vas deferens, which opens into the exterior via the cloaca. The male copulatory organ typically consists of paired spicules along with a guiding gubernaculum. The excretory system of nematodes consists of a uninucleate gland cell connected ventrally to the excretory pore which is usually located in the oesophageal region but maybe posteriorly located in some nematode species (e.g. *Tylenchulus*). The nervous system consists of the nerve ring which might be a circumoesophageal or circumintestinal commissure together with a network of nerves that are connected to the body organs and various sensory organs. These sense organs are mostly situated in the labial area (sensillae and amphids), the oesophageal region (cephalids, deirids, hemizonid and hemizonion) and on the tail (phasmids and caudalids) (Luc *et al.*, 2005; Decraemer and Hunt, 2013; Hunt *et al.*, 2018).

Meloidogyne spp. exhibit an exceptional variety of reproductive strategies, which range from obligatory mitotic parthenogenesis to amphimixis (Chitwood and Perry, 2009). Most tropical species of *Meloidogyne*, e.g. *Meloidogyne arenaria* (Neal 1889) Chitwood, 1949; *Meloidogyne enterolobii* Yang & Eisenback, 1983; *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 and *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949, exhibit obligatory mitotic parthenogenesis as the only reproduction mechanism (Chitwood and Perry 2009) and their life cycle is usually completed between 4 to 8 weeks (Noling, 2015).

1.2.2.2 Feeding habits/trophic groups of nematodes

Nematodes occupy several trophic levels in soil food webs since they feed on a wide range of food sources (Yeates, 2003). These organisms are hence grouped according to the type of food they consume, which is largely dependent on the morphology of their mouthparts (Figure 1.4). The plant feeders (plant-parasitic nematodes) or herbivores are widespread and feed with a needle-like stylet that are present in the mouth area of the body. Other nematode groups represent the bacterial feeders (bacterivores), fungal feeders (fungivores) as well as omnivores and predators (Ferris *et al.* 2001; Yeates and Stirling, 2008). The latter nematode groups each have mouth parts that are particularly adapted for feeding on bacteria (bacteria), fungi (fungivores), omnivores (feed on algae, bacteria, fungi and other soil biota including nematodes) and predators (feed on mesofaunal organisms such protozoa, rotifers, tardigrades, arthropoda as well as other nematodes) (Yeates and Stirling, 2008).

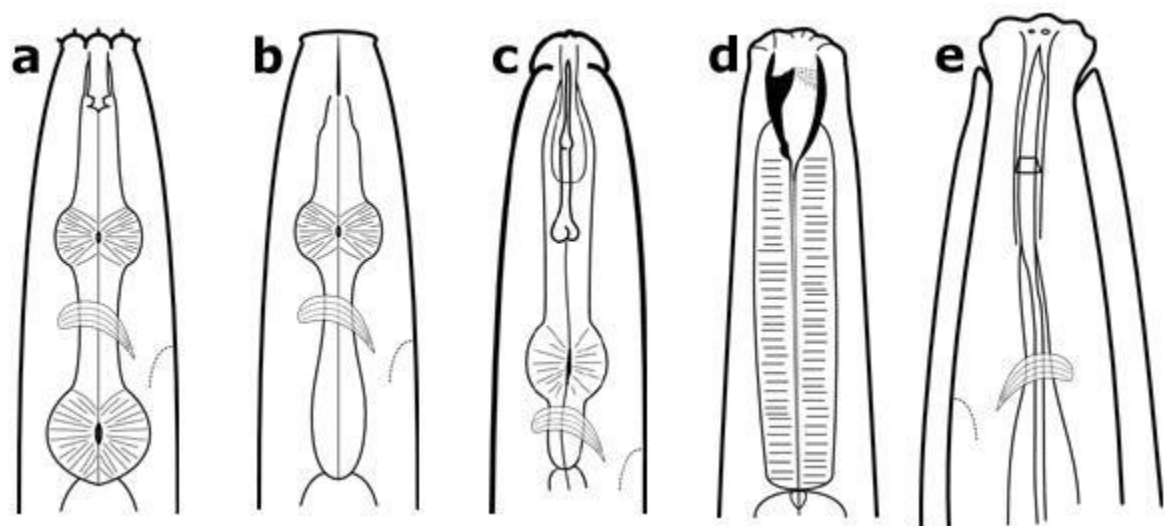


Figure 1.4. The anterior/head regions of (a) bacterial feeder, (b) fungal feeder, (c) plant feeder, (d) predator, (e) omnivore. Figure credit: Ed Zaborski, University of Illinois. <http://nsismke.blogspot.com/2012/10/functional-feeding-groups.html>.

1.2.2.2.1 Bacterivores

This group consists of many genera of the Order Rhabditida (Decraemer and Hunt, 2013; Hunt *et al.*, 2018) and feed either saprophytically or on bacteria (including pathogenic bacteria that damage plants), which are extremely abundant in soil. The mouth of a bacterivore represents a tube-like stoma (Figure 1.4A), which can be equipped with or without anterior probolae or

setae; used to draw bacterial suspensions into the alimentary canal by the sucking action of the esophagus. This group of nematodes are termed beneficial due to their ability to stimulate mineralization in soils by consuming and dispersing microorganisms in the soil (McSorley, 2007; Yeates and Stirling, 2008). Bacterivores commonly form part of the nematode communities in soils in different parts of the world and often constitute up to 20% of nematode communities that occupy a broad range of soil habitats (De Goede and Bongers, 1998; Doroszuk *et al.*, 2007).

1.2.2.2.2 Fungivores

This group of nematodes, comprising many members of the order Aphelenchida, feeds on fungi in soils/associated with plant roots/other parts by puncturing hyphae with a fragile stylet (Decraemer and Hunt, 2013) (Figure 1.4B). The most common genera of fungivores represent the genera *Aphelenchus* Bastian, 1865, *Aphelenchoides* Fischer, 1894, *Ditylenchus* Filipjev, 1936 and *Tylenchus* Bastian, 1865 (Ferris *et al.*, 2001; Sieriebriennikov *et al.*, 2014). Like bacterivores, fungivores are also very important in decomposition of organic materials in soils, having the ability to control some plant-pathogenic fungi but may also suppress some beneficial mycorrhizal fungi (McSorley, 2007; Yeates and Stirling, 2008). The feeding habit of fungivores have been found to impact differently on the soil ecology; an applicable example is grazing of fungivores on mycorrhizal fungi, which may restrict mycorrhizal development and thus limit nutrient uptake by host plants (Baynes *et al.*, 2012). Fungivores are reported to be abundant in organically amended soils and they have the potential to suppress nematode-trapping fungi. However, the availability of fungi food sources again significantly influences fungivores population densities (Jaffee, 2006). Fungivores are generally present in lower population densities in the soil than the bacterivores and plant-parasitic nematodes (Freckman and Caswell, 1985; Bae and Knudsen, 2001).

1.2.2.2.3 Omnivores

Members of the order Dorylaimida, which may feed on algae, bacteria, fungi and other soil biota, including other nematodes are referred to as omnivorous nematodes and are characterised by having a strong, protrusible and hollow stylet (Figure 1.4E) (Yeates and Stirling, 2008). A common example of an omnivore dorylaimid is that of *Aporcelaimus* (Thorne & Swanger, 1936) Makatinus Heyns, 1965; the most predominant omnivore found in

south-west Nigerian agricultural soils as a result of this study (see Chapter 2; Article 1) and regarded as an omnivore and predator according to Yeates *et al.* (1993). Its omnivory was hinged on its feeding on algae, but Wood (1973) observed a species of *Aporcelaimus* feeding on algae, moss and nematodes.

1.2.2.2.4 Predators

All members of the Order Mononchida are exclusively predacious, although a few predators are also found in the Order Dorylaimida (McSorley, 2007; Yeates and Stirling, 2008). The mouthparts of the predators are characterised by a strong stylet (in Dorylamida) or a triangular- shaped tooth with or without denticles/minute teeth (in the Order Monochida) (Fig. 1.4D). This group of nematodes feed on protozoa, rotifers, tardigrades, arthropods, as well as other nematodes. Predaceous nematodes that possess one or several teeth, e.g. *Mylonchulus* Cobb, 1916, ingest the whole body of its prey using the denticles to tear open the cuticle. Those that have a stylet, e.g. *Seinura* Fuchs, 1931 feed much like the fungal and plant feeders by piercing and sucking out the body contents of their prey (Yeates and Stirling, 2008).

1.2.2.3 The role of free-living nematodes in soil quality

Nematodes respond rapidly to external influences, such as disturbance or enrichment, within their environments (Neher, 2001). Colonizer-persister (c-p) values were developed to allow for practical studies of soil nematode community dynamics (Bongers & Bongers, 1998; Yeates *et al.*, 1993). In addition, functional guilds have been assigned to different free-living nematode genera representing the integration of their feeding habits. A concise summary of the c-p value scale is included in Table 1.1, while the functional guilds assigned to free-living genera can be found in Ferris & Bongers (2009) and Cesarz *et al.* (2015).

Table 1.1. The colonizer-persister (c-p) scale for free-living nematodes as developed by Bongers (1990) and expanded, and refined by Ferris *et al.* (2001).

c-p values	Description	Examples
c-p 1	This group mainly include the bacteri, but also fungivores with high fecundity, within a short life cycle and have dauer larvae as a survival stage.	<i>Panagrolaimus</i> , <i>Rhabditis</i>

c-p 2	Consist of mainly bacteri- and fungivores with a longer life cycle, and lower fecundity than the cp-1 group. Nematodes in this group are highly tolerant to adverse conditions, they feed continuously irrespective of the level of availability of resources.	<i>Aphelenchus</i> , <i>Cephalobus</i> , <i>Monhystera</i> , <i>Plectus</i>
c-p 3	Includes bacteri-, fungi- and carnivores with longer life cycles. Nematodes in this group are very sensitive to adverse environmental disturbance.	<i>Chromodera</i> , <i>Prismatolaimus</i> , <i>Tobrilus</i> , <i>Tripyla</i>
c-p 4	Includes bacteri-, fungi-, and carnivores as well as smaller omnivore species with longer life cycles and that are more sensitive to disturbances.	<i>Alaimus</i> , <i>Ironus</i> , <i>Prodorylaimus</i> ,
c-p 5	Mainly consists of carni- and omnivores with the longest life cycles, large bodies size, lowest fecundity and most sensitive to disturbances and/or instabilities.	<i>Aporcelaimellus</i> <i>Aporcelaimus</i> , <i>Thornia</i> .

Bacterivores are characterised as colonisers with low c-p values of 1 or 2 (Bongers and Bongers, 1998; Ferris *et al.*, 2001). The proportion of opportunistic bacterivores in the soil is largely dependent on the level of microbial activity. Fungivores having c-p values of 1, 2 or 3 are regarded as more general opportunistic nematodes. These opportunistic low c-p scale bacterivores and fungivores indicate enrichment of soils (Bongers and Bongers, 1998; Ferris *et al.*, 2001). By contrast, the presence of nematodes with c-p values from 3 to 5 is an indication of the stable structure of soil (Ferris *et al.*, 2001). The succession of beneficial nematodes plays a highly significant role in mineralisation of plant nutrients, decomposition of soil organic matter and ultimately, nutrient cycling (Neher, 2001). Nematode community structure has also served as a useful and popular indicator of soil quality (also referred to as ‘soil condition’ or ‘soil health’) since different nematode taxa and trophic groups differ in sensitivity and response to disturbances, and pollutants within their environment (Ferris *et al.*, 2001; Neher, 2001).

A highly effective and useful approach to classify soils in one of four quadrats in terms of its quality is based on free-living nematode assemblages. These assemblages represent enrichment and structure that is again based on the different c-p scale values that represent the

beneficial nematodes identified (Figure 1.5). This is representative of the faunal analyses, which is illustrated along an enrichment (EI) and structure (SI) trajectory (Bongers and Bongers, 1998; Ferris *et al.*, 2001). This tool has been upscaled and refined, and is nowadays available online as the Nematode Indicator Joint Analysis (NINJA) application: a nematode-based automated biological monitoring system (Sieriebriennikov *et al.*, 2014). The NINJA package utilises the nematode faunal composition to provide information on changes in decomposition pathways along the soil food-web, indicating succession, soil acidity, nutrient and fertility status as well as extent of contamination within the soil (Gruzdeva and Sushchuk, 2010).

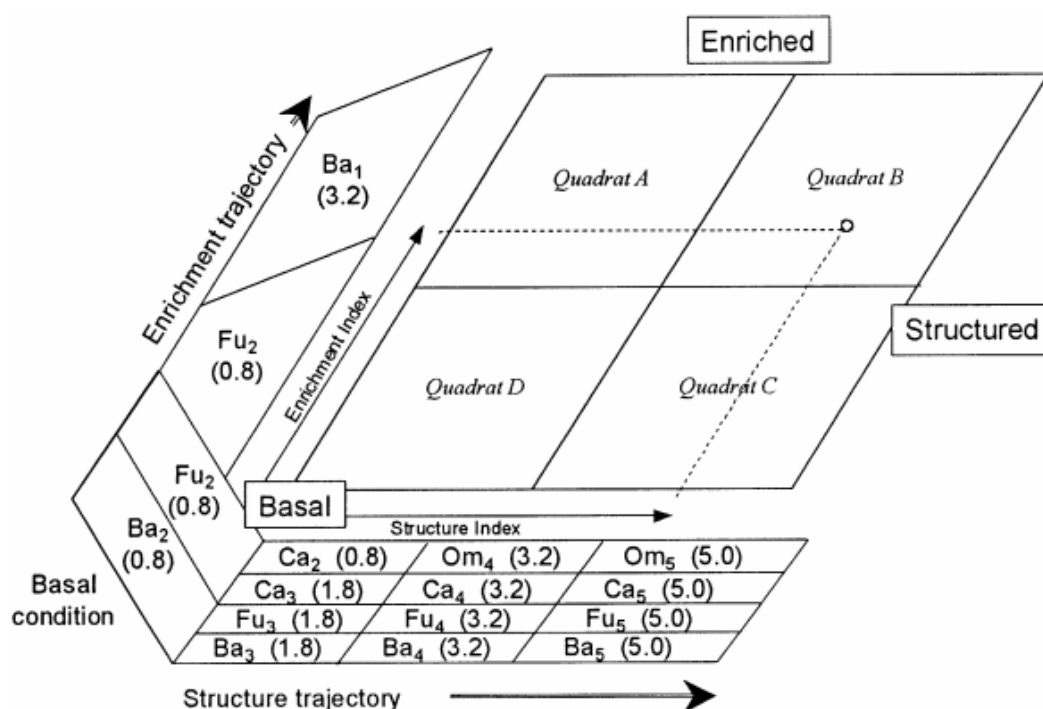


Figure 1.5. The classification of soils into four quadrats based on guilds assigned to free-living soil nematodes according to their feeding habits and demonstrated along a coloniser-persister (c-p) scale (Bongers and Bongers 1998) and food-web structure: represented by the enrichment (EI) and structure (SI) indices. Ba = bacterivores; Fu = fungivores, Om = omnivores; Ca = carnivores; Numbers after each guild indicates the c-p value of a particular nematode trophic group (Ferris *et al.*, 2001).

1.2.3 Plant-parasitic nematodes

Plant-parasitic nematodes are representative of the orders Rhabditida (formerly known as Tylenchida), Dorylaimida and Triplonchida (De Ley and Blaxter, 2002; Decraemer and Hunt, 2013). These pests have a characteristic protrusible stylet (Figure 1.4A), which they use to penetrate the cell walls of plant cells and ingest the contents. The stylet of plant-parasitic nematodes are hollow in all nematode orders except in representatives of the Triplonchida, which have a solid stylet. Some plant-parasitic nematode genera cause great economic losses (qualitative and/or quantitative) in agriculture (Jones *et al.*, 2013). In addition to the direct adverse effects such nematode pests inflict on plant health, they also play a role in disease complexes by acting either as i) vectors (e.g. for several plant-pathogenic viruses); ii) wounding agents (e.g. for fungi and bacteria); iii) host modifiers (modify the host cell content to enable nematodes and other pathogens to feed on it); iv) resistance breakers (virile populations that render nematode resistance genes ineffective); and v) rhizosphere modifiers (causing increased root exudation, thereby destabilizing microbial communities within the rhizosphere) (Bardgett *et al.*, 1999; Brussaard *et al.*, 2001). Different plant-parasitic nematode genera/species do not all have equal effects on their plant hosts. For example, those that feed shallowly on/just below the epidermis or in cortex tissue are migratory semi-endoparasites and/or ectoparasities, such as *Helicotylenchus* Steiner, 1945 (spiral nematodes), *Paratylenchus* Filipjev, 1936 (pin nematodes) and others usually affect plant productivity and energetics to a lesser extent than those feeding in the vascular system. The latter include sedentary endoparasites, such as *Meloidogyne* Goeldi, 1887 (root-knot nematodes), *Heterodera* Schmidt, 1871 (cyst nematodes) and others (Decraemer and Hunt, 2013).

1.2.3.1 Nematode pests of watermelon

Unlike some other cucurbits, only a few nematode pest genera/species have been reported to cause damage to watermelon. By large, *Meloidogyne* spp., the No-1 rated nematode pest of crops worldwide (Jones *et al.*, 2013), have been listed as the most prevalent and widespread nematode pests of watermelon, causing considerable yield losses throughout the world (Luc *et al.*, 2005; Liu Bin *et al.*, 2015; Thies *et al.*, 2015). Other plant-parasitic nematodes, apart from *Meloidogyne*, associated with watermelon from different parts of the world include are listed in Table 1.2

Table 1.2. Other plant-parasitic nematodes, apart from *Meloidogyne*, associated with watermelon from different parts of the world.

Countries	Nematodes identified
Egypt	<i>Criconemoides</i> Taylor, 1936, <i>Heterodera</i> , <i>Hirshmaniella</i> Luc and Googey, 1964, <i>Hoplolaimus</i> von Daday, 1905, <i>Pratylenchus</i> , <i>Trichodorus</i> Cobb, 1913 and <i>Tylenchorynchus</i> Cobb 1913 (Abd-Elgawad <i>et al.</i> , 2007)
India	<i>Belonolaimus</i> Steiner, 1949), <i>Helicotylenchus</i> Steiner, 1945, <i>Pratylenchus</i> and <i>Tylenchorynchus</i> (Anwar and McKenry, 2012)
South Africa	<i>Criconema mutabile</i> Taylor, 1936, <i>Criconemoides sphaerocephalus</i> Taylor, 1936, <i>Helicotylenchus dihystra</i> (Cobb, 1893) Sher, 1961, <i>Hoplolaimus pararobustus</i> (Schuurmans Stekhoven & Teunissen, 1938) Sher, 1936, <i>Pratylenchus</i> spp., <i>Pratylenchus zeae</i> Graham, 1951, <i>Rotylenchulus parvus</i> Sher, 1961, <i>Scutellonema brachyurus</i> (Steiner, 1936) Andrassy, 1958, <i>Tylenchorhynchus mashoodi</i> Siddiqi & Basir, 1959 and <i>Tylenchorhynchus ventralis</i> Loof, 1963 (Marais, 2019)
USA	<i>Rotylenchulus</i> Steiner, 1945, <i>Belonolaimus</i> , <i>Helicotylenchus</i> , <i>Xiphinema</i> Cobb, 1913 and <i>Tylenchorhynchus</i> (Maynard, 2001; Noling, 2015).

In the eastern part of Nigeria, species of *Heterodera* Schmidt, 1871, *Pratylenchus* (lesion nematodes) as well as the fungivore *Aphelenchus* have been recorded from watermelon fields (Mbaukwu *et al.*, 2016). This is the only reference of nematode pests of the crop from Nigeria.

1.2.3.2 *Meloidogyne* spp. pests of watermelon

Meloidogyne spp. are cosmopolitan and extremely polyphagous with an extensive host range (>3 000 plant species) that include almost every known plant (Jones *et al.*, 2013). They are obligate sedentary endoparasites and establish a complex trophic relationship with their host by induce anatomical, biochemical and physiological changes in host plants during feeding (Abad *et al.*, 2009). The infective second-stage juvenile stage (J2) penetrate roots/other below-ground parts of thousands of plant species (including ornamentals, herbaceous and woody plants) by moving intercellularly through the infected tissues (Jones *et al.*, 2013). Subsequently the anatomy, gene expression and physiology of their hosts are altered by formation of specialised feeding sites (giant cells), necrosis or eliciting of defence reactions (Abad *et al.*, 2009). Giant cell formation is essential to the nutrition, development and reproduction of *Meloidogyne* spp. (Hemprabha and Balasaraswathi, 2008), with females withdrawing nutrients from such feeding sites until the completion of their life cycle (Abad *et*

al., 2009). Deformation of and damage to vascular tissues due to giant cell development tends to limit translocation of water and nutrients, which contributes towards the suppression of plant growth (stunting of plants, yellowing of leaves) and even death of plants (Figure 1.6C); ultimately resulting in crop yield losses. The formation of galls results in expansion of cortical cells and division of pericycle tissues in the area of giant cell formation (Figures 1.6A & B). The feeding J2 secretes a plant growth regulator and glycoproteins from its subventral esophageal glands, which stimulate the hypertrophy (cell enlargement) and hyperplasia (cell multiplication) of the infected host cells (Abad *et al.*, 2009). Galls vary in sizes and shapes, depending on the host plant species, root-knot nematode species and the population density of the nematodes within the galls (Sardanelli and Ellison, 2005). Hence, identification of the root-knot nematode species using gall size and form is not recommended since it may vary among and within species (Sardanelli and Ellison, 2005). Unlike the giant cells, galls are not a prerequisite for optimal development of the nematode (Davis, 2007).

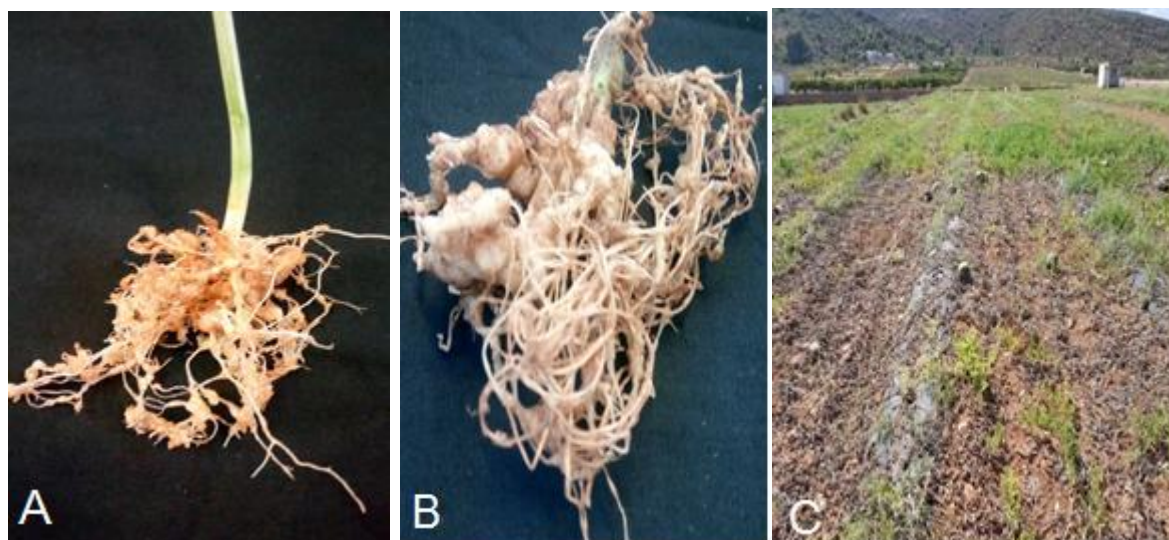


Figure 1.6 A, B and C. Galled roots of a watermelon plant due to infection by *Meloidogyne* spp. (A and B) (Source: Tesleem Bello, NWU) and an aerial view showing above ground symptoms of root-knot nematode damage (C) (Source: NEVAL). <http://www.ne-val.com/la-problematica-del-cultivo-de-la-sandia-hongos-y-nematodos>.

Root-knot nematode females lay eggs into gelatinous matrixes found around the perineal region at the posterior end of the saccate body (Karssen *et al.*, 2013). Two kinds of egg masses (white and brown) are formed by *Meloidogyne* spp., depending on environmental stress. This

strategy ensures that all J2 do not hatch at once since about 10% of eggs will enter diapause and can hatch later in a growing season or under unfavourable conditions (Perry *et al.*, 2009). White egg masses are usually produced early in the host growing cycle, with J2 hatching from eggs immediately upon development to ensure more than one generation per growing season. As the growing season progresses and/or adverse environmental stresses are imposed on the host, the egg masses turn brown and the eggs go into dormancy. These eggs are ‘carried over’ from one season to another to ensure survival of the species (Perry *et al.*, 2009).

The embryonic stages as well as the first-stage (J1) juvenile are in the egg. The J1 molts in the egg and the vermiform J2 hatches from the egg and infects the plant host, usually penetrating roots/other subterranean plant parts of the host just below the root tip (Perry *et al.*, 2009). Initially it was suggested that *Meloidogyne* spp. are generally unaffected by the presence of the host with the J2 hatching freely at appropriate temperatures and when water is available (Eisenback and Triantaphyllou, 1991). Recent research, however, shows that J2 hatching is dependent on root exudates (Curtis *et al.*, 2009; Perry *et al.*, 2013).

The J2 moves intercellularly through the plant cells in the cortex and vascular cylinder where it establishes a specialised feeding site (referred to as a giant cell). Here the J2 undergo morphological changes and gradually assume a fusiform or flask-shaped structure within 10 days after root penetration. Moulting of such J2 into third (J3) and fourth (J4) stage juveniles then follows, with no feeding taking place by the latter two juvenile stages since they do not have stylets. The J3 and J4 moult and eventually become sessile, reproducing adult females that again developed stylets and resume with feeding, or they develop into vermiform males that do not feed (Karssen *et al.*, 2013). Male formation is generally rare and uncommon, but occurs most often when the plants are heavily galled and the population density is extremely high (Stirling *et al.*, 2002) or adverse environmental conditions occur (Karssen *et al.*, 2013). When food is in abundance, most juveniles however develop into females (Karssen *et al.*, 2013). The relationship between the life stage development rate of *Meloidogyne* spp. and temperature is linear. Egg development, host root invasion by J2 and subsequent development to mature adults in host tissue all have different temperature requirements, e.g for *M. javanica* development occurs between 23-30 °C, optimal development is at about 29 °C while a

temperature range of between 21-28 °C has been found to be optimal for *M. incognita* (Osunlola and Fawole, 2014).

The reproduction potential of *Meloidogyne* spp. refers to the ability of a particular population (containing a single species) or community (containing mixed species) to reproduce in roots of a susceptible host plant (Karssen *et al.*, 2013) and is associated with injuriousness. Depending on the degree of injuriousness of a particular *Meloidogyne* sp., host plants are classified as either non, poor or good hosts (Karssen *et al.*, 2013). Variability has been observed among *Meloidogyne* spp. in terms of the penetration rates of J2, the degree of root galling inflicted by feeding J2 and females, and final population densities (Pf) (Winstead and Riggs, 1959; Edelstein *et al.*, 2010). In some studies, *M. enterolobii* was observed to have a wider host range, higher pathogenicity and increased reproduction potential compared to the other economically important *Meloidogyne* spp. species (Brito *et al.*, 2007; Cetintas *et al.*, 2007). With regard to watermelon, *M. javanica* has for example been reported to show lower root galling and egg production than *M. incognita* in roots of some genotypes in Israel (Cohen *et al.*, 2014) and Spain (López-Gómez *et al.*, 2016).

In economic terms, an estimated annual global losses of \$157 billion dollars was associated with *Meloidogyne* spp. (Singh and Kumar, 2015), while in Africa concise quantification of economical losses due to root knot nematodes is difficult even though the detection and distribution of species have been widely reported (Onkendi *et al.*, 2014). Also, statistics on crop losses due to root-knot nematode parasitism have not been generated for some countries because galled roots were considered as being a normal phenomenon by farmers (Adesiyun *et al.*, 1990). In commercial watermelon where no genotypes have been associated with a reasonable level of resistance to *Meloidogyne* spp., crop losses of up to 50% to complete crop failures have been reported (Oda *et al.*, 1997). In the USA, yield losses of 30% have been reported (Davis, 2007), while 12% has been logged for India (Singh and Kumar 2015). However, yield loss data for watermelon in Nigeria due to root-knot nematode parasitism is not available.

On a worldwide basis, the economically most important species in order of distribution and crop damage inflicted are in descending order: *M. incognita*, *M. javanica*, *M. hapla*

(Chitwood, 1949) and *M. arenaria* (Jones *et al.*, 2013). Upcoming threat species in the tropics are reported to be *M. enterolobii*; *M. paranaensis* (Carneiro *et al.*, 1996); and *M. minor* (Karssen *et al.*, 2004) (Wesemael *et al.*, 2011; Onkendi *et al.*, 2014). In Nigeria, the three commonly known and economically important *Meloidogyne* spp. species in order of abundance and economical importance are *M. incognita* races 1 and 2, *M. javanica* and *M. arenaria* race 2. Interestingly, these species are found in all parts of the country with *M. javanica* predominantly occurring in the northern states; *M. arenaria* in both the northern and middle-belt states; and *M. incognita* mostly in the southern parts of Nigeria (Adesiyun *et al.*, 1990; Olowe, 1992). Recently, *M. enterolobii* has been reported from the middle and southern parts of the country. (Kolombia *et al.*, 2017; dos Santos *et al.*, 2019).

1.2.4. Species identification of nematodes

1.2.4.1 Free-living nematode identification

Nematode identification traditionally was based mostly on the use of morphology and morphometrics using light microscopy. This morphology-based technique is time consuming and requires extensive knowledge and expertise which often limits identification to the higher taxonomic ranks such as family, or genus levels (Powers *et al.*, 2011). Most species-rich soils may contain more than 200 species, with a relatively small numbers of species dominating the community and with rare species also being present. The complex morphological structures of free-living nematodes and the lack of knowledgeable personnel to identify such species poses a great challenge to identify these nematode groups compared to their plant-parasitic counterparts that have been extensively studied (Neher, 2010; Guardiola *et al.*, 2015). Identification of rare species may be particularly challenging since it might be difficult to find sufficient representative samples to study (mostly adult females and males) (Fonseca *et al.*, 2010). Recently however, DNA-based methods have provided opportunities to solve the problem and has continuously played a critical role in biodiversity studies especially of microscopic organisms like nematodes (Guardiola *et al.*, 2015). Hence DNA based techniques have proved to be complimentary in providing increased taxonomic resolution, particularly in terms of rare or emerging threat species that may have been misidentified or missed when using morphological methods (Treonis *et al.*, 2018).

1.2.4.2 Plant-parasitic nematodes with focus on *Meloidogyne*

Complex features such as conservative morphology, varying life stages due to different habitats, wide host range, species complexes, species polyploidy with a potential hybrid origin, sexual dimorphism, and over a century of human-aided dispersal have made identification of especially *Meloidogyne* spp. a challenging task (Blok and Powers, 2009). The success of any management strategy depends largely on the knowledge about the causative organism of plant disease and this cannot be achieved without correct identification. Thus, species differentiation by the use of diagnostic techniques is a crucial component of management of economically important pests such as the *Meloidogyne* spp.. Conventional methods for nematode identification rely on time-consuming morphological and morphometric analysis of several specimens of the nematode species in question. The accuracy and reliability of such identification depends largely on the experience and skill of the person making the diagnosis, with the number of such qualified and experienced nematode taxonomists being limited and declining (Coomans, 2002; Nega, 2014). However, the recent increased access to new technologies has provided useful opportunities in nematode identification and has resulted in an increase in the level of development and reliance on molecular-based nematodes diagnostic protocols (Nega, 2014). Since more than half of the *Meloidogyne* spp. that are characterised to date have been described during the last 20 years, there is the possibility of encountering more new species. This is particularly true for the tropical regions, where a rich nematode diversity is experienced (Blok and Powers, 2009).

The most commonly used morphological diagnostic features for identification of *Meloidogyne* spp. as described by Kleynhans (1991), Brito *et al.* (2004), Eisenback and Hunt (2009) and Karssen *et al.* (2013) are listed in Table 1.3. The use of this traditional approach has a lot of challenges. Apart from the fact that it requires a high level of expertise (Hunt and Handoo, 2009; Karssen *et al.*, 2013), some features, such as perineal-pattern morphology of females as well as morphometrics of the life stages of several *Meloidogyne* spp. are similar, which further complicates accurate identification (Adam *et al.*, 2007). The overlap of many morphological features of *M. incognita* with those of *M. enterolobii* has, for example, contributed to its inaccurate identification in the past (Brito *et al.*, 2004; Hunt and Handoo, 2009). A good example is the female head region and various structures/organs of J2 of the two species which are also very similar, making differentiation between them even more difficult. For example,

the female of *M. incognita* has a stylet of 15-16 μm long whereas that for *M. enterolobii* is 14-17 μm in length (Brito *et al.*, 2004; Hunt and Handoo, 2009). Another similarity between these two species is that the mean length of *M. incognita* J2 ranges between 350-450 μm , while that of *M. enterolobii* ranges between 377-528 μm (Hunt and Handoo, 2009). These are just a few examples of similarities between life stages of the two said *Meloidogyne* spp., with various other indistinguishable characteristics existing among the major species, that complicates identification.

In Nigeria, the most widely used method used to discriminate among *Meloidogyne* spp. has been the morphological approach using the use of the perineal patterns of females (Bem *et al.*, 2014). As discussed above, this particular approach poses a great challenge to nematologists, and in this case those in Nigeria (Daramola *et al.*, 2015). However, recently molecular approaches have been used increasingly as is referred to below.

Table 1.3. The most commonly used morphological and morphometrical diagnostic features for identification and distinguishing between *Meloidogyne* spp.

Life stages	Morphological features	Morphometrical parameters
Female	Body shape/form, shape of head region and annulation, stylet shape/form, stylet knobs, form/shape, shape and form of procarpus and metacarpus. nature of perineal pattern (shape of dorsal arch presence/absence of wings, shape of lateral field, presence/ absence of punctuations or phasmids).	Body length and width, distance of DGO (dorsal gland orifice) from base of stylet, position of excretory pore, length of vulva slit, position of structures dorsally and ventrally positioned to vulva.
Male	Shape and form of the entire body, shape of head region and annulation, stylet shape/form, stylet knobs, form/shape, presence/absence of labial disc,	Length of body, stylet length, position of DGO in relation to base of stylet knobs

	shape/form of spicule, lateral field.	
Second stage juveniles (J2)	Shape and form of the entire body, shape of head region and form of annulation, stylet shape/form, stylet knobs, overlap of pharyngeal gland, form of rectum, tail shape, form of hyaline region.	Body length, distance of DGO from stylet, stylet knob length, position of excretory pore, location of hemozonid in relation to excretory pore, length of hyaline region

As opposed to the difficulty experienced with morphological and molecular approaches when used to identify some *Meloidogyne* spp., the development of biochemical and molecular techniques resulted in quicker and more accurate identification of such species (Pagan *et al.*, 2015). Generally, the two biochemical approaches that have been reported for identification of *Meloidogyne* spp. are isozymes and antibodies (Blok and Powers, 2009). The use of isozyme phenotypes to differentiate *Meloidogyne* spp. was first published by Esbenshade and Triantaphyllou (1985). These authors identified approximately 300 populations originating from 65 countries across the continents using esterase patterns. Since then, phenotypes for several species have been published (Blok & Powers 2009; Onkendi *et al.*, 2014), with carboxylesterase/esterase being identified as the most effective to distinguish between different *Meloidogyne* spp. from Nigeria (Kolombia *et al.*, 2017) and recently from various continents including Africa (dos Santos *et al.*, 2019).

Unlike isozymes, the use of antibodies for nematode identification relies on the use of an antibody that recognizes the surface of the target nematode by incubating it with the target nematode's tissue content suspension by using an immuno-magnetic capturing system (Chen *et al.*, 2001; Blok and Powers, 2009; Nega, 2014). This method has been used effectively with up to 80% of the target nematode species being identified from field samples (Chen *et al.*, 2001, 2003).

After the development of esterase and antibody techniques, DNA-based approaches became popular. The DNA of the target nematode could be easily extracted using just one or a few

individuals of any life stage. (Adam *et al.*, 2007; Subbotin *et al.*, 2013). Several DNA-based methods have been used in nematode identification including the polymerase chain reaction, (PCR), sequence characterised amplified regions (SCAR), real time PCR, random amplified polymorphic DNA (RAPD), microarrays and DNA sequencing (Berry *et al.*, 2008; Blok & Powers, 2009; Subbotin *et al.*, 2013). For this study, SCAR-PCR technique was used for identification of *Meloidogyne* spp. (see Chapter 4) and a summary of this techniques follows.

The SCAR-PCR technique has been used successfully to identify several root-knot nematode species since the early 2000s (Zijlstra *et al.*, 2000). Using this method, identification of multiple *Meloidogyne* spp. within the same sample can be done in a single reaction (Blok and Powers, 2009; Ye *et al.*, 2012). This method involves the use of specific primers that are amplified by means of PCR, with repetitive regions of a sequence referred to as SCARs being obtained (Blok and Powers, 2009). This technique has been able to effectively distinguish among the three thermophilic species (*M. arenaria*, *M. incognita* and *M. javanica*) as well as for *M. enterolobii* (Long *et al.*, 2006). Furthermore, the other cryophilic species (*M. hapla*, *Meloidogyne fallax* Karssen, 1996 and *Meloidogyne chitwoodi* Santo and Finley, 1980) have been differentiated using this method (Zijlstra *et al.*, 2000).

The SCAR-PCR technique is a widely used, cost-efficient technique that is reproducible (Zijlstra *et al.*, 2000; Tigano *et al.*, 2010; Nischwitz *et al.*, 2013; Visagie *et al.*, 2018). An advantage of these methods is that just a single, or a few individuals of any life stage are sufficient to obtain positive identifications (Adam *et al.*, 2007). The technique is however prone to problems of interference between primers especially in a multiplex reaction which may lead to compromised specificity since multiplexing works only with a limited number of primers (Blok and Powers, 2009). The sensitivity and specificity of these primers may, however, vary with respect to the different *Meloidogyne* spp. and isolates tested (Ye *et al.* 2012). Furthermore SCAR-PCR technique has been adjudged as being labour intensive (Ahmed *et al.*, 2016).

In Nigeria, the use of advance biochemical and DNA-based methods to identify *Meloidogyne* spp. has increased during the past few years. For example, the isozyme technique was applied to identify *Meloidogyne* spp. (*M. arenaria*, *M. enterolobii*, *M. incognita* and *M. javanica*)

from Nigeria and Ghana as reported by Kolombia *et al.* (2017). Also, DNA-based *Meloidogyne* spp. identification was recently reported from Nigeria by the use of SCAR-PCR by Daramola *et al.* (2015). Pagan *et al.* (2015) utilised the mitochondrial haplotype based technique to distinguish among the predominant *Meloidogyne* spp. from Benin, Kenya, Nigeria and Tanzania, while dos Santos *et al.* (2019) utilized a combination of isozyme, SCAR-PCR and RAPD techniques to successfully identify the commonly occurring *Meloidogyne* spp. that damage crops in Benin, Kenya, Nigeria, Tanzania and Uganda. However, the use of molecular identification through DNA-based techniques such as RAPD, SCAR-PCR, PCR assays, RFLPs, AFLP, microsatellites and real-time PCR (qPCR) is still scanty in Nigeria and should be used on a wider basis to ensure accurate identification of *Meloidogyne* spp.

1.2.5 Management of *Meloidogyne* spp.

Management of *Meloidogyne* spp. poses a major challenge worldwide because most species of this genus have an extensive host range (Moens *et al.*, 2009; Jones *et al.*, 2013). Monoculturing, poor management practices, the presence of weeds that act as hosts of nematode pests are all factors that cause their population densities to build up in roots/other below-ground parts of susceptible crops grown by producers season after season (Rich *et al.*, 2009; Wesemeal *et al.*, 2011).

The ultimate goal of any management strategy is, therefore, reducing the population densities of *Meloidogyne* spp. in the soil to protect crops from either direct attack or being predisposed to secondary infections. This way producers can achieve maximum crop yields at a reasonable cost (Norshie *et al.*, 2011). Nematode pest management strategies that are commonly practiced in most parts of Africa can be categorized broadly as cultural, chemical and biological. These strategies are either applied singly or combined as deemed fit by the farmers to achieve the desired result (Onkendi *et al.*, 2014).

1.2.5.1 Cultural and physical control measures

Cultural practices include a wide range of strategies of which only a few are mentioned here such as crop rotation, the use of organic soil ammendments, solarisation, the development and use of genetic host-plant resistance, the use of clean planting materials, intercropping with

poor or non-hosts of the target nematode pest, and practicing of good farm hygiene (Brown *et al.*, 2006; Coyne *et al.*, 2009). Some of these methods are used in Nigeria and in the wider SSA region to reduce *Meloidogyne* spp. densities (Onkendi *et al.*, 2014; Mashela *et al.*, 2017).

1.2.5.2 Crop rotation

Although crop rotation, which is part of Good Agricultural Practices (GAP), was not developed originally with nematode management in mind, over time, it has been one of the most cost effective nematode management strategies to protect crops against nematode pests (Siddiqui 2003). The rotation of susceptible with resistant or immune crops may lead to the death or inability of weakened *Meloidogyne* spp. J2 to infect a host plant because of starvation and attack by parasites and/or predators (Westerdahl, 2008).

A watermelon production system in Indiana involving rotations with soybean (*Glycine max* L. Merrill) and maize (*Zea mays* L.) did not suppress *M. incognita* densities. This was largely because all the crops used in succession were good hosts of the latter root-knot nematode species (Westphal, 2011). This confirms that root-knot problems are generally aggravated when susceptible crops are grown successively during follow-up seasons. In southwest Nigeria, the most commonly practiced rotation systems involve maize and cassava (*Manihot esculenta* Crantz) intercropped with yam (*Dioscorea* spp. L.) (Oyekale and Adepoju, 2012). Watermelon is usually grown as a monocrop or intercropped with either cassava or maize (Adeoye *et al.*, 2011; Adojutelegan *et al.*, 2015). However, the use of crop rotation to manage nematodes is a rare practice in Nigeria since most high yielding cultivars of major food crops such as maize, cassava, yam, cowpea (*Vigna unguiculata* (L.) Walp), rice (*Oryza sativa* L.) and groundnut (*Arachis hypogea* L.), as well as vegetables grown are susceptible to *Meloidogyne* spp. This is in agreement with Moens *et al.* (2009) that the use of crop rotation to manage *Meloidogyne* spp. is still a challenge due to the polyphagous nature of these organisms. Furthermore, a major limitation of crop rotation is that long fallow periods may result in financial losses to the farmers who have limited area of land available for cultivation. This is particularly applicable to subsistence farmers in southwest Nigeria (where our study was done) where watermelon is planted as a high income cash crop and for food security purposes. Challenges associated with the increasing human population as well as rapid

urbanisation has continuously limited the availability of land for agricultural activities in certain parts of the continent (Onkendi *et al.*, 2014).

Cover crops have also been used in crop rotation systems to reduce or suppress *Meloidogyne* spp. numbers (Zasada *et al.*, 2005). Commonly used cover crops such as *Crotolaria* spp. has been known to reduce the populations of *Meloidogyne* spp. by acting either as a non or poor host. In addition some cover crops produce allelochemicals that are toxic or inhibitory to nematode development and/or produce a niche for antagonistic flora and fauna, trapping the nematode and microbial formation of nematicidal compounds in the soil (Sikora and Fernandez, 2005; Zasada *et al.*, 2005). In Nigeria, *Crotolaria juncea* L. and *Tagetes erecta* L. have proved to be effective in the management of *M. incognita* on several crops like yam (Claudius-Cole *et al.*, 2014) and cowpea (Olabiyi and Oyedunmade, 2007) under field and greenhouse conditions. However, in South Africa the inclusion of *C. juncea* in a cotton-based rotation resulted in a decline in *M. incognita* race 4 densities but increased *Pratylenchus* sp. numbers (Van Biljon *et al.*, 2015). Care must thus be taken that use of such a cover crop does not result in creating a problem other than that the target nematode pest posed.

In Spain, *Tagetes patula* L., when used as a trap crop showed a hypersensitive necrotic response to *M. incognita* J2, subsequently preventing giant cell development for feeding by the nematode and completion of its life cycle (Buena *et al.*, 2008a). Finding a poor or non-host crop that has the same economic value as a susceptible crop, and that can be used as cover crop especially if the target nematode pest has a wide host like *Meloidogyne* spp. is, however, considered a major limitation of this approach (Zasada *et al.*, 2005).

1.2.5.3 Organic soil amendments

Organic amendments are derived from a variety of sources, including agriculture, forestry and urban areas. Of those generated by agriculture, livestock manure (fresh, composted, solid fractions from anaerobic digesters) from various species (cattle, hogs, poultry) is the most prevalent. Other amendments derived from agriculture include crop residues (straw, legumes) and spent mushroom compost. Forestry produced organic amendments on the other hand include deinking sludges, wood chips and shavings (Larney and Angers, 2012).

Nematode control resulting from the use of organic amendments have been attributed to several factors including toxic effects of nitrogen, organic acids, substances and compounds like ammonia, nitrate, hydrogen sulphide, phenolic compounds, amino acids, aldehydes, carbohydrates, fatty acids that are released during the decomposition process, and also due to the presence of predatory fungi, nematodes, insects and mites. Thoden *et al.* (2011) assumed that the variations in the efficiency of organic amendments to reduce nematode pest population densities was a function of interactions amongst the microbial populations that pre-exist in the soil, in particular the non-parasitic nematodes. Apart from being cost effective, the use of organic material also improves the efficiency of antagonistic bacteria present in soils by providing them with nutrients which are essential for their survival and growth (Onkendi *et al.*, 2014).

Soil amendment with different organic materials to reduce nematode population densities is gaining wide acceptance as an alternative control method (Oka, 2010; Udo and Ugwoke, 2010). The addition and incorporation of organic matter to soils generally result in an increase in population densities of parasites and predators of plant-parasitic nematodes and contribute to reducing their populations (Nwanguma *et al.*, 2011). Animal manures, for example have been used in various studies in Nigeria to reduce root-knot nematode population densities (Oka *et al.*, 2007; Nwanguma *et al.*, 2011). Chicken litter was reported as being the most promising organic amendment for root-knot nematode control on pepper (*Capsicum* spp. L) in a study conducted in south-west Nigeria (Nwanguma *et al.*, 2011). The latter authors reported that poultry manure at 20 MT/ha had the highest suppressive effect on soil and root nematode populations as well as root damage of pepper caused by *M. incognita*. Poultry manure has also been reported to suppress population densities and the hatching of root-knot nematode J2 that infect okra (*Abelmoschus esculentus* (L) Moench) roots in south-west Nigeria (Tanimola and Akarekor, 2014). Furthermore, efficacy of three organic amendments (virgin olive pomace, olive pomace based compost and chicken manure) in reducing *M. incognita* population densities on melon was reported from Italy by Abdeldaym *et al.* (2014).

For watermelon, only a few reports are available that showed that several organic amendments have been used to manage *Meloidogyne* spp. on the crop worldwide. In Bangladesh, Faruk *et al.* (2011) reported poultry manure and mustard oil cake as been

effective in suppressing *M. incognita* on watermelon. Also, Baloch *et al.* (2013) observed a significant reduction in *Meloidogyne* spp. penetration of watermelon roots planted in soil treated with organic ammendments of decomposed seaweed (*Spatoglossum variabile* Figari and De Notaris (1853) in Pakistan. Since poultry manure has proved to be efficient in managing *Meloidogyne* spp. on some major crops in Nigeria and on watermelon in other countries, it might also be a viable option for managing the pest on watermelon in southwest Nigeria too.

1.2.5.4 Solarisation

Physical methods such as solarisation or heat treatment of the soil when combined with some other cultural methods have been reported to be effective in the management of *Meloidogyne* spp. (Védie *et al.*, 2014). Solarisation involves the process of trapping solar radiation by placing transparent plastic films on the soil to maximise conversion and conservation of heat. It was first reported by Katan *et al.* (1976) and since then solarisation has been widely studied. Solarisation has the ability to increase soil temperature by 2-15 °C in warm climate conditions and its efficacy depends on the combination of high soil temperatures and the duration of such a condition (Védie *et al.*, 2014). Candido *et al.* (2018) reported effective suppression of *M. javanica* on melon and tomato using solarisation under screenhouse conditions in Italy. In a field experiment conducted in Nigeria, Ogwulumba and Ugwuoke (2011) utilized black plastic bags as mulch to suppress the reproduction of *M. javanica* in tomato roots. Several other authors from South Africa and west African countries have also shown the efficacy of solarization in managing *Meloidogyne* spp. (Carson and Otoo, 1996; Mashela *et al.*, 2017)

1.2.5.5 Biological control

Biological control encompasses any ecologically based strategy that ultimately results in the reduction in pest populations (Striling, 2014). This involves the use of living organisms (natural enemies of the target pest or disease) to manage pest populations and relies on predation, parasitism, herbivory or natural mechanisms but typically involves an active human role (Flint and Dreistadt, 1998). Several micro-organisms are known to possess nematophagous properties. These organisms have been identified and classified as trappers, endoparasites, egg-parasites and toxin producers (Liu *et al.*, 2009). A few commercial biocontrol agents, mostly containing fungi as the active ingredient are used to reduce plant-

parasitic nematode population densities with varying degrees of success. A promising strategy for the biocontrol of soil-borne plant pathogens reported from Nigeria is the exploitation of plant-growth promoting rhizobacteria (PGPR) (Udo *et al.*, 2013). The fungus *Purpureocillium lilacinum* (previously named *Paecilomyces lilacinum*) was applied to tomato roots in a Nigerian field experiment and showed promising results for the control of *Meloidogyne* spp. since it reduced galling and the number of eggs and J2 in the roots (Udo *et al.*, 2013). Furthermore, *Trichoderma* spp., e.g. *T. pseudokoningii* have been used to control *Meloidogyne* spp. on rice, tomato, maize in Nigeria (Oyekanmi *et al.*, 2008) and on egg plant (*Solanum melongena* L.) in Egypt (El-Nagdi and Abd-El-Khair, 2008). The rhizobacterium *Paenibacillus polymyxa* was reported to significantly reduce *M. incognita* egg and J2 densities in tomato roots in a controlled experiment in India (Sidiqqi and Akhtar, 2009). Formulations of *Pochonia chlamydosporia* in combination with *Pseudomonas fluorescens* have also been reported to significantly reduce the number of galls on roots of bell pepper induced by *M. incognita* in India (Rao *et al.*, 2004).

1.2.5.6 Genetic host plant resistance

Host-plant resistance to nematodes has been defined as the inherent characteristics of a plant which makes it able to avoid damage (due to reduced reproduction and development of the target nematode pest) or to recover from attacks inflicted on it by plant-parasitic nematode species (Moens *et al.*, 2009; Karssen *et al.*, 2013). Resistant cultivars retard or prevent the development and reproduction of *Meloidogyne* spp., depending on the level and type of resistance, and thus reduce populations significantly in comparison to that of a susceptible variety (Sardanelli and Ellison, 2005). Resistance could be broadly classified as either qualitative or quantitative. Qualitative resistance refers to resistance variation that is due to allelic differences at just one or two *R* genes (resistance genes) with allele effects large enough so that one can reliably determine an individual's resistance genotype from its phenotype at the single plant level regardless of environmental variation (St Clair, 2010). Quantitative resistance has also been defined in different ways, phenotypically as the reduction but not complete elimination of disease compared with the most susceptible phenotypes, and genetically as resistance based on the combined action of many genes of modest effect (Niks *et al.*, 2015).

Several mechanisms are involved in genetic host plant resistance. Tolerance is for example a very important mechanism of resistance which relates to the ability of a host genotype to withstand or recover from the damaging effects of nematode attack and still yield well compared to a less tolerant plant (Horber, 1980; Trudgill, 1991; Smith, 2005). Hypersensitive response (HR) is another common resistance mechanism which results in necrosis of the feeding site within a couple of days post infection (Bakker *et al.*, 2006). Antibiosis is used to describe adverse effects of resistant plants on pest physiology and life history such as reduced growth, survival, and fecundity (Horber, 1980), while antixenosis denotes plant traits affecting pest behaviour in ways that reduce the preference for, or acceptance of, a plant as a host by a pest (Kogan & Ortman, 1978). Immunity could be seen as the most superior mechanism of resistance and refers to capacity of a plant to prevent or withstand biological attack by invading biological agent (Miller *et al.*, 2017).

Resistance to nematodes in plants is generally achieved by failure of the nematodes to produce functional feeding sites in the host after invasion and to develop subsequently as reproducing females. The utilisation of cultivars resistant to *Meloidogyne* spp. has been identified as a potential alternative for methyl bromide as part of an integrated pest management approach (Giannakou and Anastasiadis, 2005). The use of host plant resistance is usually very effective, environmentally friendly and inexpensive to the farmer since it does not require additional inputs or technology (Djian-Caporallino *et al.*, 2007; Kamuya *et al.*, 2008). If available, the use of resistant germplasm will be one of the best nematode control options for Africa because, except for being cost effective, its use does not have any damaging effect on the environment.

A concern when using a resistant host plant to reduce root-knot nematode infection is, however, that resistance-breaking populations of *Meloidogyne* spp. exist; rendering this strategy ineffective (Buena *et al.*, 2008b). The species *M. enterolobii* has particularly been found to show virulence against several available sources of *Meloidogyne* spp. resistance genes and is therefore considered very injurious. The species was reported to render the resistance genes (*Mi-1*, *N* and *Tabasco*) ineffective in terms of the resistance they confer to the major *Meloidogyne* spp. (*viz.* *M. incognita*, *M. javanica* and *M. arenaria*) in tomato and pepper, respectively (Brito *et al.*, 2007; Thies *et al.*, 2008). Furthermore, *M. enterolobii* was also reported to have a wider host range, higher pathogenicity and increased reproduction potential compared to its thermophilic counterpart species (Cetintas *et al.*, 2007; Brito *et al.*,

2007). *Meloidogyne javanica* is another highly injurious species as previously reported from Nigeria (Ogbuji, 1981; Nzeako *et al.*, 2013) where it caused destruction of pepper and tobacco (*Nicotiana tabacum* L.) in field greenhouse trials. Reports from South Africa again also showed that local *M. javanica* populations were the most injurious on tomato in glasshouse and microplot trials (Fourie *et al.*, 2012; Visagie *et al.*, 2018; Rashidifard *et al.*, 2019). Studies from the USA also reported *M. javanica* as the most injurious *Meloidogyne* spp. causing damage on tobacco when compared to *M. arenaria* and *M. incognita* (Arens and Rich, 1981). Furthermore, Ornat *et al.* (2001) identified a highly injurious population of *M. javanica* that rendered the *Mi* resistance gene in tomato plants in Spain ineffective. Managing these species, but also virulent types of other thermophilic *Meloidogyne* spp., in particular thus pose a challenge to producers, especially on the African continent where subsistence agriculture constitute a major segment of the agricultural sector (Coyne *et al.*, 2009).

In Nigeria, a few cultivars of major crops like maize (e.g. genotypes ART98SW6-OB and ILE1-OB) were reported to be resistant to *M. incognita*, while the cassava cultivar TME B419 was recently reported to be tolerant to *M. incognita* (Akinsanya and Afolami, 2019). In addition, the cowpea cultivar 1T845-2246-4 was identified with resistance to *M. incognita* in field and screenhouse trials (Adegbite *et al.*, 2005; Adegbite 2011). These resistant cultivars can be harnessed and used either as intercrops or in rotation with watermelon to manage *M. incognita* on the crop in Nigeria. However, crop cultivars that are resistant to nematodes are few and most nematode pests are polyphagous (Wang *et al.*, 2008).

For watermelon in particular, no commercial cultivar worldwide has been reported to show resistance to *Meloidogyne* spp. Thies *et al.* (2016) evaluated 19 accessions of *C. lanatus* var *citroides* and some wild watermelon accessions in the USA, with results showing lower levels of *M. incognita* reproduction and higher vigour and root mass when compared with reference entries of commercial cultivars. This study suggested that wild watermelon accessions may serve as useful sources of resistance to *M. incognita*.

In Nigeria, the most commonly cultivated watermelon cultivars are Sugarbaby, Sugar dragon, Crimson Sweet, Kaolak, Charleston Grey and Kollos. However, no information exists regarding the host status of these cultivars to the commonly occurring *Meloidogyne* spp.

prevalent in watermelon production areas in the country. Producers in Nigeria are poor and cannot afford expensive chemicals and genetic host plant resistance may offer them a realistic way to prevent their watermelon crops from being damaged by *Meloidogyne* spp. Moreover, should cultivars with resistance against *Meloidogyne* spp. become available for use in Nigeria, the follow-up crops of such farmers could also be protected against high population densities of these pests. However, breeding for genetic resistance in crops is a tedious process which can take years to in the end result in a product that can be used by farmers.

1.2.5.7 Chemical control

Although chemical control is the most effective and most rapid method to reduce nematode pest population densities over the short term and is achieved by the use of nematicides (Radwan *et al.*, 2007; 2012), it is just mentioned as a management strategy but not discussed in depth. Chemically-derived nematicides, however, aside from being toxic to the farmer during application, also leave residues in the food chain which has led to increased environmental concerns. Therefore, increasing pressure from consumers and other related groups have been experienced to ban the use of highly toxic products such as methylbromide and others (Wesemael *et al.*, 2011).

In Nigeria, the four registered and most widely used nematicides on major crops are carbamates, viz. aldicarb (2-methyl-2-[methylthio] proionaldehyde O-[methylcarbamoyl]oxime), oxamyl (methyl-N', N'-dimethyl-N-[(methylcarbamoyl)oxy]-1-thioxamidate), carbofuran (2, 3-dihydro-2,2-dimethyl-7-benzofuranyl methycarbamate), and miral (0-[5-chloro-1-[1-methylethyl]-1H-1, 2, 4-triazol-3-yl] O,O-diethylphosphorothioate) (Erhunmwunse *et al.*, 2012). While a range of nematicides have been registered for use on watermelon in the USA (Noling, 2009), no nematicide is registered for use on watermelon in Nigeria.

1.2.5.8 Preventative management strategies

A range of already described preventative strategies exist to limit nematode damage and are applied worldwide, including Nigeria, to protect the crops of farmers (Moens *et al.*, 2009). Exclusion is for example a very effective and economical means of preventing nematode damage and is achieved successfully through quarantine. Various quarantine measures have

assisted to reduce the risk of spreading *Meloidogyne* spp. through international trade routes since various species (e.g. *M. fallax* and *M. chitwoodi*) are listed as quarantine pest threats (Moens *et al.*, 2009; Karssen *et al.*, 2013). *Meloidogyne enterolobii*, a species that is also present in Nigeria (Kolombia *et al.*, 2017) and to which this strategy applies has for example been added onto the EPPO quarantine list (EPPO/PQR 2017).

Planting of healthy and nematode-free planting materials (e.g hot water treatment) is another effective means towards ensuring good crop yields by protecting it against nematode pests. The development of an environmentally-friendly and good practice seed production system can significantly reduce nematode problems and effectively represent a long-term strategy for establishing good quality pest and disease-free planting material within SSA (Coyne *et al.*, 2009). Such an exciting new approach represents the wrap and plant technology consisting of a field-deployable nutrient-rich biodegradable product, made from banana fibre impregnated with abamectin (a nematicide), that has been developed in Kenya. Growing of seed potato wrapped in such a matrix significantly reduced potato cyst nematode populations in the soil and resulted in a 4-fold increase in potato yield compared to untreated seed potato (Ochola *et al.*, 2019). The use of such techniques will be one of the best future nematode control options for Africa because it is simple, easily adaptable and eco-friendly.

The above-mentioned nematode management approaches can be used singly to manage or reduce nematode damage on watermelon, but the future of nematode management in Africa will have to depend on an integrated approach involving a combination of two or more compatible strategies. Only this way will *Meloidogyne* spp. and other nematodes affecting watermelon adversely be effectively managed over the long-term and will sustainable production of the crop be made possible.

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CHAPTER 2: ARTICLE 1

Free-living nematode assemblages in the rhizosphere of watermelon plants in Nigeria: a baseline study

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Free-living nematode assemblages in the rhizosphere of watermelon plants in Nigeria: a baseline study

Tesleem T. BELLO^{1,2,3,*}, Danny L. COYNE² and Hendrika FOURIE¹

¹North-West University, Unit for Environmental Sciences and Management, Integrated Pest Management, Private Bag X6001, Potchefstroom 2520, South Africa

²International Institute of Tropical Agriculture, IITA, PMB 5320, Ibadan, Nigeria

³Federal College of Education, Abeokuta, PMB 2096, Ogun State, Nigeria

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Summary – Watermelon is increasingly produced and consumed in Nigeria and sub-Saharan Africa. However, limited information exists regarding nematode pests and beneficial/free-living nematodes associated with the crop. The present study recorded the abundance and diversity of free-living nematodes from 50 watermelon fields across south-west Nigeria during 2016/2017. Of the 30 genera identified from soil samples, *Cephalobus*, followed by *Rhabditis*, *Aphelenchus* and *Aporcelaimus*, were predominant. Variation in nematode community structures across the 50 fields was apparent for mean maturity indices, metabolic footprints, feeding-type composition and coloniser-persister (c-p) structure. Faunal analyses characterised 52% of the fields as having stable and enriched soil food webs, which is beneficial for crop production. Significant correlations were apparent between some nematode genera and selected soil properties, and rainfall. This study provides the first information of free-living nematodes associated with watermelon from sub-Saharan Africa, offering novel and baseline information on their abundance and diversity in south-west Nigeria.

Keywords – beneficial nematodes, *Citrullus lanatus*, faunal analysis, prominence values, soil health.

Watermelon (*Citrullus lanatus* L.) is among the world's most important vegetable crops, grown for the fruits it produces and the high economic returns to farmers (Schippers, 2000). Of the global annual production of 118.5 million metric tonnes (MT) recorded for 2016, 6.2 million MT is produced in Africa (FAO, 2018). Watermelon cultivation in Nigeria, originally confined to the drier north, is gradually extending to the south west. This expansion in production is stimulated by increasing demand and driven partly by enhanced consumer awareness of the dietary and health benefits of fresh vegetable consumption (Iheke, 2009). In south-west Nigeria, yields between 26.4 and 38.7 MT ha⁻¹ (Okunlola *et al.*, 2011) are lower than those obtained elsewhere in the country (Abdulrahman & Yahaya, 2009), due to various constraints, including low soil fertility and, especially, soil-borne diseases and pests. Nematode communities are known to have substantial effects on soil productivity, which ultimately affects crops. Functional guilds, representing the integration of nematode feeding habits (Yeates *et al.*, 1993; Bongers & Bongers, 1998), and coloniser-persister

(c-p) scales (Bongers, 1990; Ferris *et al.*, 2001) have been developed to allow for practical studies of soil nematode community dynamics (Johnson, 2000). Association of soil nematodes with the cycling of nutrients due to their ability to regulate soil bacterial and fungal populations (Ingham *et al.*, 1985; Perez-Moreno & Read, 2001; Hoorman, 2011) lead to a more positive view of the role of nematodes in soil processes being adopted (Yeates, 2003). However, the importance and ecological value of terrestrial free-living nematodes have been recognised since the early 1960s (Banage, 1963). Terrestrial free-living nematode communities and their usefulness have been studied on a number of crops (Neher & Campbell, 1994; Porazinska & Coleman, 1995; Dong *et al.*, 2008; Chandra & Khan, 2011), but only limited information is available for their abundance and distribution, as well as the ecological services they provide, within sub-Saharan Africa (SSA) agro-ecological systems (Durand *et al.*, 2012).

Unlike some other cucurbits, just a few nematode pest genera/species have been reported to cause damage to watermelon. *Meloidogyne* spp. (root-knot), *Belonolaimus*

* Corresponding author, e-mail: tesleembello@yahoo.com

spp. (sting nematodes) and *Rotylenchus* spp. (spiral nematodes) are reported as the most economically important in the USA (Maynard, 2001; Noling, 2015), whilst root-knot nematodes appear to be the most prevalent and widespread nematode pests of watermelon globally, causing considerable yield losses (Li *et al.*, 2015; Thies *et al.*, 2015). In Nigeria, species of *Heterodera*, *Meloidogyne*, *Pratylenchus* (lesion nematodes) as well as the fungivorous *Aphelenchus* are reported from watermelon fields in the eastern part but with no indication of their importance to production locally (Mbaukwu *et al.*, 2016).

Watermelon production, just like many other crops in Nigeria, is characterised by low input farming systems where crop nutrition relies primarily on soil organic matter, since inorganic fertilisers are not readily available in most rural agrarian communities. Hence any soil health based strategy towards enhancing soil productivity would require adequate knowledge of soil fauna of which nematodes constitute a very important component. Should watermelon production be sustained in Nigeria, a good understanding of the nematode community structures and the soil processes would be useful since nematodes can be regarded as superior bio-indicators of soil conditions due to these organisms reflecting the function and/or structure of ecological processes; responding to changes in soil conditions resulting from land management practices and anthropogenic influences; being abundant in soils; and studied extensively, and in detail, in terms of their taxonomy (Neher, 2001; Ferris, 2010). Therefore, identification of nematodes, both plant-parasitic and free-living, associated with watermelon production becomes important. To date, no information is available on the abundance, distribution and diversity of free-living nematodes associated with watermelon in SSA.

The current study was conducted to investigate these aspects in providing a good understanding of free-living nematode diversity and soil health dynamics using watermelon agroecosystems as a case study. This knowledge is important in making decisions and formulating policies that will facilitate the maintenance of soils to optimise sustainable food production.

Materials and methods

The survey was conducted in 25 zones of the six states of south-west Nigeria: Lagos, Ogun, Ondo, Oyo, Osun and Ekiti (divided as stipulated in the Agricultural Development Programmes: ADP) (Fig. 1). During the 2016/2017 growing season 50 watermelon fields, two

fields per zone, were sampled, primarily from peri-urban areas (Table 1). Rhizosphere soil samples were removed from actively growing, fruit-bearing watermelon plants. Eighteen plants per field were sampled along a 'W-shaped' pattern (Coyne *et al.*, 2007). Three plant samples were bulked together ultimately to provide six composite samples per field. These were placed in polythene sample bags, labelled accordingly and transported in cool boxes to the nematology laboratory of International Institute of Tropical Agriculture (IITA) for analyses. Information regarding field location (coordinates) was obtained using a Global Positioning System (GPS) device and crop history by means of a basic questionnaire that farmers completed (Table 1). The pH (H₂O) of soil from each field, as well as the percentages of sand, silt and clay were determined by personnel of the Soil Microbiology Unit of IITA using standard protocols (Bouyoucos, 1962), and the percentages of soil organic matter were determined using the Degtjareff method (Walkley & Black, 1934). Rainfall and temperature data were obtained from the Nigerian Meteorological Agency (NIMET; <https://nimet.gov.ng>).

NEMATODE EXTRACTION, COUNTING AND IDENTIFICATION

Each of the six composite soil samples per field were thoroughly mixed and nematodes were extracted from a 200 ml sub-sample of each sample using a modified pie-pan method (Coyne *et al.*, 2007). The nematodes were collected in tap water after extraction, identified to genus level and counted using a Doncaster counting dish (Doncaster, 1962). Nematode individuals were fixed in a 4% formaldehyde solution (Nico *et al.*, 2002) and mounted on glass microscope slides in anhydrous glycerin (De Grisse, 1963) to verify genus identification using morphological characteristics. This was undertaken with the aid of interactive diagnostic keys of University of Nebraska- Lincoln UNL Nematology Laboratory, plus pictorial keys of Andr  ssy (2005), Holovachov *et al.* (2009) and Jairajpuri & Ahmad (1992).

STATISTICAL ANALYSES

Nematode abundance and their frequency of occurrence/prevalence were expressed per field as well as collectively, by calculating the Prominence Values (PV) for each genus according to the equation: $PV = \text{population density} \times \sqrt{\text{frequency of occurrence}}/10$ (De Waele & Jordaan, 1988).

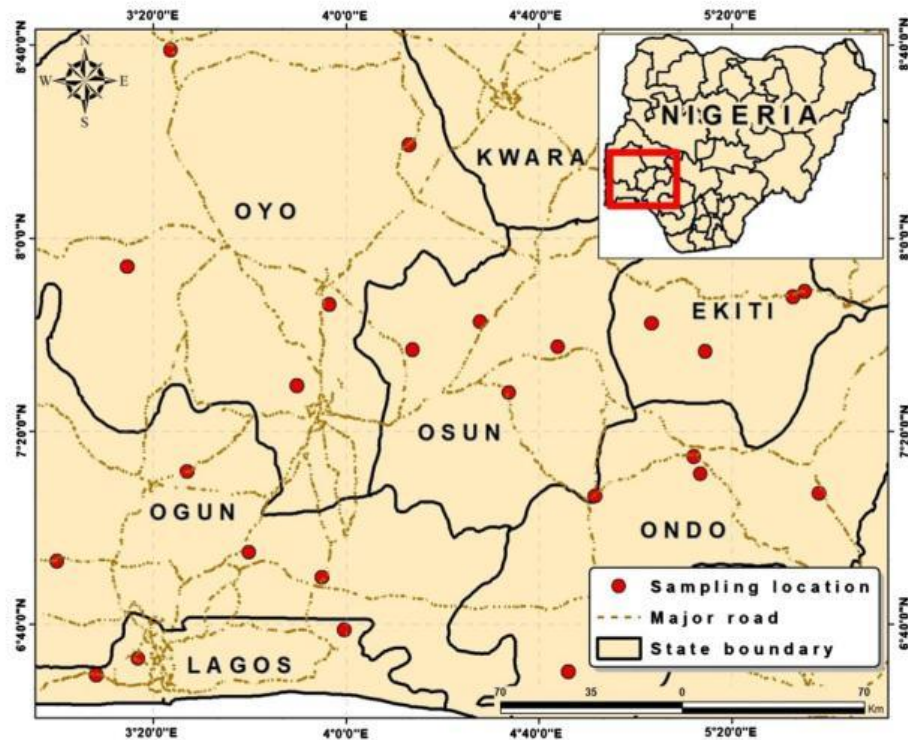


Fig. 1. Map of Nigeria that highlights the south-western states, showing the localities (red circles) where nematode samples were obtained from watermelon fields during 2016 and 2017. Courtesy Omodele Taiwo, Institute of Agricultural Research and Training, IAR&T, Nigeria.

Furthermore, nematode data were subjected to faunal (food web) analysis (Ferris *et al.*, 2001; Ferris, 2010). The enrichment and structure trajectories are calculated independently from the weighted abundance of nematodes in guilds representing the following food web components: basal (b), enrichment (e) and structure (s). Calculation of the three components were done as follows: the b component was calculated as $\sum k_b n_b$ where k_b represents the weightings assigned to guilds indicating basal characteristics of the food web (Ba_2 , Fu_2) and n_b equals nematode abundance in those guilds; the e and s components were calculated using the nematode guilds indicating enrichment (Ba_1 , Fu_2) and structure (Ba_3 - Ba_5 , Fu_3 - Fu_5 , Om_3 - Om_5 , Ca_2 - Ca_5), respectively. Ultimately the Enrichment Indices (EI) were calculated as $100 \times (e/(e+b))$ and Structural Indices (SI) as $100 \times (s/(s+b))$. Calculations for total biomass and metabolic footprints were also done

using the Nematode Indicator Joint Analysis (NINJA) on-line program (Sieriebriennikov *et al.*, 2014). This way the soil condition of each field was categorised into quadrats as being either stressed and enriched (Quadrat A), stable and enriched (Quadrat B), stable and depleted (Quadrat C) or stressed and depleted (Quadrat D) based on where the nematode faunal composition of each sampled field mapped in the faunal profile (Ferris *et al.*, 2001). Nematode diversity was expressed in terms of nematode feeding groups as well as coloniser-persister values. Data were also subjected to Pearson's Correlation Analysis and to Principal Component Analysis (PCA) (Hotelling, 1933; Thioulouse *et al.*, 1997) to establish whether any correlations existed between the frequency of occurrence/prevalence of nematode genera identified and soil properties, as well as rainfall.

Table 1. Field site details of 50 watermelon fields sampled for nematodes across south-west Nigeria during March 2016-February 2017.

State	Zone	Site	GPS coordinates	Soil properties					Rainfall (mm)	Average annual temperature (°C)	Cropping history (2014 and 2015)
				pH (H ₂ O)	Sand (%)	Silt (%)	Clay (%)	OM (%)			
Ekiti	Aramoko	Aramoko	7°42'23.148"N 5°3'22.967"E	6.23	73	19	8	1.96	1345	26.7	Vegetables
	Aramoko	Oye	7°47'52.84"N 5°19'42.628"E	5.30	70	18	12	4.48			Vegetables
	Ado-Ekiti	Ado	7°36'37.944"N 5°14'31.512"E	6.14	71	20	9	3.77	1371		Vegetables
	Ado-Ekiti	Farm settlement	7°42'37.62"N 5°14'54.126"E	5.81	72	17	11	2.11			Maize/ vegetables
	Ikole	Ifaki	7°47'53.812"N 5°32'41.798"E	5.87	68	15	7	4.42	1348		Maize/ cassava
	Ikole	Ikole	7°46'57.586"N 5°30'52.503"E	6.17	70	18	12	2.24			Watermelon
	Ikere	Ayedun	7°48'58.721"N 5°35'7.279"E	6.77	74	12	14	2.69	1432		Maize/ Cassava
	Ikere	Ikere	7°38'32.304"N 5°18'10.944"E	5.99	65	18	17	9.84			Vegetables
Lagos	Lagos east	Onilu	6°32'53.763"N 3°16'51.279"E	5.85	78	15	7	1.94	1851	27.1	Watermelon
	Lagos east	Ilado	6°26'40.476"N 3°26'34.947"E	6.15	82	11	7	3.64			Vegetables
	Lagos west	Ijanikin	6°29'32.364"N 3°8'5.631"E	5.73	83	12	5	3.72	1730		Cucurbits
	Lagos west	Ojo	6°27'42.829"N 3°9'28.384"E	7.01	79	14	7	2.85			Pineapple/ Vegetables
	Lagos far east	Ilara Epe	6°38'56.424"N 3°59'34.464"E	5.95	85	11	4	6.71	1741		Vegetables
	Lagos far east	Ikorodu	6°36'36.15"N 3°55'55.642"E	5.96	77	16	7	5.44			Vegetables/ maize
Ogun	Abeokuta	Osiele	7°11'36.688"N 3°26'56.714"E	7.20	71	19	10	4.68	1307	28.2	Cassava/ vegetables
	Abeokuta	Alabata	7°10'22.84"N 3°22'7.313"E	6.26	68	11	21	13.11			Pepper/ cassava
	Ilaro	Kajola	6°53'5.972"N 3°0'3.786"E	6.04	74	20	6	4.27	1626		Melons
	Ilaro	Erinja	6°52'41.642"N 3°2'45.711"E	5.22	71	21	8	4.84			Maize
	Ijebu	Ijebu ode	6°49'47.928"N 3°54'59.245"E	6.14	76	13	11	12.40	1521		Vegetables/ Yam
	Ijebu	Ago iwoye	6°56'54.232"N 3°57'44.944"E	6.05	74	16	10	3.36			Yam/s Vegetable
	Ikenne	Iperu	6°54'54.755"N 3°39'53.492"E	6.71	72	19	9	5.78	1636		Cassava
	Ikenne	Ilisan	6°52'36.114"N 3°42'33.907"E	5.89	77	13	10	9.87			Vegetables
Ondo	Okitipupa	Okitipupa	6°30'10.872"N 4°46'4.223"E	6.23	70	11	19	12.68	1762	26.8	Maize
	Okitipupa	Igbotako	6°38'52.778"N 4°38'22.146"E	5.64	72	10	18	8.66			Pineapple/ Maize

Table 1. (Continued.)

State	Zone	Site	GPS coordinates	Soil properties					Rainfall (mm)	Average annual temperature (°C)	Cropping history (2014 and 2015)
				pH (H ₂ O)	Sand (%)	Silt (%)	Clay (%)	OM (%)			
	Akoko	Ilepa	7°11'11.171"N 5°13'28.467"E	6.06	71	12	17	12.82	1432		Vegetables
	Akoko	Akungba	7°13'52.176"N 5°33'0.611"E	6.51	74	14	12	8.46			Maize/ Cassava
	Akure	Akure	7°14'46.068"N 5°12'12.312"E	5.56	72	16	12	3.64	1377		Maize
	Akure	Oda	7°11'11.71"N 5°13'28.46"E	6.26	70	13	17	8.74			Watermelon
	Owo	Emure	7°7'4.772"N 5°38'4.344"E	5.54	72	15	13	3.63	1530		Maize
	Owo	Owo	7°13'25.104"N 5°27'43.399"E	6.08	71	14	15	2.16			Vegetables
	Ondo	Owena	7°6'32.904"N 4°51'35.316"E	5.76	75	18	7	3.45	1548		Yam/ Pepper Vegetables
	Ondo	Ondo	7°5'6.842"N 4°57'52.738"E	5.98	70	20	10	3.71			
Osun	Osogbo	Alagba	7°42'40.428"N 4°27'51.16"E	6.34	63	22	15	8.04	1313	25.7	Maize
	Osogbo	Jagun	7°46'28.668"N 4°34'23.627"E	6.21	70	20	10	4.41			Vegetables
	Ife	Ipetumodu	7°28'0.048"N 4°33'47.34"E	7.81	76	15	9	3.61	1441		Cassava
	Ife	Modakeke	7°24'1.548"N 4°38'40.199"E	6.11	76	10	14	4.77			Vegetables
	Ilesha	Imo	7°37'27.592"N 4°43'57.396"E	8.33	80	12	8	2.18	1396		Maize
	Ilesha	Ijamo	7°35'7.114"N 4°48'3.558"E	5.88	78	10	12	6.15			Vegetables
	Iwo	Okeoba	7°36'54.716"N 4°13'50.948"E	6.87	78	13	9	2.09	1393		Vegetables
	Iwo	Isaleoba	7°30'50.536"N 4°14'39.839"E	6.71	62	20	18	13.62			Yam/ Maize
Oyo	Saki	Agoamodu	8°38'56.112"N 3°23'32.423"E	6.63	71	16	13	7.46	1215	27.3	Yam/ Cassava Watermelon
	Saki	Sepeteri	8°30'6.553"N 3°24'14.472"E	6.21	74	15	11	12.86			
	Ogbomoso	Abogunde	8°19'23.952"N 4°12'59.151"E	6.11	74	15	11	3.77	1254		Vegetables
	Ogbomoso	Ijeru	8°20'44.667"N 4°11'9.686"E	6.45	70	16	14	6.63			Curcubits
	Oyo	Isokun	7°46'19.668"N 3°56'34.439"E	5.15	80	9	11	3.19	1334		Maize/ Vegetables
	Oyo	Jobele	7°53'23.663"N 3°50'14.610"E	5.67	78	13	9	4.79			Watermelon
	Ibadan	Ijaye	7°29'29.76"N 3°49'48.101"E	5.86	72	15	13	9.88	1194		Pepper
	Ibadan	Erunmu	7°30'22.84"N 3°54'40.319"E	5.87	72	18	10	5.21			Pineapple/ Vegetables

Table 1. (Continued.)

State	Zone	Site	GPS coordinates	Soil properties					Rainfall (mm)	Average annual temperature (°C)	Cropping history (2014 and 2015)
				pH (H ₂ O)	Sand (%)	Silt (%)	Clay (%)	OM (%)			
	Ibarapa	Okeodo	7°30'55.872"N 3°27'51.274"E	6.05	68	12	20	14.17	13.99		Vegetables
	Ibarapa	Okebaa	7°54'5.317"N 3°14'34.799"E	5.97	70	18	12	5.88			Maize

OM: Organic matter (C). Annual rainfall and temperature data from Nigerian Meteorological Agency (www.nimet.gov.ng).

Results

Thirty genera of terrestrial free-living nematodes (Tables 2, 3) were identified in association with watermelon in south-west Nigeria. Of these, bacterivores were most abundant and were represented by 14 genera (Fig. 2A; Table 3), followed by omnivores with eight genera, predators with seven genera and a single fungivore genus. Guilds were represented by c-p1-5 nematodes, with those classified as c-p2 (ten genera) generally dominating (present in 54% of fields; Fig. 2B; Table 3).

Data pooled across the 50 fields showed that the four predominant genera were *Cephalobus* (Ba₁), *Rhabditis* (Ba₂), *Aphelenchus* (Fu₂) and *Aporcelaimus* (Om₅) (in descending order) with high PVs (674, 519, 483 and 426, respectively). These genera occurred in 58, 54, 32 and 42%, respectively, of the fields sampled (Table 2). *Cephalobus* was predominant in the state of Oyo (PV = 863; MPD = 1114), occurring in 60% of the samples and least dominant in Lagos (PV = 283; MPD = 849), occurring in 33% of the samples (Table 3). *Rhabditis* was predominant in Ekiti (PV = 824, MPD = 592), where it occurred in 75% of samples (Table 3) but was the least dominant in Lagos (PV = 314, MPD = 628) where it occurred in 50% of samples (Table 3). *Aporcelaimus* was predominant in Ekiti (PV = 949; MPD = 1 550) occurring in 58% of the samples, and least dominant in Osun (PV = 87; MPD = 142) where it occurred in 38% of the samples (Table 3). *Aphelenchus* was predominant in Ekiti (PV = 679; MPD = 859) occurring in 63% of the samples, and least dominant in Oyo (PV = 140; MPD = 312), occurring in 20% of samples.

Of the 30 genera identified, *Tripyla* (reported from Ondo and Oyo only) was the least dominant (PV = 13, MPD = 46) with individuals being present in soil samples from only 8% of fields (Table 2).

PCA revealed significant associations between the frequency of occurrence of the four prevalent genera *Cephalobus*, *Rhabditis*, *Aphelenchus* and *Aporcelaimus* and selected soil properties, and rainfall (Fig. 3; Table 4). Occurrence of *Cephalobus* was significantly and positively correlated with percent silt and percent clay, but negatively with percent sand and rainfall, while that of *Rhabditis* was significantly and positively correlated with percent silt and percent clay, and rainfall but significantly negatively correlated with percent sand. Significant positive correlations were also evident between the occurrence of *Aporcelaimus* and percent clay and percent organic matter, and rainfall. However, occurrence of *Aphelenchus* significantly and positively correlated with percent silt but showed significant and negative correlations with percent sand and percent organic matter.

The frequencies of occurrence of four genera (*Mesorhabditis*, *Panagrolaimus*, *Prodorylaimus* and *Wilsonema*) were associated with high soil pH, while that of seven genera (*Aporcelaimus*, *Chromadora*, *Mononchus*, *Plectus*, *Prionchulus*, *Tobrilus* and *Tripyla*) were associated with high percent organic matter. Occurrence of *Aporcelaimellus*, *Aporcelinus* and *Cephalobus* was linked to high percent clay, while that of *Labronema*, *Pseudacrobeles* and *Rhabditis* was associated with high percent silt. The occurrence of *Acrobeloides*, *Eucephalobus*, *Laimydorus*, *Rhabditella* and *Seinura* was associated with high rainfall as well as high percent sand.

The metabolic footprints of the free-living nematode assemblages for each of the 50 fields sampled are plotted on graphs that represent their soil food web status (Fig. 4), enabling quantification of the amplitude of carbon utilisation by different food web components (Sieriebriennikov *et al.*, 2014).

To avoid replication of results, fields with high EI values refer to soils that contain high abundance Ba₁

Table 2. Pooled data, representing prominence values (PV), mean population densities (MPD) and frequencies of occurrence (FO: %) for terrestrial free-living nematode genera identified from 50 watermelon fields sampled during 2016/2017 across south-west Nigeria.

Genus	Guild ¹	No. of fields recorded from	PV ²	MPD ³	FO (%) ⁴
<i>Acrobeles</i>	Ba ₂	14	170	321	28
<i>Acrobeloides</i>	Ba ₂	12	223	455	24
<i>Alaimus</i>	Ba ₄	24	241	349	48
<i>Cephalobus</i>	Ba ₂	29	674	885	58
<i>Eucephalobus</i>	Ba ₂	19	262	425	38
<i>Mesorhabditis</i>	Ba ₁	2	64	318	4
<i>Monhystera</i>	Ba ₂	18	199	332	36
<i>Panagrolaimus</i>	Ba ₁	7	39	104	14
<i>Plectus</i>	Ba ₂	2	12	61	4
<i>Prismatolaimus</i>	Ba ₃	17	140	240	34
<i>Pseudacrobeles</i>	Ba ₂	22	285	429	44
<i>Rhabditella</i>	Ba ₁	14	191	361	28
<i>Rhabditis</i>	Ba ₁	27	519	707	54
<i>Wilsonema</i>	Ba ₂	18	90	149	36
<i>Aporcelinus</i>	Om ₅	27	302	410	54
<i>Aporcelaimus</i>	Om ₅	21	426	657	42
<i>Aporcelaimellus</i>	Om ₅	7	25	66	14
<i>Chromadora</i>	Om ₃	9	82	194	18
<i>Labronema</i>	Om ₄	11	173	368	22
<i>Laimydorus</i>	Om ₅	4	75	265	8
<i>Prodorylaimus</i>	Om ₄	2	17	87	4
<i>Thornia</i>	Om ₄	10	89	199	20
<i>Ironus</i>	Pr ₄	22	176	265	44
<i>Mononchus</i>	Pr ₄	20	218	345	40
<i>Mylonchulus</i>	Pr ₄	17	212	364	34
<i>Prionchulus</i>	Pr ₄	23	113	167	46
<i>Seinura</i>	Pr ₂	11	50	107	22
<i>Tobrilus</i>	Pr ₃	12	143	292	24
<i>Tripyla</i>	Pr ₃	4	13	46	8
<i>Aphelenchus</i>	Fu ₂	16	483	854	32

Coloniser-persister (c-p) values and trophic groups of the nematode genera are also listed.

¹According to the classification of Ferris *et al.* (2001).

²Prominence value (PV) = Population density $\times \sqrt{(\text{Frequency of occurrence})/10}$.

³Mean population density = total number of nematodes in the six samples taken per field divided by the number of samples in which the nematodes occur.

⁴Frequency of occurrence = (number of samples containing a nematode genus divided by the number of samples collected) $\times 100$.

and Fu₂, whilst fields with zero and/or low SI values are generally due to the absence or low abundance of c-p3-5 predators and omnivores (Fig. 4A, B).

Fifty-two percent of the fields plotted in Quadrat B (Fig. 4), which represents stable and enriched soil food-web conditions, had intermediate to high EI and SI values. Soils from 40% of the fields plotted in Quadrat C, which had intermediately high EI and zero or low SI values and was described as stressed and enriched in terms of

its soil nematode food webs. However, 4% of the fields were plotted in Quadrat A and 2% in Quadrat D (Fig. 4); the former quadrat represents stressed and enriched soil conditions in terms of its soil nematode food webs, which had intermediately high EI and zero or low SI values, while the latter quadrat represents stressed and depleted soil conditions having low to intermediate EI and SI values.

Table 3. Data, representing prominence values (PV), mean population densities (MPD) and frequencies of occurrence (FO: %), for terrestrial free-living nematode genera identified from rhizosphere samples of watermelon plants sampled during 2016/2017 from 50 fields across six states in south-west Nigeria.

Nematode genus	Guild ¹	Ekiti			Lagos			Ogun			Ondo			Osun			Oyo		
		PV ²	MPD ³	FO (%) ⁴	PV ²	MPD ³	FO (%) ⁴	PV ²	MPD ³	FO (%) ⁴	PV ²	MPD ³	FO (%) ⁴	PV ²	MPD ³	FO (%) ⁴	PV ²	MPD ³	FO (%) ⁴
Bacterivores																			
<i>Acrobeles</i>	Ba ₂	150	300	25	110	330	33	242	396	38	176	555	10	166	271	38	124	226	30
<i>Acrobeloides</i>	Ba ₂	173	345	25	194	582	33	307	501	38	212	474	20	–	–	–	210	383	30
<i>Alaimus</i>	Ba ₄	192	314	38	100	299	33	377	477	63	163	297	30	197	278	50	291	348	70
<i>Cephalobus</i>	Ba ₂	692	740	88	283	849	33	786	1283	38	723	1022	50	453	523	75	863	1114	60
<i>Eucephalobus</i>	Ba ₂	218	308	50	298	447	67	364	515	50	217	485	20	86	171	25	309	564	30
<i>Mesorhabditis</i>	Ba ₁	–	–	–	–	–	–	159	318	25	–	–	–	–	–	–	–	–	–
<i>Monhystera</i>	Ba ₂	129	258	25	107	321	33	213	347	38	134	300	20	85	139	38	356	460	60
<i>Plectus</i>	Ba ₂	–	–	–	–	–	–	–	–	–	66	120	30	51	103	25	–	–	–
<i>Panagrolaimus</i>	Ba ₁	–	–	–	–	–	–	41	82	25	–	–	–	–	–	–	27	61	20
<i>Prismatolaimus</i>	Ba ₃	81	162	25	118	353	33	322	407	63	72	132	30	68	112	38	64	143	20
<i>Pseudacrobeles</i>	Ba ₂	273	386	50	222	443	50	277	453	38	289	409	50	201	329	38	308	436	50
<i>Rhabditella</i>	Ba ₁	288	576	25	71	212	33	252	504	25	340	538	40	81	162	25	–	–	–
<i>Rhabditis</i>	Ba ₁	824	952	75	314	628	50	356	504	50	597	944	40	393	556	50	490	693	50
<i>Wilsonema</i>	Ba ₂	279	558	25	110	331	33	94	133	50	37	67	30	28	56	25	10	17	40
Omnivores																			
<i>Aporcelinus</i>	Om ₅	393	497	63	139	417	33	279	455	38	285	368	60	116	189	38	406	453	80
<i>Aporcelaimellus</i>	Om ₅	18	36	25	–	–	–	–	–	–	–	–	–	88	175	25	10	22	20
<i>Aporcelaimus</i>	Om ₅	949	1550	58	445	771	33	548	895	38	198	361	30	87	142	38	382	427	80
<i>Chromadora</i>	Om ₃	–	–	–	–	–	–	175	350	25	38	86	20	36	72	25	134	245	30
<i>Labronema</i>	Om ₄	257	419	38	–	–	–	146	291	25	225	356	40	–	–	–	176	393	20
<i>Laimydorus</i>	Om ₅	–	–	–	63	190	33	170	339	25	–	–	–	–	–	–	–	–	–
<i>Prodorylaimus</i>	Om ₄	–	–	–	–	–	–	44	87	25	–	–	–	–	–	–	–	–	–
<i>Thornia</i>	Om ₄	–	–	–	–	–	–	114	228	25	69	155	20	184	368	25	88	161	30
Predators																			
<i>Ironus</i>	Pr ₄	13	26	25	43	129	33	251	355	50	191	302	40	200	284	50	250	354	50
<i>Mononchus</i>	Pr ₄	181	362	25	33	100	33	184	300	38	185	414	20	239	302	63	328	392	70
<i>Mylonchulus</i>	Pr ₄	194	387	25	67	201	33	211	344	38	201	367	30	297	485	38	226	358	40
<i>Prionchulus</i>	Pr ₄	86	171	25	29	86	33	113	161	50	151	196	60	93	132	50	126	163	60
<i>Seinura</i>	Pr ₂	51	102	25	99	296	33	30	61	25	37	68	30	–	–	–	12	28	20
<i>Tobrilus</i>	Pr ₃	175	350	25	–	–	–	152	249	38	148	330	20	82	165	25	195	356	30
<i>Tripyla</i>	Pr ₃	–	–	–	–	–	–	14	–	–	14	32	20	–	–	–	27	61	20
Fungivores																			
<i>Aphelenchus</i>	Fu ₂	679	859	63	238	713	33	581	949	38	561	1254	20	494	987	25	140	312	20

Coloniser persister (c-p) values and trophic groups of the nematode genera are also listed. A dash (–) indicates the absence of nematodes in those samples.

¹According to the classification of Ferris *et al.* (2001).

²Prominence value (PV) = Population density $\times \sqrt{\text{Frequency of occurrence}}/10$.

³Mean population density = total number of nematodes in the six samples taken per field divided by the number of samples in which the nematodes occur.

⁴Frequency of occurrence = (number of samples containing a nematode genus divided by the number of samples collected) $\times 100$.

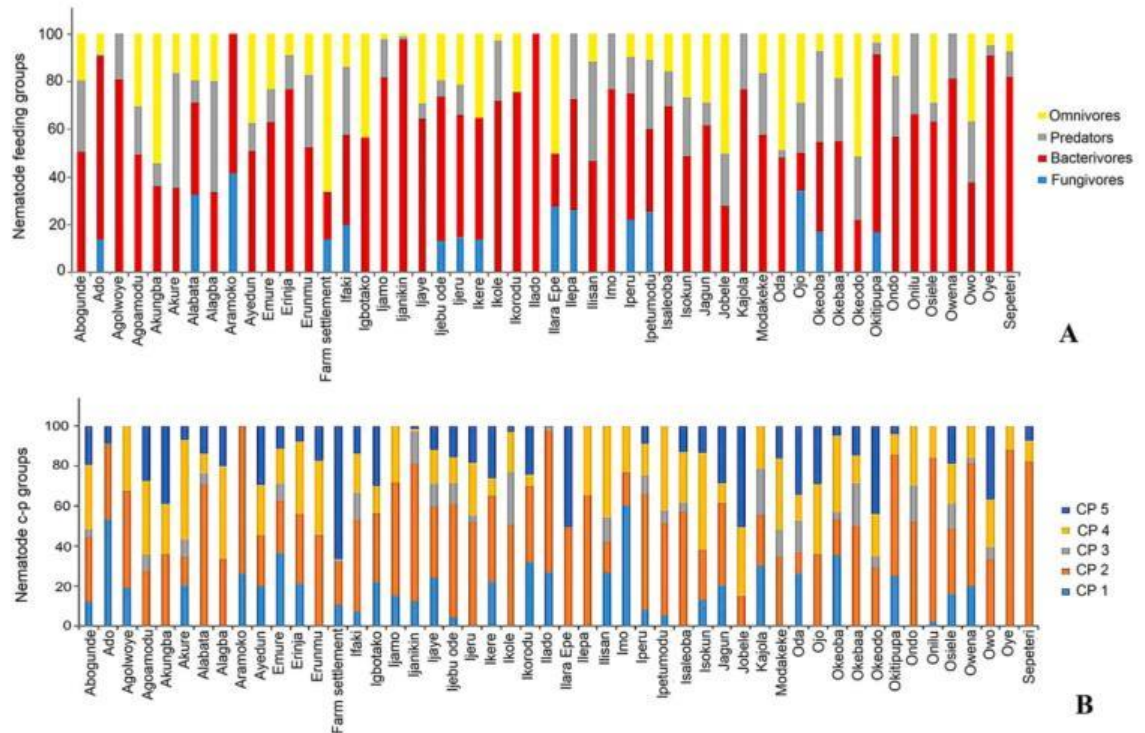


Fig. 2. A: Feeding type composition (as %) of terrestrial free-living nematode assemblages identified from 50 watermelon fields sampled across south-west Nigeria during the 2016/17 growing season. B: The coloniser-persister structure (as %) of terrestrial free-living nematode assemblages identified from 50 watermelon fields sampled across south-west Nigeria during the 2016/2017 growing season.

Discussion

In Africa, as in many other areas, there is only fragmented and limited information available concerning terrestrial free-living nematode assemblages in crop fields. Within SSA, there are, however, substantially more reports on plant-parasitic nematodes in Nigeria than for other countries in that part of Africa (Coyle *et al.*, 2018). Results of the current study, therefore, constitute a valuable addition to the available data that deal with terrestrial, free-living nematodes in SSA. Other known studies for Nigeria include the assessment of nematodes associated with monoculture maize (*Zea mays*) (Eche *et al.*, 2013), yam (*Dioscorea* spp.) (Alabi *et al.*, 2017) and one for weeds (Eche *et al.*, 2015). The 30 terrestrial free-living nematode genera identified from watermelon fields during the current study are first reports for West African watermelon agroecosystems.

A high diversity of 30 terrestrial free-living nematode genera recorded for watermelon fields in Nigeria during this study is consistent with other similar studies. Porazinska & Coleman (1995) reported 17 free-living nematode genera from 21 families from cucurbit fields in Georgia, while Li *et al.* (2015) identified 18 genera from monoculture peanut (*Arachis hypogaea*) farms in China. Furthermore, 43 free-living nematode genera were listed for 30 weeds species growing in mango (*Mangifera indica*), oil palm (*Elaeis guineensis*), citrus (*Citrus sinensis*), maize, cassava (*Manihot esculentus*) and yam (*Dioscorea alata*) fields in north central region of Nigeria (Eche *et al.*, 2015). Alabi *et al.* (2017), however, reported only four genera (*Aphelenchus*, *Cephalobus*, *Monhystera* and *Prisma-tolaimus*) from yam fields in Nigeria (Ogun State) while Eche *et al.* (2013) did not list the genera identified from the rhizosphere of maize samples for their study.

From the available literature, it appears that of the 30 identified genera from watermelon fields, four (*Aphelen-*

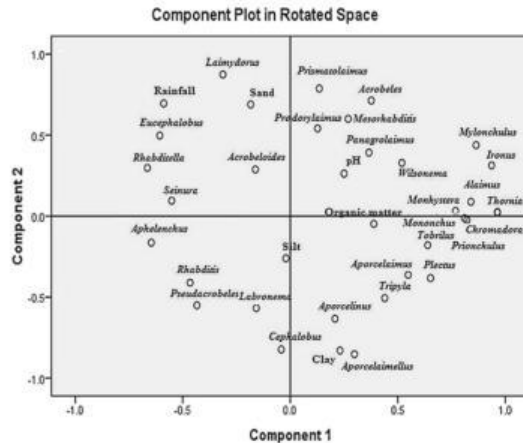


Fig. 3. Principal Component Analysis (PCA) showing correlations among the occurrence of terrestrial free-living nematode genera and rainfall as well as selected soil properties of 50 watermelon fields sampled in south-west Nigeria during the 2016/2017 cropping season. Eigenvalues were 0.945 and 0.327 for PC1 and PC2, respectively, while PC1 and PC2 represented 30.9 and 24.1% of the variations observed, respectively.

cus, *Cephalobus*, *Monhystera* and *Prismatolaimus*) had previously been recorded in south-west Nigeria from yam fields (Alabi *et al.*, 2017), whilst 14 genera were reported from rhizosphere soil of 30 weed species sampled in Makurdi, North Central region of Nigeria (Eche *et al.*, 2015).

Bacterivores were dominant in soils from watermelon fields sampled during this study, agreeing with findings of previous studies. For example, Eche *et al.* (2013) identified bacterivores, represented mainly by the family Rhabditidae, as the predominant trophic group present in soils of maize-cropping systems in northern Nigeria. Also, Eche *et al.* (2015) identified bacterivores (represented by Cephalobidae, Monhysteridae, Plectidae, Alaimoidea and Rhabditidae) as the predominant trophic group in the rhizospheres of weed species sampled from the North Central agroecological zone of Nigeria. The abundance of c-p2 bacterivores (Ferris *et al.*, 2001), also the predominant guild across the 50 fields sampled during the current study, has been associated with soils that contain high levels of organic matter and with high C/N ratios, which is common within organically amended agroecosystems (Ettema & Bongers, 1993). This supports the concept that c-p values of nematode taxa are useful in interpreting the status of soil food webs in different habitats (Bongers, 1990). It furthermore indicates that the high abundance

and diversity of c-p3 to c-p5 guilds recorded in the current study may be a result of high soil organic matter content. Musinguzi *et al.* (2013) proposed a 3.4% organic matter threshold as high for most tropical soils. However, the soil organic matter contents across the 50 fields sampled in this study ranged between 1.94–14.17%, with 82% of the fields sampled having an organic matter content higher than the threshold of 3.4% (Krull *et al.*, 2004; Musinguzi *et al.*, 2013). Ultimately, the majority of fields sampled (52%) during this study plotted in Quadrat B, which refers to stable and enriched food webs in terms of terrestrial free-living nematode assemblages. This implies that soils of such fields are maturing and characterised by low to moderate disturbance; such soils are also N-enriched with low C:N ratios while their decomposition channels are balanced (Ferris *et al.*, 2001; Sieriebriennikov *et al.*, 2014). By contrast, Bekker (2016) and Mbatyoti *et al.* (2018) reported the opposite (low abundance and diversity of c-p3-5 nematode guilds) in South African agricultural soils (where soybean and maize are cultivated) with low organic contents (generally less than 1%). Most of these fields were hence characterised as degraded and depleted (Quadrat D).

Association of soil nematodes with the cycling of nutrients due to their ability to regulate soil bacterial and fungal populations (Ingham *et al.*, 1985; Perez-Moreno & Read, 2001; Hoorman, 2011) led to adoption of a more positive view of the role of nematodes in soil processes (Yeates, 2003). However, the importance and ecological value of terrestrial free-living nematode composition has been recognised since the early 1960s (Banage, 1963). Nonetheless, information about their abundance and distribution as well as the ecological services they provide within SSA agro-ecological systems remain limited (Durand *et al.*, 2012) but depend on many factors, *e.g.*, soil characteristics, as well as climatic and environmental conditions (Bakonyi *et al.*, 2007; Godefroid *et al.*, 2013).

Nematode community structures have been linked to soil and environmental factors (Neher *et al.*, 2005; Fajardo *et al.*, 2011), although opposing results have been obtained in this regard. For example, in our study, the occurrence of the three predominant genera (*Cephalobus*, followed by *Rhabditis* and *Aphelenchus*) was found to be associated with high soil silt contents and rainfall. By contrast, Geraert (1967) reported that *Aphelenchus*, for example, showed no preference for soil type (soil vs clay), while Norton *et al.* (1971) found it more associated with sandy than clay soils. Walker (1984), however, suggested

Table 4. Correlation data for the frequency of occurrence of the four predominant terrestrial free-living nematode genera and rainfall, and selected soil properties of 50 watermelon fields sampled across six states in south-west Nigeria during the 2016/2017 cropping season.

Nematode genera	Parameters	Pearson correlation value	P value
<i>Cephalobus</i>	Frequency of occurrence × pH	0.157	0.463
	Frequency of occurrence × % sand	−0.859*	0.0001
	Frequency of occurrence × % silt	0.460*	0.024
	Frequency of occurrence × % clay	0.925*	0.0001
	Frequency of occurrence × % organic matter	0.399	0.540
	Frequency of occurrence × rainfall	−0.885*	0.0001
<i>Rhabditis</i>	Frequency of occurrence × pH	−0.298	0.176
	Frequency of occurrence × % sand	−0.611*	0.002
	Frequency of occurrence × % silt	0.511*	0.011
	Frequency of occurrence × % clay	0.444*	0.030
	Frequency of occurrence × % organic matter	0.031	0.887
	Frequency of occurrence × rainfall	0.590*	0.002
<i>Aporcelaimus</i>	Frequency of occurrence × pH	−0.101	0.637
	Frequency of occurrence × % sand	−0.382	0.066
	Frequency of occurrence × % silt	0.221	0.299
	Frequency of occurrence × % clay	0.455*	0.026
	Frequency of occurrence × % organic matter	0.660*	0.0001
	Frequency of occurrence × rainfall	0.801*	0.000
<i>Aphelenchus</i>	Frequency of occurrence × pH	−0.286	0.176
	Frequency of occurrence × % sand	−0.622*	0.001
	Frequency of occurrence × % silt	0.784*	0.0001
	Frequency of occurrence × % clay	0.320	0.128
	Frequency of occurrence × % organic matter	−0.452*	0.027
	Frequency of occurrence × rainfall	−0.218	0.307

* Significant correlations at $P \leq 0.05$ (Pearson's Correlation Analysis).

that the rarity of *Aphelenchus* in sandy loam forest soils opposed to preference of a clay loam soil (wheat field) was not related to soil type but rather to biotic factors, such as food type and presence of competitors. Similarly, Sanchez-Moreno *et al.* (2006) suggested that although the abundance of predatory nematodes was sensitive to soil physical perturbation, other soil forces can drive their preference. This should be considered with the occurrence of *Aporcelaimus* (fourth in predominance) being associated with high soil clay and organic matter contents in our study. In general, our results agree with that of Wang *et al.* (2004), who reported that the abundance of various bacterivores and predators was positively correlated with organic matter, while that of fungivores (agreeing with *Aphelenchus* in our study) was negatively correlated.

It is agreed with Palomares-Rius *et al.* (2015) that the interaction between a specific crop and soil physicochemical characteristics impacts on the distribution of certain groups of nematodes, which in effect elevates some ne-

matode functional groups to be superior indicators of soil conditions more than others.

Interestingly, the dominant Ba₂ Cephalobidae and Fu₂ Aphelenchidae are usually regarded as widely distributed general opportunists that can be found even in stressed environments, whereas Ba₁ Rhabditidae are enrichment opportunists, thriving in high N-content systems. Om₅ *Aporcelaimus* is at the other end of the spectrum, indicative of foodweb structure and complexity, in stable and healthy environments. A more ecological interpretation of the distribution of these nematode genera, merged with potential driving factors (OM, pH, rainfall, texture, temperature, previous crops), can help understand the biological state of soils in watermelon production, and also provide some indication as to whether some agricultural practices might improve soil health in the region (*e.g.*, optimal level of organic fertilisation, liming, irrigation and crop rotation). Interpretation of faunal profiles is also a very useful tool and since most soils plotted in Quadrats

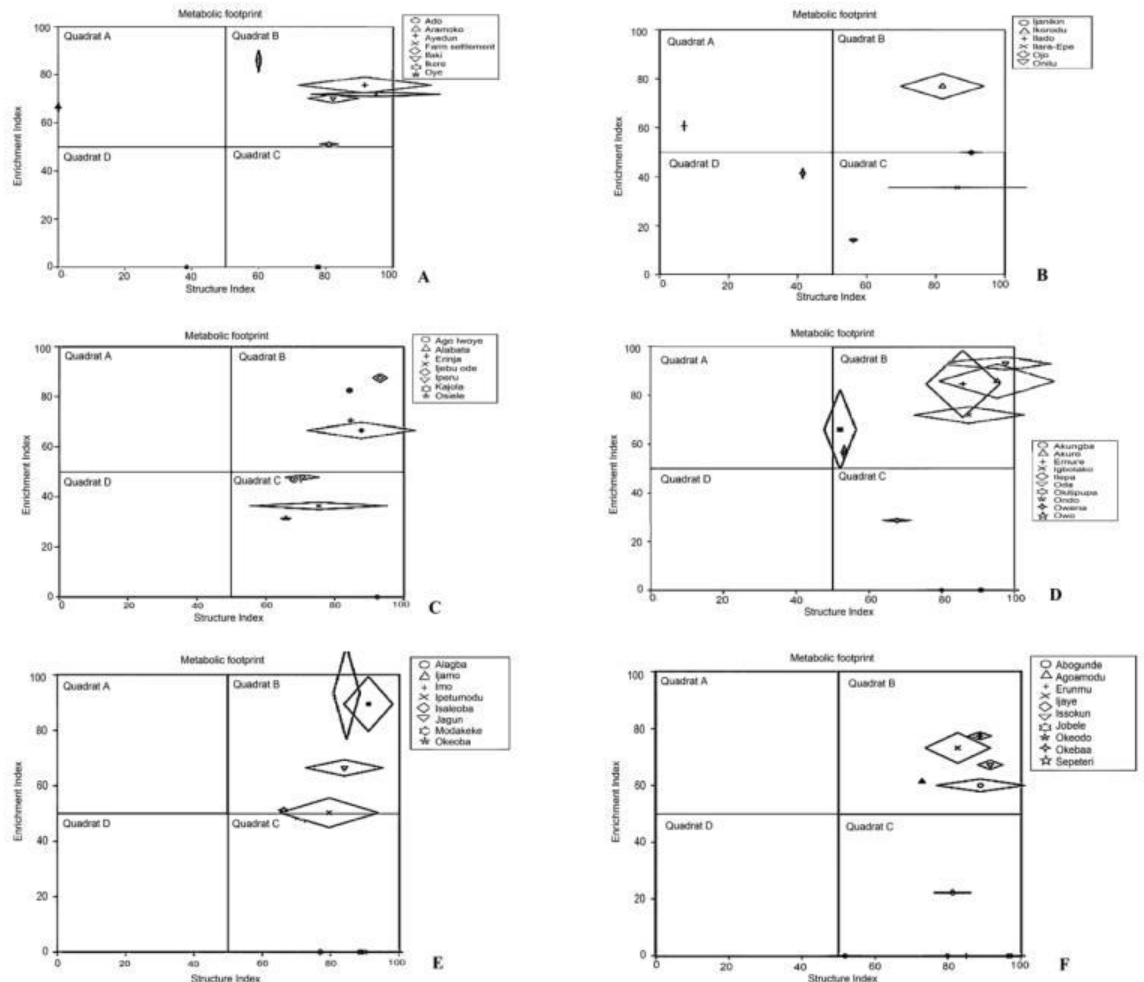


Fig. 4. The metabolic footprints of terrestrial free-living nematode assemblages present in eight watermelon fields in Ekiti state (A), six watermelon fields in Lagos State (B), eight watermelon fields in Ogun State (C), ten watermelon fields in Ondo state (D), eight watermelon fields in Osun state (E) and ten watermelon fields in Oyo State (F) in south-west Nigeria, sampled during 2016/2017, indicating their representative food-web conditions (the point in the middle of a metabolic footprint rhombus represents the intersection of EI and SI values, while the length of the vertical and horizontal axes of a rhombus corresponds to the footprints of enrichment and structure components, respectively) (Sieriebrennikov *et al.*, 2014).

B or C, watermelon farming seems to be fairly sustainable in the sampled fields, but possibly enrichment could be reduced. Also, larger structure footprints as recorded for the majority of the sites sampled during this study are indicative of a higher abundance of omnivores and predators, reflecting food web complexity and regulatory roles. Hence, a good understanding of nematode diversity and

soil health dynamics within agroecosystems is important in making decisions and formulating policies that will facilitate the maintenance of soil health towards optimising sustainable food production. The current study contributes to our knowledge of this and furthermore provides the first known study in West Africa on terrestrial free-living nematode assemblages and their implication in soil health.

The data provided by this study serve as a baseline for further diversity studies on terrestrial free-living nematodes present in West African agroecosystems. Ultimately, useful information in terms of crop-related associations, in this case watermelon, with specific nematode assemblages can be generated to characterise nematode communities that can contribute to our understanding of soil quality.

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CHAPTER 3: ARTICLE 2

Morphological and molecular characterisation of *Aporcelaimellus nigeriensis* sp. n. (Dorylaimida, Aporcelaimidae), a remarkable dorylaim from Nigeria

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Milad RASHIDIFARD^{*1}, Tesleem T. BELLO^{1,2,3}, Hendrika FOURIE¹, Danny L. COYNE² and Reyes PEÑA-SANTIAGO⁴

¹*North-West University, Unit for Environmental Sciences and Management, Integrated Pest Management, Private Bag X6001, Potchefstroom, 2520, South Africa.*

²*International Institute of Tropical Agriculture, IITA, PMB 5320, Ibadan, Nigeria.*

³*Federal College of Education, Abeokuta, PMB 2096, Ogun State, Nigeria.*

⁴*Departamento de Biología Animal, Biología Vegetal y Ecología, Universidad de Jaén, Campus 'Las Lagunillas' s/n, Edificio B3, 23071- Jaén, Spain.*

*Corresponding author, e-mail: Milad.rashidifard@nwu.ac.za

3.1 Abstract

A new species of the genus *Aporcelaimellus*, collected in a watermelon field in Nigeria is described, including its morphological and molecular (D2-D3 28S-rDNA, 18r-DNA) characterisation. *Aporcelaimellus nigeriensis* sp. n. is distinguishable by its 2.76-3.55 mm length, very coarse ventral body pores, lip region offset by deep constriction and 24-27 μm broad, odontostyle 30-36 μm long at its dorsal side, neck 648-779 μm long, pharyngeal expansion occupying 54-60% of total neck length, uterus 300-473 μm or 2.1-3.2 body diameters long and tripartite, $V = 49-54$, tail short and convex conoid (27-41 μm , $c = 72-115$, $c' = 0.5-0.7$), spicules 108-137 μm long, and 9-10 μm spaced ventromedian supplements with hiatus. LSU analysis revealed a close relationship of *A. nigeriensis* sp. n. with other *Aporcelaimellus* species and questioned, once more, the monophyly of Aporcelaimidae. SSU phylogenetic tree was not able to resolve the relationship between the new species and other closely related species.

Keywords – Description, LSU, morphology, morphometry, phylogeny, SSU.

With more than 3300 described species, dorylaims are probably the most diverse nematode Order, despite their distribution being restricted to terrestrial and freshwater habitats. With the exception of species identified from South African, which have been extensively studied for over half a century, the dorylaimid fauna of Africa remains poorly explored. However, plant parasitic and virus vector species of the family Longidoridae have received more attention due to their applied aspects. Nigerian dorylaimid fauna is no exception of this general panorama as no monographic contribution was devoted to characterise it. Nevertheless, two dozen species from 20 genera are currently recorded from Nigeria by means of 15 reports (Table 3.1), proving its tentatively high nematode diversity.

During a nematological survey conducted to characterise the nematode community associated with watermelon fields in Nigeria, interesting specimens belonging to the genus *Aporcelaimellus* Heyns, 1965 were collected. A detailed study revealed that such specimens represent a non-described species that is herein presented.

3.2 **Material and methods**

3.2.1 NEMATODE EXTRACTION AND PROCESSING

Rhizosphere soil samples were collected from watermelon fields during a survey conducted in the south-western agricultural areas of Nigeria during 2016. Nematodes were extracted from soil using a modified pie-pan method (Coyne *et al.*, 2007), fixed in a hot 4% formaldehyde solution (Nico *et al.*, 2002), and subsequently mounted in anhydrous glycerine as permanent slides (De Grisse, 1963). Specimens for molecular analysis were stored in DESS solution.

Nematodes were observed, measured and photographed using a Nikon Eclipse 80i microscope equipped with DIC optics, a drawing tube (*camera lucida*) and a Nikon DS digital camera. Morphometrics include Demanian indices and other usual measurements and ratios. Position of pharyngeal gland nuclei presented according with Loof and Coomans (1970). Spicules were described following Peña-Santiago *et al.* (2014). Microphotographs were edited using Adobe® Photoshop® CS.

3.2.2 MOLECULAR IDENTIFICATION

Fixed specimens in DESS solution were rinsed using double distilled water (ddH₂O), after which one specimen was then transferred into 1.5 ml Eppendorf tube containing 20 µl ddH₂O for molecular characterisation. DNA of the specimen was extracted using the chelex-100 protocol as described by Rashidifard *et al.* (2019). And amplified using a Vacutec thermocycler (www.vacutec.co.za). The amplification reaction was made up by adding 12.5 µl ready to use master mix (Promega Corporation), 1 µl forward primer (10 µM), 1 µl reverse primer (10 µM), 5 µl DNA and 5.5 µl ddH₂O. The following primers were used for amplification of partial large subunits (LSU) (D2-D3) rDNA: D2A (5'-ACAAGTACCGTGAGGGAAAGTTG-3'), D3B (5'-TCGGAAGGAACCAGCTACTA-3') (Subbotin *et al.*, 2006) and partial SSU: SSU F04 (GCTTGTCTCAAAGATTAAGCC), and small subunits (SSU) R26 (CATTCCTTGGCAAATGCTTTCG) (Blaxter *et al.*, 1998). Polymerase chain reaction (PCR) amplification was carried out using the following steps: 3 min initial denaturation at 94 °C, 35 cycles of denaturation for 45 s at 94 °C, annealing temperature (54 °C and 56 °C for SSU and LSU, respectively) for 45 s and finally a 6 min extension cycle at 72 °C followed by a holding temperature of 4 °C.

3.2.3 PHYLOGENETIC ANALYSES

The newly obtained sequences for LSU and SSU were compared using the BLASTN search with those of other species available in GenBank. Taxa selection for reconstruction of the LSU tree, was conducted based on Álvarez-Ortega *et al.* (2013a) and for SSU the currently available SSU rDNA sequences of the genera within the family Aporcelaimidae were obtained from GenBank. The sequences of selected taxa as well as outgroups were aligned using the MUSCLE alignment tool (Edgar, 2004) implemented in Geneious version 7.1 (Kearse *et al.*, 2012). The jModelTest 2.1.10 (Darriba *et al.*, 2012) programme was used to identify the most appropriate nucleotide substitution model. The identified model was General Time Reversible with proportion of invariable sites and a Gamma distribution (GTR+I+G) for LSU and SSU genes. Bayesian inference (BI) was performed using MrBayes 3.2.2 (Huelsenbeck & Ronquist, 2001) implemented in Geneious 7.1, running the chain for 3×10⁶ generations. Markov Chains Monte Carlo (MCMC) algorithm was used to estimate the posterior

probabilities of the Bayesian phylogenetic trees (Larget & Simon, 1999) using the 50% majority rule. The Markov chain was sampled every 100 generations and a 25% burn-in samples was implemented.

3.3 Results

*Aporcelaimellus nigeriensis*¹ sp. n.

(Figs 3.1-3.3)

MATERIAL EXAMINED

Five females and four males from two locations.

MEASUREMENTS

See Table 3.2.

3.3.1 DESCRIPTION

Adult

Moderately slender ($a = 20-28$) nematodes of medium to large size, 2.76-3.55 mm long. Body cylindrical, tapering towards both ends but substantially towards the anterior region as the tail is short. Upon fixation, habitus regularly curved ventrad, C-shaped in females, J-shaped in males. Cuticle three-layered, very thick throughout the entire body, 5.5-7.5 μm in anterior region, 8.5-14.5 μm at mid-body and 11-15 μm on tail, consisting of a very thin outer layer with nearly smooth surface, and two much thicker intermediate and inner layers; both intermediate and inner layers are equally thick at caudal and mid-body regions but the former is distinctly thicker than the latter in the anterior region; the intermediate layer bears very conspicuous radial striation that it is not perceptible in the inner layer. Lateral chord very narrow, 5.5-11.5 μm wide or 4-8% of mid-body diam. Ventral body pores coarse, very conspicuous, 56-64 μm in total: 19-20 μm at neck region, 13-20 μm from neck base to vulva,

¹ The specific epithet refers to the geographical origin of the new species in Nigeria.

and 22-25 μm from vulva to anus ($n = 5$ females); dorsal pores reduced to cervical region, not so coarse as the ventral ones; lateral pores small. Lip region offset by a distinct constriction, 2.7-3.0 times wider than high, and 17-24% of body diam. at neck base; lips mostly separate, with moderately protruding papillae. Amphid fovea stirrup-shaped, its opening 11-12.5 μm or nearly one-half (42-52%) of lip region diam. Odontostyle strong, 3.9-4.6 times longer than wide, hardly longer (1.1-1.3 times) than lip region diam., and 0.87-1.18% of total body length, with aperture 20-22 μm long or two-thirds (64-71%) of its total length. Guiding ring simple but distinct, plicate. Odontophore linear, rod-like, 1.8-2.2 times longer than odontostyle. Pharynx entirely muscular, enlarging very gradually, with its basal expansion 5.4-6.9 times as long as wide, 2.7-3.7 times the body diam. at neck base, and occupying 54-60% of total neck length; pharyngeal gland nuclei located as follows ($n = 3$): DO = 48-52, DN = 51-57, S₁N₁ = 65-70, S₁N₂ = 72-75, S₂N = 84-85. Cardia conical, 20-47 x 14-23 μm , surrounded by intestinal tissue. Tail short, convex conoid, its ventral side visibly straighter than the dorsal side; caudal pores two pairs, subdorsal, at the middle of tail.

Female

Genital system didelphic-amphidelphic, with well-developed genital branches, the anterior 440-678 μm and the posterior 498-726 μm long. Ovaries comparatively large, reaching and surpassing the oviduct-uterus junction, the anterior 162-310 μm long, the posterior 185-335 μm long. Oviduct 112-203 μm or 0.8-1.4 body diameters long, consisting of a slender distal portion made of prismatic cells and a distinct *pars dilatata* with perceptible lumen. Oviduct and uterus separate by a distinct sphincter. Uterus 300-473 μm or 2.1-3.2 body diameters long, and tripartite as it consists of a narrower intermediate section between the proximal and distal parts, these both more dilate. Uterine egg ovoid, 119-135 x 64-72 μm ($n = 4$). Vagina extending inwards 56-60 μm , occupying two-fifths (38-43%) of body diam.: *pars proximalis* 38-44 x 20-25 μm , with somewhat sigmoid walls and surrounded by a relatively weak musculature, *pars refringens* consisting of two drop-shaped sclerotized pieces measuring 9-11 x 6.5-7.5 μm and a combined width of 13.5-16 μm , and *pars distalis* very short, 5-6 μm long. Vulva a transverse slit. Prerectum 1.7-2.4, rectum 1.1-1.4 times the anal body diam. long.

Table 3.1. Dorylaimid species recorded in Nigeria.

Species	Habitat	Reference
<i>Afronygus longicaudatus</i> Heyns, 1968	Bush, maize	Heyns (1968)
<i>Discolaimoides bulbiferus</i> (Cobb, 1906) Heyns, 1963	?	Das <i>et al.</i> (1969)
<i>Dorylaimellus (Ibadanus) brevidens</i> Siddiqi, 1983	Maize	Siddiqi (1983a)
<i>Glochidorella brevicula</i> Siddiqi, 1982	Forest	Siddiqi (1982a)
<i>Helmabia campyla</i> Siddiqi, 1995	Rubber tree	Siddiqi (1995)
<i>Helmabia falcata</i> Siddiqi, 1995	Rainforest	Siddiqi (1995)
<i>Laimydorus keilini</i> (Lee, 1961) Andr�ssy, 1986	Freshwater-pool	Lee (1961)
<i>Leptonchus transvaalensis</i> Heyns, 1963	?	Goseco and Ferris (1979)
<i>Longidorus attenuatus</i> Hooper, 1961	?	Loof and Coomans (1970)
<i>Longidorus laevicapitatus</i> Williams, 1959	?	Loof and Coomans (1970)
<i>Longidorus macrosoma</i> Hooper, 1961	?	Loof and Coomans (1970)
<i>Longidorus pisi</i> Edward, Misra & Singh, 1964 ¹	?	Loof and Coomans (1972)
<i>Moshajia cultristyla</i> Siddiqi, 1982	Rice	Siddiqi (1982)
<i>Neoactinolaimus hintoni</i> (Lee, 1961) Thorne, 1967	Freshwater-pool	Lee (1961)
<i>Opisthodorylaimus cavalcantii</i> (Lordello, 1955) Carbonell & Coomans, 1986		Carbonell and Coomans (1986)
<i>Oriverutus lobatus</i> Siddiqi, 1971	Rice	Siddiqi (1971)
<i>Oxydirus gangeticus</i> Siddiqi, 1966	?	Andr�ssy (2009)
<i>Paraoxydirus cavenessi</i> (Ferris, Goseco & Ferris, 1980) Siddiqi, 1983	Banana	Ferris <i>et al.</i> (1980)
<i>Sicorinema sericatum</i> Siddiqi, 1982	Rubber tree	Siddiqi (1982b)
<i>Solididens bisexualis</i> (Thorne, 1930) Heyns, 1968	?	Heyns (1968)
<i>Thornedia nigerica</i> (Jairajpuri, 1968) Jairajpuri & Ahmad, 1992	<i>Mucuna utilis</i>	Jairajpuri (1968)
<i>Thornenema mauritianum</i> (Williams, 1959) Baqri & Jairajpuri, 1968	?	Carbonell and Coomans (1987)
<i>Tylencholaimellus cylindricus</i> Pe�a-Santiago, Peralta & Siddiqi, 1993	Sugarcane	Pe�a-Santiago <i>et al.</i> (1993)
<i>Zetalaimus blepheronchus</i> Siddiqi, 1983	Rainforest	Siddiqi (1983b)

¹Recorded as *Longidorus siddiqii* Aboul-Eid, 1970.

Table 3.2. Morphometrics of *Aporcelaimellus nigeriensis* sp. n. Measurements in μm except L in mm.

Population	Type			Modakeke	
Character n	Holotype	Paratypes		2♀♀	3♂♂
	♀	2♀♀	♂		
L	2.98	3.34, 3.41	3.17	2.76, 3.11	2.83-3.55
A	22	21, 24	26	20, 21	22-28
B	4.2	4.8, 4.8	4.2	4.0, 4.0	4.1-4.6
C	90	108, 97	77	89, 115	72-98
V	50	51, 50	-	54, 49	-
c'	0.6	0.5, 0.5	0.7	0.5, 0.5	0.5-0.7
Lip region diam.	26	24, 26	27	?, 27	26-27
Odontostyle length-dorsal side	34	33, 31	30	35, 36	35
ventral side	31	29, 28	28	30, 32	29-31
Odontophore length	63	62, 62	63	64, 60	58-64
Neck length	706	700, 708	758	691, 779	648-764
Pharyngeal expansion length	401	423, 399	418	392, 383	347-424
Body diam. at neck base	126	141, 123	113	129, 141	115-126
mid-body	135	158, 141	123	138, 148	127-130
anus/cloaca	59	60, 68	56	58, 54	56-59
Distance vulva – anterior end	1493	1706, 1718	-	1486, 1536	-
Prerectum length	128	143, 123	174	99, 126	143-210
Rectum/cloaca length	65	71, 79	83	78, 75	81-88
Tail length	33	31, 35	41	31, 27	30-41
Spicules length	-	-	137	-	108-132
Ventromedian supplements	-	-	10	-	9-10

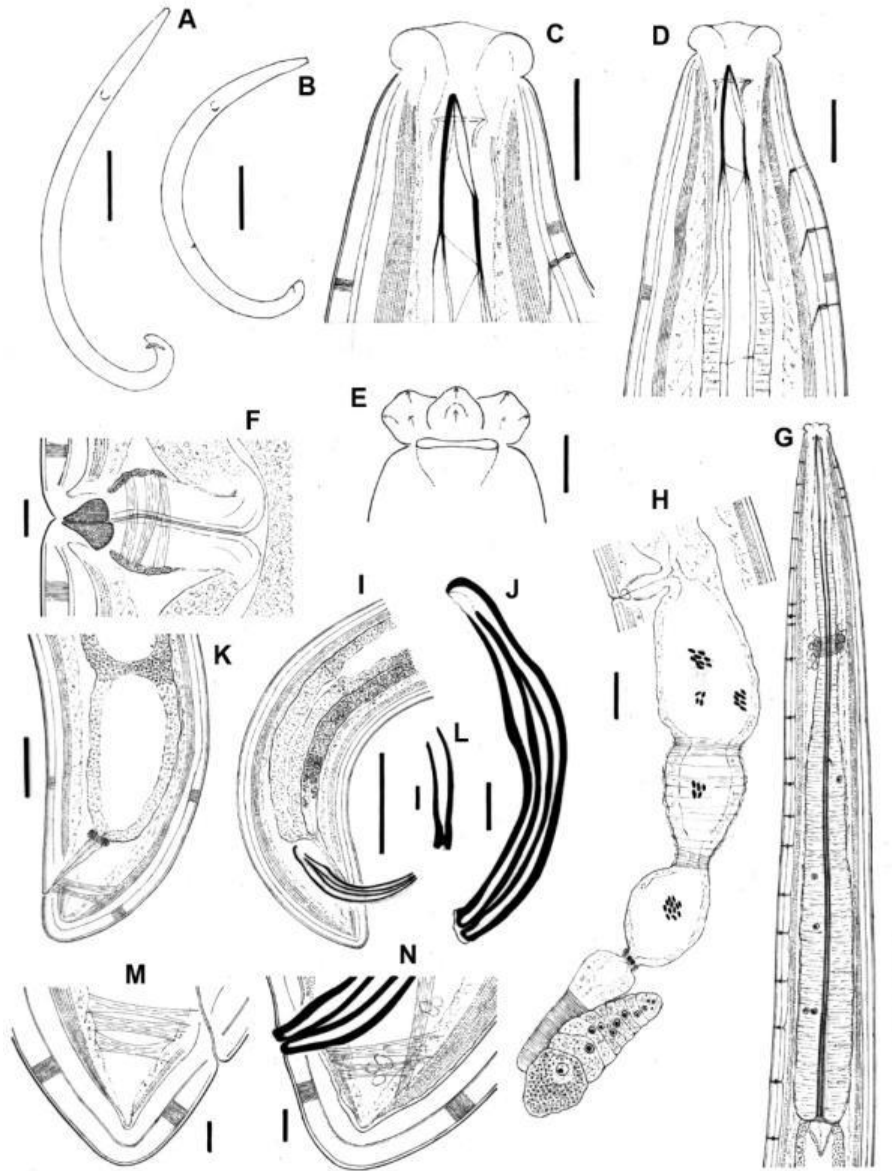


Figure 3.1. *Aporcelaimellus nigeriensis* sp. n. A: Entire body (male). B: Entire body (female). C, D: Anterior region in lateral median view. E: Lip region in lateral surface view. F: Vagina. G: Neck region. H: Female, posterior genital branch. I: Male, posterior body region. J: Spicule. K: Female, posterior body region. L: Lateral guiding piece. M: Female, caudal region. N: Male, caudal region. (Scale bars: A, B = 500 μ m; C, D, J = 20 μ m; E, F, M, N = 10 μ m; G, I = 100 μ m; H, K = 50 μ m; L = 5 μ m.)

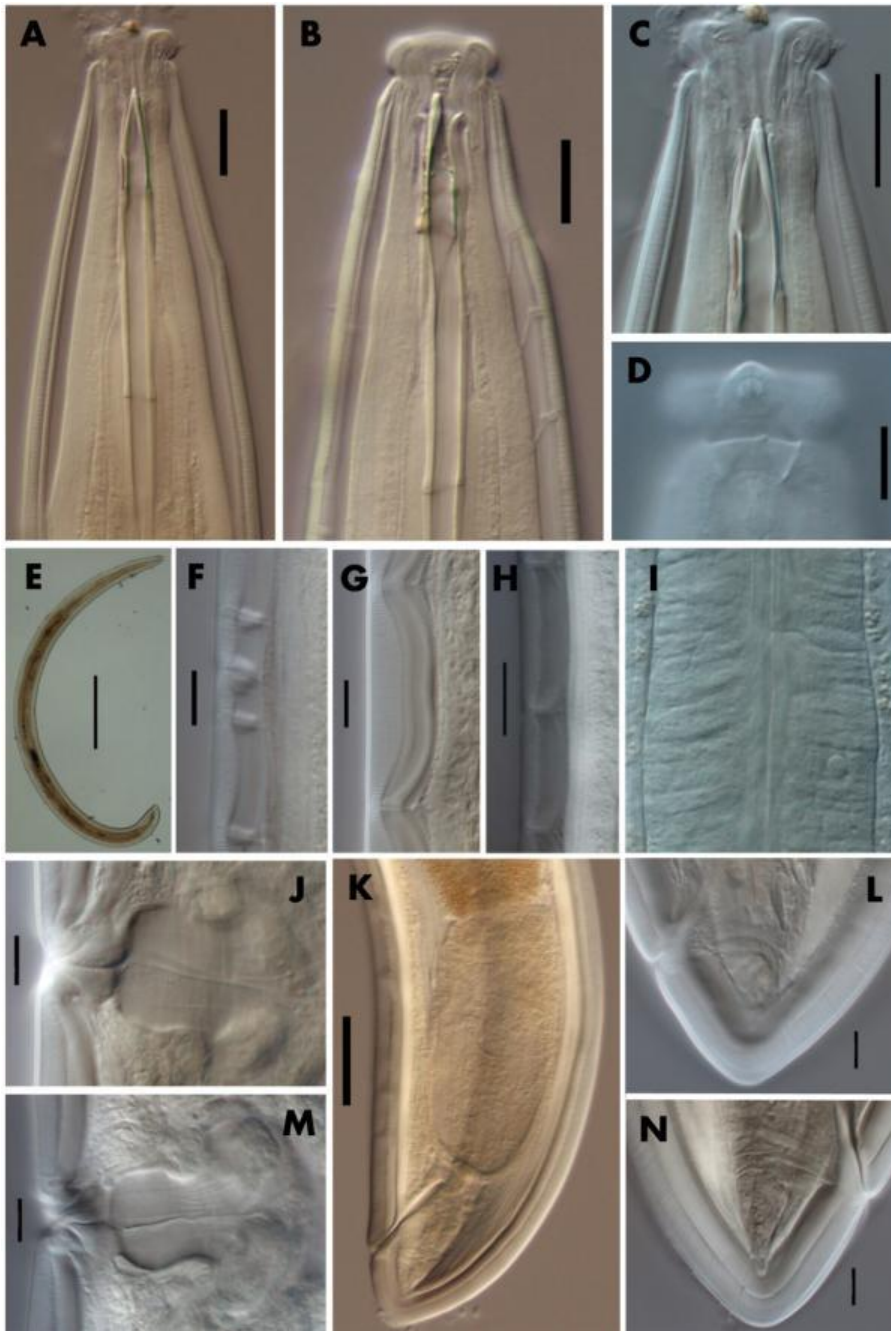


Figure 3.2. *Aporcelaimellus nigeriensis* sp. n. (LM). A-C: Anterior region in lateral median view. D: Lip region in lateral surface view. E: Entire body (female). F-H: Coarse ventral pores at different regions of body. I: Pharyngeal enlargement showing DO and DN. J, M: Vagina. K: Female, posterior body region. L, N: Female, caudal region. (Scale bars: A-C = 20 μ m; D, F-H, J, L-N = 10 μ m; E = 500 μ m, K = 50 μ m.)

Male

Genital system diorchic, with opposed testes. Prerectum 2.4-3.6, cloaca 1.4-1.5 times the body diam. at level of cloacal aperture. In addition to the ad-cloacal pair, situated at 16-19 μm from the cloacal aperture, there is a series of 9-10 widely but irregularly spaced, 23-51 μm apart, ventromedian supplements, the most posterior of which is located at 70-85 μm from the ad-cloacal pair, a short distance behind the level of anterior end of spicules. Spicule dorylaimid, 5.9-7.3 times as long as wide and 1.9-2.4 times longer than body diam. at level of cloacal aperture: head 11-13 x 8-9.5 μm , occupying 9-10% of total spicule length, with its dorsal side strongly curved at its anterior tip, whereas the ventral side is very short and straight; median piece occupying 31-39% of spicule maximum width; posterior tip 7.5-9.5 μm broad; ventral hump and hollow weak but perceptible, the former situated at 31-41% of total spicule length; curvature 110-126°. Lateral guiding piece 25-30 μm long, 7.1-9.3 times longer than wide, with furcate tip.

3.3.2 MOLECULAR CHARACTERISATION

One 769 bp long D2-D3 28S rDNA sequence and one 776 bp long partial 18S rDNA sequence (GenBank access codes MN685820 and MN505320, respectively) were obtained. Their analysis allowed the elucidation of evolutionary relationships of the new species. The results are presented in Figs. 3.4 and 3.5, and discussed below.

3.3.2.1 DIAGNOSIS AND RELATIONSHIPS

The new species is characterised by its 2.76-3.55 mm long body, very coarse ventral body pores, lip region offset by deep constriction and 24-27 μm broad, odontostyle 30-36 μm long at its dorsal side and 28-32 μm at its ventral side, neck 648-779 μm long, pharyngeal expansion 347-424 μm long or 54-60% of total neck length, female genital system didelphic-amphidelphic, uterus 300-473 μm or 2.1-3.2 body diameters long and tripartite, $V = 49-54$, tail short and convex conoid (27-41 μm , $c = 72-115$, $c' = 0.5-0.7$), spicules 108-137 μm long, and 9-10 spaced ventromedian supplements with hiatus.

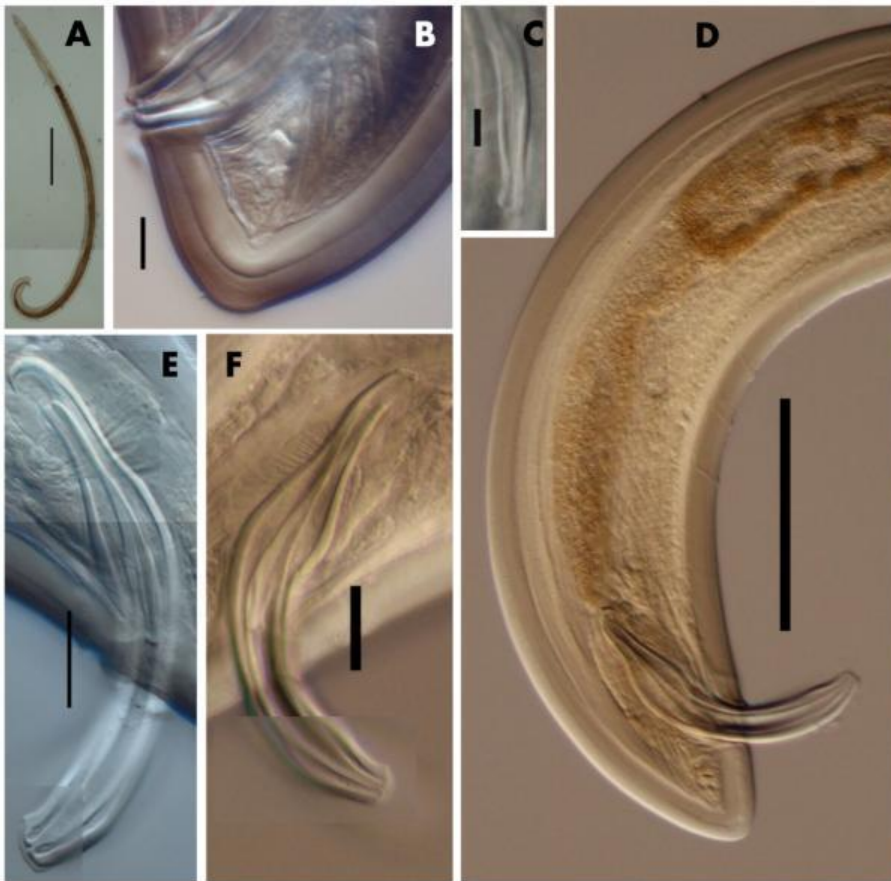


Figure 3.3. *Aporcelaimellus nigeriensis* sp. n. (LM). Male. A: Entire. B: Caudal region. C: Lateral guiding piece. D: Posterior body region. E, F: Spicules. (Scale bars: A = 500 μm ; B = 10 μm ; C = 5 μm , D = 100 μm , E, F = 20 μm .)

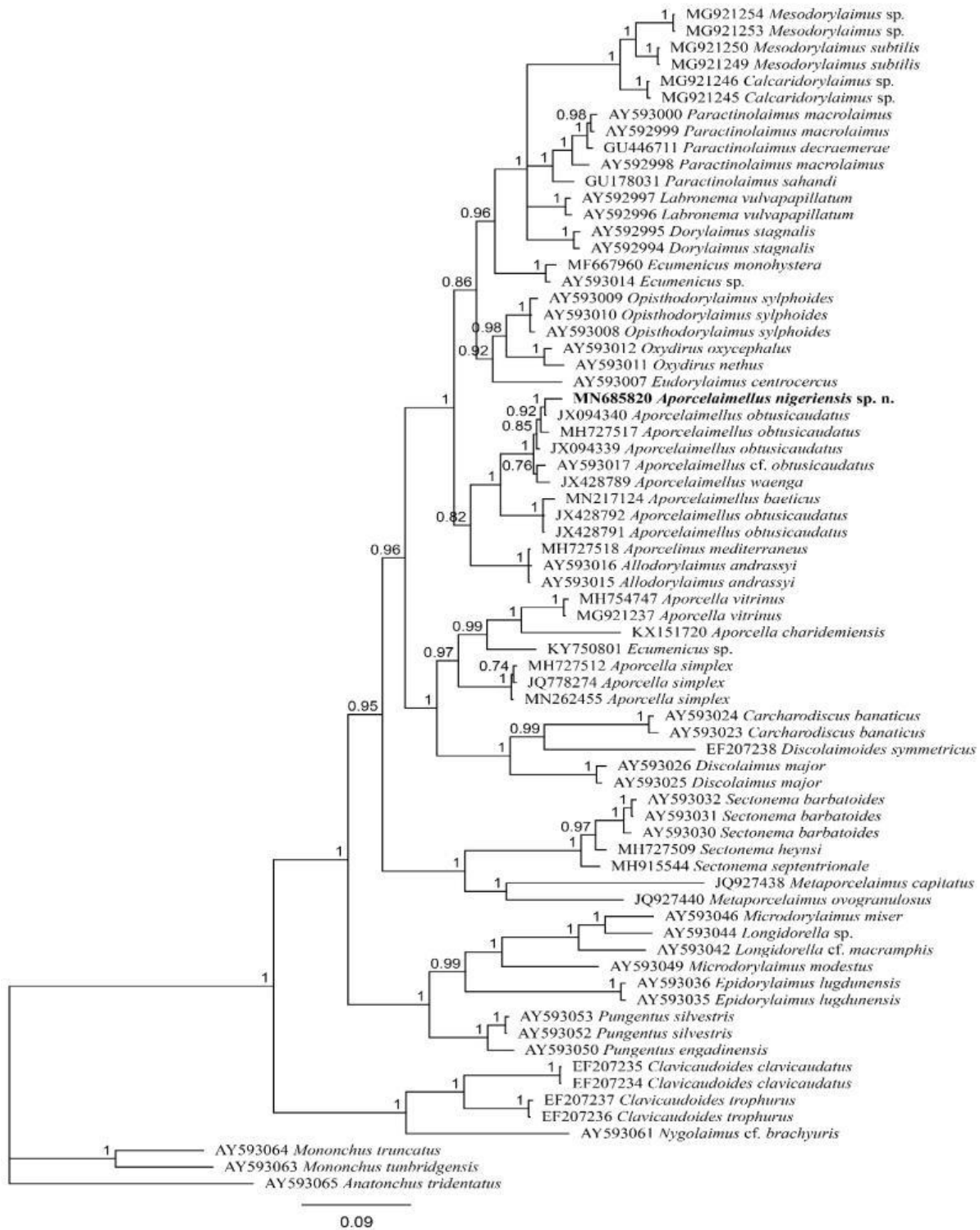


Figure 3.4. Bayesian inference (BI) with 50% majority rule of *Aporcelaimellus nigeriensis* sp. n. using LSU (D2-D3) ribosomal DNA under GTR+I+G model. Posterior probabilities more than 50% are given for the appropriate clade. Original sequence is indicated by bold font.

In its general morphology and particularly in having coarse ventral body pores, the new species resembles *A. porosus* Álvarez-Ortega, Ahmad & Peña-Santiago, 2011, known to occur in West Africa too, but it significantly differs from this in its larger general size (body 2.76-3.55 vs 2.51-2.81 mm long, neck 648-779 vs 589-626 μm long), thicker cuticle (for instance, 11-15 vs 6.5-10.5 μm on caudal region) with different layering (intermediate and inner layers with equal thickness vs inner layer much thicker than the others), wider lip region (24-27 vs 19.5-21.5 μm), longer odontostyle (30-36 vs 26-30 μm), and much longer spicules (108-137 vs 81-87 μm). In having a comparatively strong odontostyle, broad lip region and tripartite uterus, it resembles *A. castaneanus* Álvarez-Ortega, Abolafia, Liébanas & Peña-Santiago, 2012, from Spain but it can be easily distinguished from this in its larger general size (body 2.76-3.55 vs 2.18-2.83 mm long), presence (vs absence) of coarse ventral body pores, much thicker cuticle (for instance, 11-15 vs 5-7 μm on caudal region) with different layering (intermediate and inner layers with equal thickness vs inner layer much thicker than the others), broader lip region (24-27 vs 21-22 μm), much longer odontostyle (30-36 vs 22-24 μm), shorter caudal region (27-41 vs 44-52 μm , $c = 72-115$ vs 48-60, $c' = 0.5-0.7$ vs 0.8-1.1), longer spicules (108-137 vs 94-103 μm), and less ventromedian supplements (9-10 vs 14-15).

Evolutionary relationships of *A. nigeriensis* sp. n. as obtained using Bayesian Inference based on partial LSU and SSU sequences are shown in two trees (Figs. 3.4 & 3.5). The most relevant results of LSU analysis (Fig. 3.4) is the inclusion of the new species sequence in a very highly supported clade with other *Aporcelaimellus* sequences; *A. obtusicaudatus* (Bastian, 1865) Alther, 1968 being its closest relative. Results from the current study agree with those of previous studies (Álvarez-Ortega *et al.*, 2013a,b) concerning the non-monophyly of the family Aporcelaimidae Heyns, 1965 as sequences of the genera *Aporcella* Andrásy, 2002, *Metaporcelaimus* Lordello, 1965 and *Sectonema* Thorne, 1930 form part of respective separate highly supported clades. Unfortunately, SSU analysis (Fig. 3.5) did not provide any satisfactory new insight into the phylogeny of the new species or the aporcelaims since the corresponding branching did not reach good support.

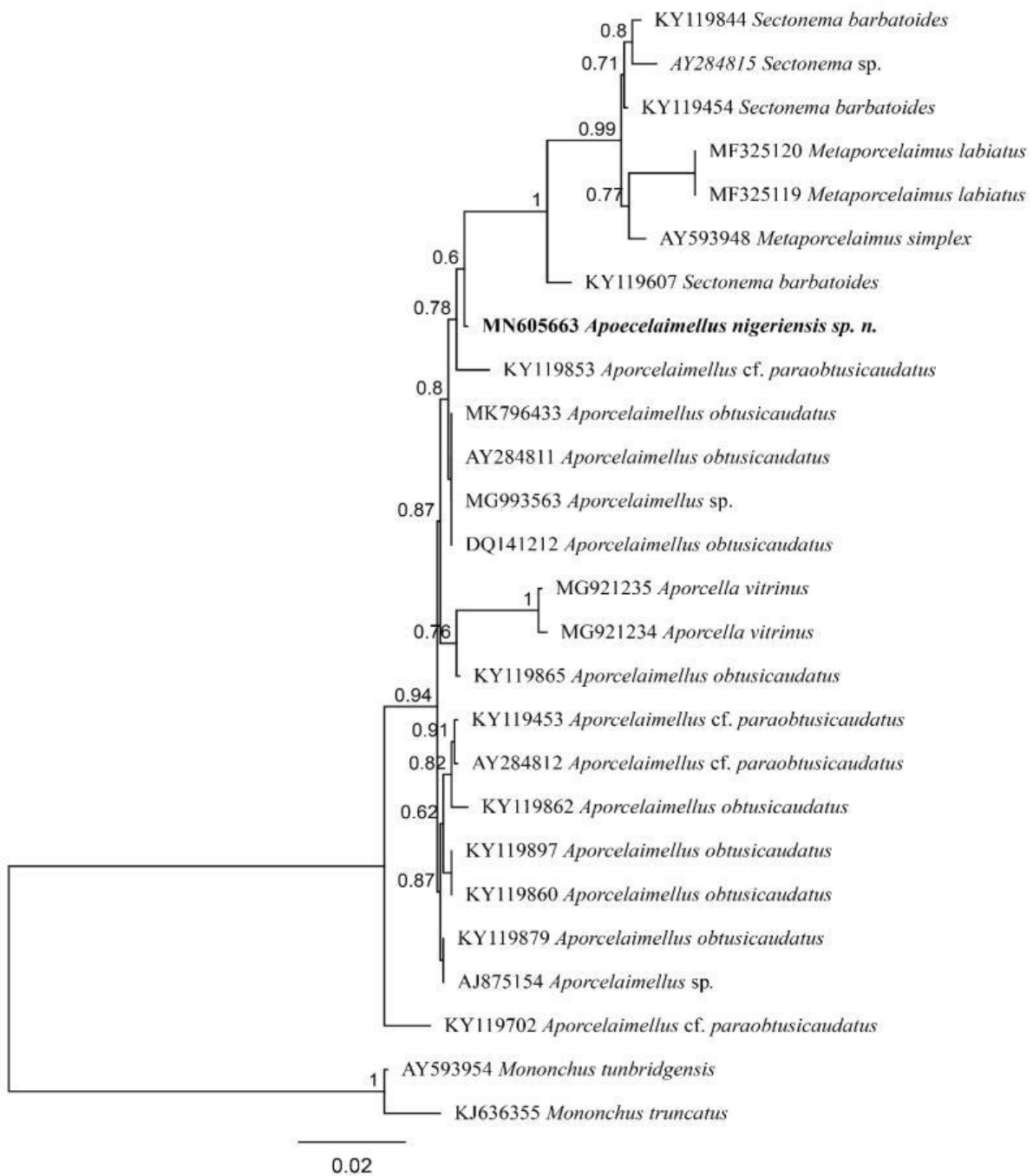


Figure 3.5. Bayesian inference (BI) with 50% majority rule of *Aporcelaimellus nigeriensis* sp. n. using SSU ribosomal DNA under GTR+I+G model. Posterior probabilities more than 50% are given for the appropriate clade. Original sequence is indicated by bold font.

3.3.3 TYPE LOCALITY AND HABITAT

Southwest Nigeria, Ibarapa, a peri-urban locality (coordinates: 7°30'55.87" N 3°27'51.27" E) with a history of maize and vegetable production, where the species was collected from a watermelon field on sandy-loam soil (sand = 68%, silt = 12%, clay = 20%, organic matter = 14.17%, pH = 6.05).

3.3.3.1 OTHER LOCALITY AND HABITAT

Southwest Nigeria, Modakeke, an agrarian locality (coordinates: 7°24'1.99" N 4°15'41.55" E) with a history of leafy vegetable production, where the species was collected from a watermelon field on sandy-loam soil (sand = 76%, silt = 10%, clay = 14%, OM = 4.73%).

3.3.4 TYPE MATERIAL

Female holotype, two female and one male paratypes deposited with nematode collection of the University of Jaén, Spain.

3.3.5 REMARKS

One of the most remarkable traits of the new species herein described is the presence of a row of coarse ventral body pores throughout the entire body, a very atypical and infrequent feature within the genus *Aporcelaimellus* and representatives of the family Aporcelaimidae. Nevertheless, this rare trait also occurs in *A. porosus*, which, interestingly, was also described from Western Africa. Unfortunately, molecular data of this second species are not available, therefore it is not possible to confirm the evolutionary relationship between them but which is proposed to be conducted in the future.

3.4 Acknowledgments

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CHAPTER 4: ARTICLE 3

Abundance and diversity of plant-parasitic nematodes associated with watermelon in Nigeria, with focus on *Meloidogyne* spp.

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Tesleem T. BELLO^{1,2,3*}, Danny L. COYNE², Milad RASHIDIFARD¹ and Hendrika FOURIE¹

¹*North-West University, Unit for Environmental Sciences and Management, Integrated Pest Management, Private Bag X6001, Potchefstroom, 2520, South Africa.*

²*International Institute of Tropical Agriculture, IITA, PMB 5320, Ibadan, Nigeria.*

³*Federal College of Education, Abeokuta, PMB 2096, Ogun State, Nigeria.*

*Corresponding author, e-mail: tesleembello@yahoo.com

4.1 Abstract

Little information is available for nematode pests associated with watermelon in sub-Saharan Africa (SSA). This study recorded the abundance and prevalence of plant-parasitic nematodes from 25 localities across south-west Nigeria during 2016/2017. Of the 11 nematode species identified, those belonging to *Meloidogyne* spp. were predominant according to Prominence Values (PVs), followed by *Helicotylenchus dihystra*, *Pratylenchus zae* and *Scutellonema bradys*. Morphological and molecular analyses revealed the identity of four *Meloidogyne* spp.: *Meloidogyne enterolobii*, *M. incognita*, *M. javanica* and *M. arenaria* (descending order of abundance and occurrence). *Meloidogyne arenaria* is reported for the first time from south-west Nigeria, while *S. bradys* and *X. nigeriense* are first reports for watermelon. Significant associations were observed between the frequency of occurrence of the predominant nematode species and soil properties as well as rainfall. Results provide baseline information on the nematode pest occurrence on watermelon in Nigeria and in a wider context for SSA.

Keywords - molecular techniques, morphology, perineal-pattern, prominence values, *Meloidogyne* spp., soil properties.

Production of watermelon (*Citrullus lanatus* (Thunb) Matsum & Nakai) in Nigeria has traditionally been confined to the drier central and northern savannah regions. Although official records about the extent of watermelon production in Nigeria is scanty, cultivation of the crop has expanded, especially to the south-western parts (Okunlola *et al.*, 2011). Increased production has been stimulated by a general increase in public demand and driven in large by enhanced consumer awareness of the dietary and health benefits of watermelon (Iheke, 2009).

Cucurbit production worldwide is adversely affected by various constraints, including plant-parasitic nematodes, which are viewed as serious pests (Netscher and Sikora, 2005; Abd-Elgawad *et al.*, 2007; Mbawukwu *et al.*, 2016). *Meloidogyne* spp. appear to be the most prevalent, widespread and damaging nematode pests of watermelon, causing considerable crop yield losses worldwide, including in Africa (Abd-Elgawad *et al.*, 2007; Mbawukwu *et al.*, 2016), the Mediterranean (Cohen *et al.*, 2014), Puerto Rico (Montalvo & Esnard, 1994), Mexico (Ramírez-Suárez *et al.*, 2014), and especially the United States of America (USA), where they have been extensively studied (Thomason and McKinney, 1959; Winstead and Riggs, 1959; Sumner and Johnson, 1973; Davis, 2007; Thies, 1996; Thies and Levi, 2003, 2007; Thies *et al.*, 2010, 2015, 2016). With regard to Nigeria, *Meloidogyne* spp. were the most commonly found nematode pests associated with watermelon in eastern Nigeria (Mbawukwu *et al.*, 2016), while this genus has also been recorded to infect the crop in South Africa (Marais, 2019). In addition to *Meloidogyne*, species of *Heterodera* Schmidt, 1871 and *Pratylenchus* Filipjev, 1936 have been previously recovered from watermelon from eastern Nigeria (Mbawukwu *et al.*, 2016). From North Africa, Abd-Elgawad *et al.* (2007) recorded seven genera viz. *Criconemoides* Taylor, 1936, *Heterodera*, *Hirshmaniella* Luc & Googey, 1964, *Hoplolaimus* von Daday, 1905, *Pratylenchus*, *Trichodorus* Cobb, 1913 and *Tylenchorhynchus* Cobb, 1913 from watermelon in Egypt. From South Africa, Marais (2019) listed *Criconema mutabile* Taylor, 1936, *Criconemoides sphaerocephalus* Taylor, 1936, *Helicotylenchus dihystra* (Cobb, 1893) Sher, 1961, *Hoplolaimus pararobustus* (Schuurmans Stekhoven & Teunissen, 1938) Sher, 1936, *Meloidogyne* spp., *Pratylenchus* spp., *Pratylenchus zae* Graham, 1951, *Rotylenchulus parvus* Sher, 1961, *Scutellonema brachyurus* (Steiner, 1936) Andrassy, 1958, *Tylenchorhynchus mashoodi* Siddiqi & Basir, 1959 and *Tylenchorhynchus ventralis* Loof, 1963 infecting watermelon crops. From India, Anwar & McKenry (2012) recovered species of *Belonolaimus* Steiner, 1949, *Helicotylenchus* Steiner, 1945, *Pratylenchus* and *Tylenchorhynchus*, while *Belonolaimus*, *Helicotylenchus*, *Xiphinema* Cobb, 1913, *Rotylenchulus* Steiner, 1945 and *Tylenchorhynchus* have been reported from the crop in the USA (Maynard, 2001; Noling, 2015).

Traditionally, the three tropical root-knot nematode species *Meloidogyne arenaria* Chitwood 1949, *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949, and *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949, have been the most widely reported root-knot nematode species from across SSA, infecting various food and ornamental crops (Caveness, 1967; Whitehead, 1969; IITA, 1981; De Waele & Elsen, 2007). More recently, the newly emerging threat *Meloidogyne enterolobii* Yang & Eisenback, 1983 is being increasingly recorded infecting an ever-broadening range of crops in SSA (Onkendi & Moleleki, 2013; Pagan *et al.*, 2015; Visagie *et al.*, 2018; Coyne *et al.* 2018; Pretorius, 2018; Rashidifard *et al.*, 2019). In Nigeria *M. enterolobii* was recently reported as the most frequently occurring root-knot nematode species on tomato (*Lycopersicon esculentum* Mill.) (dos Santos *et al.*, 2019). This species has been recorded infecting watermelon crops from several countries, such as Brazil, Mexico, Puerto Rico, the USA and the West Indies (Rammah & Hirschmann, 1988; Brito *et al.*, 2007; Gomes *et al.*, 2008; Bitencourt & Silva, 2010; Silva *et al.*, 2010; Quénéhervé *et al.*, 2011; Crozzoli *et al.*, 2011, 2012; da Silva & Krasuski, 2012; Onkendi & Moleleki, 2013; Ye *et al.*, 2013).

Although the four *Meloidogyne* spp. referred to above are found within Nigerian agroecological systems, *M. arenaria* has not, to our knowledge, been reported from south-western Nigerian crop production systems (Caveness, 1967; Nwauzor & Fawole, 1982; Daramola *et al.*, 2013; Mbawukwu *et al.*, 2016; dos Santos *et al.*, 2019).

Nematode diagnostics within SSA and other areas, where resources for morphological or molecular characterisation are limited or inaccessible, has traditionally led to the identification of root-knot and other nematodes to genus level only (Powers *et al.*, 2011; Pagan *et al.*, 2015). However, improvements in species level identification of nematodes using molecular techniques within SSA has been experienced, particularly for *Meloidogyne* spp., during recent years (Onkendi & Moleleki, 2013; Pagan *et al.*, 2015; Visagie *et al.*, 2018; Coyne *et al.*, 2018; Rashidifard *et al.*, 2019; dos Santos *et al.*, 2019). Previously, root-knot nematode species identification mainly relied on morphological taxonomy (Sasser *et al.*, 1983), isozyme polymorphisms (Esbenshade & Triantaphyllou, 1990), as well as host range tests (Hartman & Sasser, 1985). Morphological identification for certain species can be unreliable since certain diagnostic characters overlap across species (Hewlett & Tarjan 1983; Adam *et al.*, 2007; Moens *et al.*, 2009; Karssen *et al.*, 2013). The differential host test is time consuming and have become outdated, due to the required plant specificity (only a few cultivars of selected host plants known to be good and/or non-hosts), which is furthermore suggested to be epigenetic (subject to non-genetic influences) in nature (Robertson *et al.*, 2009; Perfus-Barbeoch *et al.*, 2014). In addition, economically important thermophilic (tropical) species of *Meloidogyne* that reproduce by mitotic

obligatory parthenogenesis most probably have a reticulate (hybrid) origin (Brito *et al.*, 2004; Janssen *et al.*, 2016; Rashidifard *et al.*, 2019). This implies that overlapping morphological characteristics may occur to an increased extent and hence intensifying the challenge in terms of identification. The wide host range, intraspecific variation, interspecific similarities, and indistinct species boundaries or species complexes hence complicate their identification when using traditional techniques (Pagan *et al.*, 2015). Isozyme phenotyping on the other hand is very specific and accurate but requires young females, which can be cumbersome and difficult. Complimenting these traditional approaches, DNA-based identification methods became popular and an attractive alternative during the past few decades since they are rapid, more reliable and do not require a specific nematode life stage (Powers, 2004; Powers *et al.*, 2005; Jeyaprakash *et al.*, 2006; Qiu *et al.*, 2006; Adam *et al.*, 2007). Such state-of-the-art methods include, among others, the Sequence Characterised Amplified Regions - Polymerase Chain Reaction, (SCAR-PCR), real time PCR, Random Amplified Polymorphic DNA (RAPD), microarrays and DNA sequencing of specific genes based on rDNA, mtDNA, IGS or ITS, satellite probe markers (Berry *et al.*, 2008; Blok & Powers, 2009; Kiewnick *et al.*, 2013; Subbotin *et al.*, 2013), and also genotyping by sequencing (GBS) (Rashidifard *et al.*, 2018). The SCAR-PCR technique is a widely used, cost-efficient technique that is reproducible (Zijlstra *et al.*, 2000; Tigano *et al.*, 2010; Nischwitz *et al.*, 2013; Onkendi & Moleleki, 2013; Visagie *et al.*, 2018). Specific primers have been developed for several *Meloidogyne* spp. to amplify a genomic fragment of target species (Zijlstra *et al.*, 2000; Blok and Powers, 2009; Subbotin *et al.*, 2013). An advantage of these methods is that just a single, or a few individuals, of any life stage are sufficient to obtain positive identifications (Adam *et al.*, 2007). The importance of accurate identification of root-knot nematode species cannot be over-emphasised since effective management strategies (e.g. crop rotation, host plant resistance, plant quarantine requirements) depend upon it (Adam *et al.*, 2007).

Since no research was conducted on assessing the abundance and identity of the economically important plant-parasitic nematodes associated with watermelon in Nigeria, this study focused on this facet using morphological and molecular approaches with the focus being on *Meloidogyne*.

4.2 **Materials and Methods**

4.2.1 NEMATODE SAMPLING, EXTRACTION, COUNTING AND IDENTIFICATION

Soil and root samples were collected from fruit-bearing watermelon plants across 25 zones of the six states (Ekiti, Lagos, Ogun, Ondo, Osun & Oyo) of south-west Nigeria (Figure 4.1). Soil and root samples were taken from 18 plants per field along a 'W-shaped' pattern (Coyne *et al.*, 2007). Composite samples, comprising three of the 18 samples collected per field were combined into a single bag to provide six composite samples per field. The samples were labelled appropriately and transported in insulated cool bags to the Nematology Laboratory of International Institute of Tropical Agriculture (IITA) (Ibadan, Nigeria) within 24 h, where they were stored in a refrigerator at 5-7 °C for a maximum of 5 days before extraction of nematodes commenced. During preparation of the samples, the roots and soil of each composite sample were separated. The roots from each composite sample were washed under a gentle stream of running tap water, blotted on paper, cut into 0.5 cm pieces and mixed well. A 5 g subsample of roots was then taken from each composite sample (six per field) for nematode extraction using the adapted sugar centrifugal flotation method (Hooper *et al.*, 2005). An additional 20 g root subsample was further obtained from the remaining chopped roots and used to extract root-knot nematode eggs and second-stage juveniles (J2) using the adapted NaOCl method (Riekert, 1995).

The soil from each composite sample was thoroughly mixed and a 200 ml subsample removed from each of the six composite samples and nematodes extracted using the pie-pan method (Coyne *et al.*, 2007). The nematodes extracted from each composite sample were collected into a glass flask, then counted and identified to genus level using a Doncaster counting dish (Doncaster, 1962). Nematode individuals extracted from each composite sample were fixed in a 4% formaldehyde solution (Nico *et al.*, 2002), mounted on glass microscope slides in anhydrous glycerine (De Grisse, 1963) and identified to species level with the aid of interactive diagnostic keys of Siddiqi (2000), and the CABI Crop Protection Compendium (CABI, 2007).

The percentages of sand, silt and clay of soil samples were determined in the Soil Microbiology Laboratory (IITA) using standard protocols (Bouyoucos, 1962), while organic matter content (OM) was obtained using the Degtjareff method (Walkley & Black, 1934). Soil pH (H₂O) from each field was also determined using a pH meter. Rainfall and temperature data were obtained from the Nigerian Meteorological Agency (NIMET) (<https://nimet.gov.ng>).

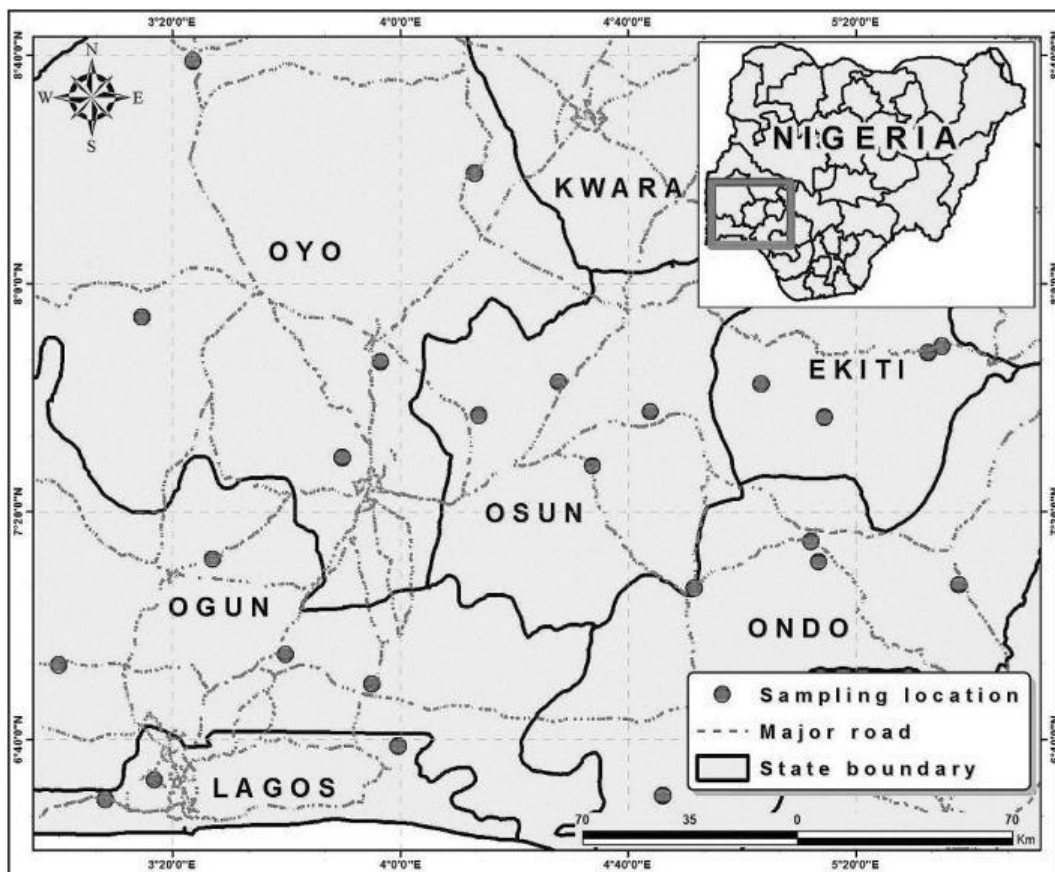


Figure 4.1: A map of Nigeria showing the communities (grey circles) where nematode samples were obtained from watermelon fields during 2016 and 2017 (Photo: Omodele Taiwo, GIS expert, Institute of Agricultural Research and Training; IAR&T, Nigeria).

4.2.2 REARING OF *MELOIDOGYNE* SPP. FOR MOLECULAR AND MORPHOLOGICAL IDENTIFICATIONS

Meloidogyne spp. eggs and J2 extracted from galled watermelon roots from each of the 25 localities (Table 4.1) were inoculated onto roots of two-leaf stage seedlings of susceptible tomato (cv. Tropimech) (Bello *et al.*, 2015) and reared *in vivo* in separate 5-l capacity pots (filled with sterilised sandy-loam soil; 7.2% clay, 91.5% sand, 1.3% silt and 0.58% OM; pH (H₂O) of 7.22) in a glasshouse at IITA. Infected tomato seedlings were manually irrigated with tap water daily. Fifty-six days after inoculation, individual tomato plants were uprooted and 20 females from each of the 25 *Meloidogyne* spp. populations (containing single species) and or communities (containing mixed species) removed using a scalpel, which was dipped in 90% ethanol after each female was isolated.

Table 4.1: Codes assigned to the *Meloidogyne* spp. populations (single species) and/or communities (mixed species) obtained from watermelon roots sampled from 25 localities across south-west Nigeria and which were reared *in vivo* in sterilised soil in pots in a glasshouse for identification purposes.

Codes	Communities	Codes	Communities
L1	Aramoko	L14	Akure
L2	Ado-Ekiti	L15	Owo
L3	Ikole	L16	Ondo
L4	Ikere	L17	Osogbo
L5	Lagos east	L18	Ife
L6	Lagos west	L19	Ilesha
L7	Lagos far east	L20	Iwo
L8	Abeokuta	L21	Saki
L9	Ilaro	L22	Ogbomoso
L10	Ijebu	L23	Oyo
L11	Ikenne	L24	Ibadan
L12	Okitipupa	L25	Ibarapa
L13	Akoko		

The 20 females isolated per locality were each transferred to a single 1.5 ml Eppendorf tube containing 70% ethanol and stored at 4-7 °C until use. Before DNA extraction commenced, using

the modified Chelex method (Rashidifard *et al.*, 2019), the females were placed in distilled water at 25 °C for 24 h to rinse off the ethanol. The quality and quantity of the female DNA extracted for each community were then verified using a Nano Drop 2000 spectrophotometer.

4.2.3 POLYMERASE CHAIN REACTION (PCR) AND SEQUENCE CHARACTERISED AMPLIFIED REGION (SCAR-PCR)

Amplification of the DNA of each of the 25 *Meloidogyne* spp. populations and/or communities was conducted by PCR using the SCAR species-specific primers for the thermophilic species *M. incognita*, *M. arenaria*, *M. javanica* and *M. enterolobii* (Table 4.2). A solution containing 12.5 µl master mix, 8 µl of nuclease free water and 5 picomol each of both forward and reverse primers for each of the four *Meloidogyne* spp. were prepared separately for the 25 communities. Then 1.5 µl DNA template of each of the 25 communities were added to individual Eppendorf tubes and centrifuged.

Table 4.2: Primer codes used for the identification of *Meloidogyne* spp. with their sequences, specificity and reference sources.

Codes for primers	Primer sequence 5'-3'	Specificity and reference source
Far	TCGGCGATAGAGGTAAATGAC	<i>Meloidogyne arenaria</i> -specific
Rar	TCGGCGATAGACACTACAACT	SCAR; Zijlstra <i>et al.</i> (2000)
Me-F	AAC TTTTGTGAAAGTGCCGCTG	<i>Meloidogyne enterolobii</i> -specific
Me-R	TCAGTTCAGGCAGGATCAACC	sequence; Long <i>et al.</i> (2006)
Finc	CTCTGCCCAATGAGCTGTCC	<i>Meloidogyne incognita</i> -specific
Rinc	CTCTGCCCTCACATTAGG	SCAR; Zijlstra <i>et al.</i> (2000)
Fjav	GGTGCGCGATTGAACTGAGC	<i>Meloidogyne javanica</i> -specific
Rjav	CAGGCCCTTCAGTGGA ACTATAC	SCAR; Zijlstra <i>et al.</i> (2000)

The 25 tubes containing the DNA solutions for each *Meloidogyne* spp. population and/or community were placed in a thermocycler (BIORAD) AC1000™ and the programme run according to the requirements for each of the species-specific primers (Tables 4.3 & 4.4). The

PCR products obtained for each of the 25 *Meloidogyne* spp. populations and/or communities were analysed by electrophoresis in a 1% (m/v) agarose gel suspended in an electrophoresis tank filled with 1 x TAE buffer. Amplified products were loaded into the slots of the submerged gel using disposable micropipettes, one for each community. The DNA of each of four (*M. arenaria*, *M. enterobii*, *M. incognita* and *M. javanica*) South African *Meloidogyne* spp. populations identified by Visagie *et al.* (2018) was used as references (Table 4.4) and were also loaded in separate slots into each gel. Electrophoresis was conducted at 120 V for 30 min. The respective gels were then removed and placed under ultraviolet (UV) illumination for observation of the DNA banding patterns of the *Meloidogyne* spp. (Figure 4.4).

Table 4.3: Polymerase chain reaction (PCR) amplification profiles used during this study with different primers for identification of *Meloidogyne* spp. (Zijlstra *et al.*, 2000; Long *et al.*, 2006).

Primers	Denaturation	Cycles	Denaturation	Annealing	Extension	Final extension
Far/Rar	94°C 2 min	45	94°C 30 sec	61°C 30 sec	72°C 1 min	72°C 5 min
MeF/MeR	94°C 2 min	35	94°C 30 sec	64°C 30 sec	72°C 1 min	72°C 5 min
Finc/Rinc	94°C 2 min	45	94°C 30 sec	54°C 30 sec	72°C 1 min	72°C 5 min
Fjav/Rjav	94°C 2 min	45	94°C 30 sec	64°C 30 sec	72°C 1 min	72°C 5 min

Table 4.4: Reference populations used for the molecular identification of *Meloidogyne* spp. obtained from watermelon roots from 25 localities situated in south-western Nigeria.

Species	Life stage	Host crop	Origin (Country; Province; Population Code)	DNA fragment size (bp)
<i>M. arenaria</i>	Second stage juveniles	Maize (<i>Zea mays</i> L.)	South Africa; Mpumalanga; ¹ Population M36	420
<i>M. enterolobii</i>	Second stage juveniles	Guava (<i>Psidium guajava</i> L.)	South Africa; Mpumalanga; ¹ Population M48	250
<i>M. incognita</i>	Mature females	Maize	South Africa; Northern Cape; ¹ Population M5	1 200
<i>M. javanica</i>	Mature females	Pumpkin (<i>Curcubita</i> <i>pepo</i> L.)	South Africa; Northern Cape; ¹ Population M76	700

¹Populations identified by Visagie *et al.* (2018).

4.2.4 PERINEAL PATTERN MORPHOLOGY AND MORPHOMETRICS

After maintaining cultures of the 25 *Meloidogyne* spp. populations and/or communities in roots of the tomato cv. Tropimech for 56 days, infected roots containing adult females were transferred to 100 ml capacity glass beakers and rinsed in tap water to remove soil and other debris. The infected roots were stained in an acid-fuchsin lactoglycerol solution (Hunt & Handoo, 2009) and 21 red-stained females from each population and/or community carefully removed from galls using a scalpel and dissection microscope. The anterior and posterior parts of each female was excised (with a scalpel) and mounted in a drop of glycerol on a glass slide to study and measure the pharyngeal morphology and perineal patterns, respectively (Marais *et al.*, 2017). A NIS Elements software programme (Version 3.07), furnished with a Nikon light microscope (Eclipse 50i), was used to measure vulval-slit lengths (VSL), anus-phasmid distances (APD), intra phasmid distances (IPD) and anus-vulva distances (AVD) of mounted specimens (Marais *et al.*, 2017). Visible morphological characteristics related to the pharyngeal morphology and perineal patterns were also recorded. Photographs were taken of the perineal patterns of females using a dedicated DS-Fi1 camera.

4.3 Data analyses

Prominence values (PV) were calculated for each nematode genus and/or species (identified from both soil and root samples) across the 25 localities sampled and also for pooled data of the six states (Ekiti, Lagos, Ogun, Ondo, Osun & Oyo) according to the following equation: $PV = \text{Population density} \times \sqrt{\text{frequency of occurrence}} \div 10$ (Bolton & De Waele, 1989). Nematode data were $\log(x+1)$ transformed before multivariate analysis was done in order to minimise variation and conform data to normal distribution (Zuur *et al.*, 2010). The association between plant-parasitic nematode species and selected soil physical, as well as environmental parameters were assessed using canonical correspondence analysis (CCA) bi-plots (Vegan in R package) (Oksanen *et al.*, 2007), and Pearson's correlation analysis (Statistica software version 12; www.statistica.com).

4.4 Results

4.4.1 SURVEY

Eleven plant-parasitic nematode species and 11 genera were identified from both soil and root samples (Tables 4.5 - 4.7A & B), viz. *Belonolaimus longicaudatus* Rau, 1958, *Criconema* sp., *H. dihystra*, *Helicotylenchus multicinctus* (Cobb, 1893) Golden, 1956, *Longidorus* sp. *Meloidogyne* spp. (*M. arenaria*, *M. enterolobii*, *M. incognita*, *M. javanica*), *Mesocriconema* sp. Andrassy, 1965, *Paratrichodorus* sp., *Pratylenchus coffeae* Goodey, 1951, *P. zae*, *Rotylenchulus* sp., *Scutellonema bradys* (Steiner & Le Hew, 1933) Andrassy, 1958 and *Xiphinema nigeriense* Luc, 1961.

Data pooled across all communities showed that the genus *Meloidogyne* had the highest PV and were the dominant genus in both roots (PVs of 2 486 individuals/5 g, and 14 330 individuals/20 g root) and soil (PV = 362 individuals/200 ml soil) (Tables 4.5A & B). *Helicotylenchus* followed by *Pratylenchus* were the second and third most prominent genera according to PVs in both soil (represented by the species *H. dihystra* and *P. zae*) and root samples (represented by the species *H. multicinctus* and *P. zae*) (Tables 4.5A & B). The least prominent species, in terms of PVs, in soil were *Longidorus* spp. (Table 4.5A) and in roots *S. bradys* (Table 4.5B).

Table 4.5A: Prominence values (PV), frequencies of occurrence (FO) and mean population densities (MPD) of plant-parasitic nematode species recovered from soil from watermelon fields in 25 localities in south-west Nigeria during 2016/2017.

Nematode species	200 ml soil		
	PV ¹	MPD ²	FO ³
<i>Meloidogyne</i> spp. (<i>M. arenaria</i> , <i>M. enterolobii</i> , <i>M. incognita</i> , <i>M. javanica</i>)	362	409	87
<i>Helicotylenchus dihystra</i>	229	405	32
<i>Pratylenchus zae</i>	103	243	18
<i>Scutellonema bradys</i>	77	221	12
<i>Helicotylenchus multicinctus</i>	73	175	15
<i>Criconea</i> sp.	64	185	12
<i>Rotylenchulus</i> sp.	53	154	12
<i>Mesocriconea</i> sp.	53	142	14
<i>Paratrichodorus</i> sp.	45	106	18
<i>Pratylenchus coffeae</i>	20	64	10
<i>Longidorus</i> sp.	10	35	9
<i>Belonolaimus longicaudatus</i>	8	27	8
<i>Xiphinema nigeriense</i>	7	22	8

¹Prominence value = Mean Population density x $\sqrt{\text{frequency of occurrence}} \div 10$; ²Mean population density = total number of nematodes $\div$ number of samples obtained; ³Frequency of occurrence = number of samples containing a nematode species $\div$ number of samples collected x 100.

Pooled soil data for the fields sampled per state show that the diversity of plant-parasitic nematode species in soil samples varied among the states (Table 4.6). The genera with the highest abundance (in terms of MPDs and PVs) as well as occurrence in fields of all six states were *Meloidogyne* spp. and *H. dihystra*. The PVs for *Meloidogyne* spp. ranged from 269 (Osun) to 617 (Ogun), and this genus was present in up to 92% of the fields. *Helicotylenchus dihystra* individuals were present in up to 67% of the fields with PVs ranging between 46 (Osun) and 436 (Ekiti). *Pratylenchus zae* was generally third in dominance with PVs ranging between 55 (Ondo) and 303 (Ogun) and occurred in up to 18% of the fields (Table 4.6). According to PVs, the species with the least abundance and occurrence were *B. longicaudatus* with PVs that ranged between 6 (Osun) to 18 (Oyo); *X. nigeriense* followed with PVs ranging from 7 (Ogun) to 20 (Ondo) and being present in up to 8% of fields.

Table 4.5B: Plant-parasitic nematodes species identified from roots of watermelon plants sampled from 25 localities across south-west Nigeria during 2016/2017.

Nematode species	5 g roots (sugar-flotation extraction)			20 g roots (NaOCl extraction)		
	PV ¹	MPD ²	FO ³	PV ¹	MPD ²	FO ³
<i>Meloidogyne</i> spp.	2 486	2 620	90	14	14 859	93
(<i>M. arenaria</i>, <i>M. enterolobii</i>, <i>M. incognita</i>, <i>M. javanica</i>)				330		
<i>Helicotylenchus multicinctus</i>	89	187	44	106	223	54
<i>Pratylenchus zae</i>	9	36	10	50	192	20
<i>Scutellonema bradys</i>	4	11	14	14	38	15

¹Prominence value = Mean Population density $\times \sqrt{\text{frequency of occurrence}} \div 10$; ²Mean population density = total number of nematodes $\div$ number of samples obtained; ³Frequency of occurrence = number of samples containing a nematode species $\div$ number of samples collected $\times 100$

In roots, the PVs for *Meloidogyne* spp. ranged between 1 400 (Lagos) and 4 112 (Ekiti) with individuals being detected in fields of all six states in up to 95% of the samples (Table 4.7). For *H. multicinctus*, PVs ranged between 3 (Ogun) and 40 (Lagos) with individuals occurring in fields of all six states in up to 40% of the root samples. For *P. zae*, PVs ranged between 9 (Ondo) and 34 (Ogun) with individuals being present in up to 25% of the root samples. *Scutellonema bradys* had low PVs (ranging between 5 for Ondo and 9 for Lagos), with individuals occurring in up to 25% of the root samples. In 20 g roots, only *Meloidogyne* was found with the highest mean population density (MPD) of 26 350 for Ekiti and the lowest of 10 116 for Oyo (Table 4.7).

Pearson's correlation analysis revealed significant correlations between the frequency of occurrence of *Meloidogyne* spp., the other three predominant plant-parasitic nematode species (*H. dihystra*, *P. zae* and *S. bradys*) and selected soil properties, as well as rainfall. Significant and positive correlations were apparent for the frequency of occurrence of *Meloidogyne* spp. and pH as well as % silt and significant negative correlations for % sand, % OM and rainfall (Table 4.8).

Table 4.6: Prominence values (PV), mean population densities (MPD) and frequencies of occurrence (FO; %) of plant-parasitic nematode species identified from 200ml of rhizosphere soil sampled from watermelon plants in 25 localities across six states of south-west Nigeria during 2016/2017.

Genus	Ekiti			Lagos			Ogun			Ondo			Osun			Oyo		
	PV ¹	MPD ²	FO ³ (%)	PV	MPD	FO (%)	PV	MPD	FO (%)	PV	MPD	FO (%)	PV	MPD	FO (%)	PV	MPD	FO (%)
<i>Belonolaimus longicaudatus</i>	-	-	-	18	43	17	10	29	13	-	-	-	6	18	13	18	56	15
<i>Criconema</i> sp.	-	-	-	165	285	33	-	-	-	80	180	23	-	-	-	41	81	20
<i>Helicotylenchus dihystra</i>	436	712	38	169	207	67	115	230	25	123	276	20	46	92	25	371	586	40
<i>Helicotylenchus multicinctus</i>	89	156	20	43	116	18	-	-	-	37	89	23	-	-	-	62	107	24
<i>Longidorus</i> sp.	-	-	-	-	-	-	8	17	25	-	-	-	-	-	-	24	46	10
<i>Meloidogyne</i> sp.	315	267	72	336	301	80	617	545	78	377	339	81	269	243	82	454	403	79
<i>Mesocriconema</i> sp.	61	122	25	-	-	-	-	-	-	119	188	40	-	-	-	-	-	-
<i>Pratylenchus zae</i>	96	191	25	-	-	-	303	605	25	55	100	32	-	-	-	81	182	20
<i>Pratylenchus coffeae</i>	-	-	-	-	-	-	48	101	20	30	73	17	-	-	-	26	86	12
<i>Rotylenchus</i> sp.	46	93	25	66	114	33	-	-	-	-	-	-	128	257	25	-	-	-
<i>Paratrichodorus</i> sp.	-	-	-	61	107	33	163	461	13	35	78	12	37	74	25	-	-	-
<i>Scutellonema bradys</i>	-	-	-	98	170	33	-	-	-	93	209	20	-	-	-	127	264	20
<i>Xiphinema nigeriense</i>	-	-	-	-	-	-	7	20	25	20	50	20	-	-	-	-	-	-

¹Prominence value = Mean population density $\times \sqrt{\text{frequency of occurrence}} \div 10$; ²Mean population density = total number of nematodes $\div$ number of samples obtained; ³Frequency of occurrence = number of samples containing a nematode genus $\div$ number of samples collected $\times 100$; - = no nematodes present

Table 4.7: Prominence values (PV), mean population densities (MPD) and frequencies of occurrence (FO; %) of plant-parasitic nematode species identified from 5- and 20-g watermelon roots from 25 localities across six states of south-west Nigeria during 2016/2017.

Genus	Ekiti			Lagos			Ogun			Ondo			Osun			Oyo		
	PV ¹	MPD ²	FO ³ (%)	PV	MPD	FO (%)	5g roots (Sugar-flotation method)			PV	MPD	FO (%)	PV	MPD	FO (%)	PV	MPD	FO (%)
<i>Helicotylenchus</i>	24	48	25	40	69	33	3	8	13	10	32	10	6	18	13	18	28	40
<i>Multicinctus</i>																		
<i>Pratylenchus</i>	24	48	25	-	-	-	34	68	25	9	21	20	-	-	-	-	-	-
<i>Zaeae</i>																		
<i>Meloidogyne</i> spp.	4 112	4335	95	1400	1518	85	2733	2964	85	3235	3372	92	4001	4 17	90	2682	3037	78
<i>Scutellonema</i>	-	-	-	9	21	17	-	-	-	5	10	25	5	10	25	-	-	-
<i>Bradys</i>																		
20g roots (adapted NaOCl method)																		
<i>Meloidogyne</i> spp.	26 350	26 617	98	18 017	18 017	100	10 275	10 275	100	12 526	12 653	98	13 741	13 741	100	10 116	10 324	96

¹Prominence value = Mean population density $\times \sqrt{\text{frequency of occurrence}} \div 10$; ²Mean population density = total number of nematodes $\div$ number of samples obtained; ³Frequency of occurrence = number of samples containing a nematode genus $\div$ number of samples collected $\times 100$; - = no nematodes present

The frequency of occurrence of *H. dihystra* was significantly and positively correlated with % sand, but significantly and negatively with % silt, clay and organic matter (Table 4.8). Occurrence of *P. zae* was significantly correlated with % silt and clay, but significantly and negatively with pH, % sand and rainfall, while that of *S. bradys* was significantly and positively correlated with pH, % sand and rainfall, but significantly and negatively with % silt and clay.

Table 4.8: Correlation data for the frequency of occurrence of the four predominant plant parasitic nematode genera (and seven species) and rainfall as well as selected soil properties of watermelon fields from 25 localities in south-west Nigeria during the cropping season of 2016/2017

Nematode species	Parameters	Pearson correlation value	P value
<i>Meloidogyne</i> spp. (<i>M. arenaria</i>, <i>M. incognita</i>, <i>M. enterolobii</i> and <i>M. javanica</i>)	Frequency of occurrence x pH	0.564*	0.004
	Frequency of occurrence x % sand	-0.444*	0.03
	Frequency of occurrence x % silt	0.757*	0.001
	Frequency of occurrence x % clay	0.235	0.270
	Frequency of occurrence x % organic matter (OM)	-0.716*	0.001
	Frequency of occurrence x Rainfall	-0.476	0.13
<i>Helicotylenchus dihystra</i>	Frequency of occurrence x pH	0.008	0.97
	Frequency of occurrence x % sand	0.540*	0.006
	Frequency of occurrence x % silt	-0.140	0.515
	Frequency of occurrence x % clay	-0.656*	0.001
	Frequency of occurrence x % OM	-0.574*	0.003
	Frequency of occurrence x Rainfall	0.200	0.349
<i>Pratylenchus zae</i>	Frequency of occurrence x pH	-0.674*	0.001
	Frequency of occurrence x % sand	-0.753*	0.001
	Frequency of occurrence x % silt	0.625*	0.001
	Frequency of occurrence x % clay	0.653*	0.001
	Frequency of occurrence x % OM	0.117	0.587
	Frequency of occurrence x Rainfall	-0.527*	0.008
<i>Scutellonema bradys</i>	Frequency of occurrence x pH	0.435*	0.034
	Frequency of occurrence x % sand	0.007*	0.002
	Frequency of occurrence x % silt	-0.567*	0.004
	Frequency of occurrence x % clay	-0.520*	0.009
	Frequency of occurrence x % OM	0.356	0.088
	Frequency of occurrence x Rainfall	0.649*	0.001

*Significant correlations at $P \leq 0.05$

Table 4.9: *Meloidogyne* spp. identified from watermelon in 25 localities in south-west Nigeria using the SCAR-PCR technique.

Population code	Locality	<i>Meloidogyne</i> spp. identified
L1	Aramoko	<i>Meloidogyne enterolobii</i> , <i>Meloidogyne incognita</i> , <i>Meloidogyne javanica</i>
L2	Ado-Ekiti	<i>Meloidogyne enterolobii</i> , <i>Meloidogyne javanica</i>
L3	Ikole	<i>Meloidogyne enterolobii</i> , <i>Meloidogyne incognita</i>
L4	Ikere	<i>Meloidogyne enterolobii</i> , <i>Meloidogyne incognita</i>
L5	Lagos east	<i>Meloidogyne incognita</i>
L6	Lagos west	<i>Meloidogyne enterolobii</i>
L7	Lagos far east	<i>Meloidogyne incognita</i>
L8	Abeokuta	<i>Meloidogyne incognita</i> , <i>Meloidogyne javanica</i>
L9	Ilaro	<i>Meloidogyne enterolobii</i> , <i>Meloidogyne incognita</i> , <i>Meloidogyne javanica</i>
L10	Ijebu	<i>Meloidogyne arenaria</i> , <i>Meloidogyne enterolobii</i>
L11	Ikenne	<i>Meloidogyne enterolobii</i>
L12	Okitipupa	<i>Meloidogyne enterolobii</i> , <i>Meloidogyne incognita</i>
L13	Akoko	<i>Meloidogyne incognita</i> , <i>Meloidogyne javanica</i>
L14	Akure	<i>Meloidogyne incognita</i>
L15	Owo	<i>Meloidogyne enterolobii</i> , <i>Meloidogyne javanica</i>
L16	Ondo	<i>Meloidogyne arenaria</i> , <i>Meloidogyne enterolobii</i>
L17	Osogbo	<i>Meloidogyne enterolobii</i> , <i>Meloidogyne incognita</i>
L18	Ife	<i>Meloidogyne enterolobii</i>
L19	Ilesha	<i>Meloidogyne enterolobii</i>
L20	Iwo	<i>Meloidogyne enterolobii</i>
L21	Saki	<i>Meloidogyne enterolobii</i> , <i>Meloidogyne incognita</i> , <i>Meloidogyne javanica</i>
L22	Ogbomoso	<i>Meloidogyne incognita</i> , <i>Meloidogyne javanica</i>
L23	Oyo	<i>Meloidogyne enterolobii</i>
L24	Ibadan	<i>Meloidogyne enterolobii</i> , <i>Meloidogyne incognita</i> , <i>Meloidogyne javanica</i>
L25	Ibarapa	<i>Meloidogyne enterolobii</i> , <i>Meloidogyne javanica</i>

Canonical correspondence analysis revealed significant relationships amongst the frequency of occurrence of the four predominant nematode species and soil characteristics, as well as rainfall (Figure 4.2). Frequency of occurrence of both *Meloidogyne* spp. and *H. dihystra* were associated with soil pH and % sand, while that of *P. zae* was associated with the % soil, clay and silt. Occurrence of *S. bradys* was associated with rainfall.

4.4.2 Identification of *Meloidogyne* spp.

4.4.2.1 MOLECULAR IDENTIFICATION: SCAR-PCR

Four *Meloidogyne* spp., viz. *M. arenaria*, *M. enterolobii*, *M. incognita* and *M. javanica* were identified using the SCAR-PCR method. These results agree with amplifications of both the species-specific markers (Table 4.3) and the standards used for each of these species (Figure 4.3A-D). Multiple *Meloidogyne* spp. were detected in 64% of the localities, while in the remaining 36% single-species populations were identified (Table 4.9). The presence of *M. enterolobii* was verified by amplification of the 250 bp fragment (Figure 4.3B). This species occurred most frequently, being detected as a single-species population at three localities (L6, L18, L19 and L20) and as mixed-species communities at 15 of the localities (Table 4.9). *Meloidogyne incognita* was identified as a single-species population at three of the localities and as mixed-species communities at 11 localities by amplification of the 1 200 bp SCAR fragment (Figure 4.3C). Amplification of the 700 bp revealed the presence of *M. javanica* (Figure 4.3D) and that of the 420 bp fragment, the presence of *M. arenaria* (Figure 4.3A) in mixed-species communities at 10 and two of the localities, respectively (Table 4.9).

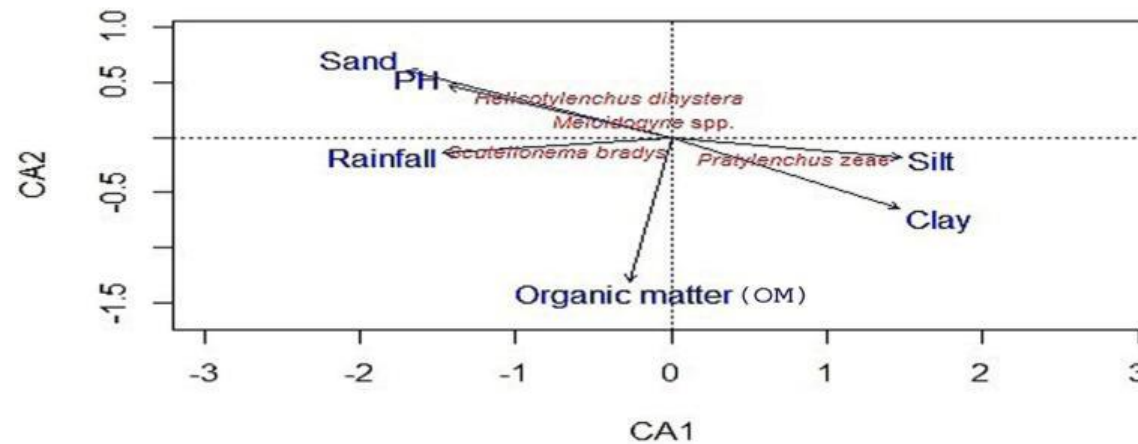


Figure 4.2: Canonical correspondence analysis (CCA) bi-plots showing relationships between selected soil physical properties viz. % silt, % clay, % sand and % organic matter (OM), as well as pH and rainfall and the frequency of occurrence of plant-parasitic nematode species from watermelon fields in south-west Nigeria during the 2016/17 growing season. Axes 1 and 2 explain 80.3% and 18.9% of the variances observed, respectively.

Table 4.10: Morphometric data (pooled) for perineal-pattern parameters (in μm) of three *Meloidogyne* spp. identified from watermelon roots obtained from south-west Nigeria.

<i>Meloidogyne</i> spp.	Parameters	Mean $\pm$ SD	Range
<i>M. incognita</i> (n = 178)	VSL ¹	26.9 $\pm$ 2.2	(22.5 - 31.2)
	AVD ²	21 $\pm$ 2.2	(18.1 - 26.8)
	IPD ³	23.4 $\pm$ 4.6	(10.2 - 39.2)
	APD ⁴	18.9 $\pm$ 2.4	(15 - 26.5)
<i>M. enterolobii</i> (n = 243)	VSL	30 $\pm$ 2.2	(25.4 - 33.4)
	AVD	23.8 $\pm$ 2.2	(18.9 - 27.1)
	IPD	26.9 $\pm$ 5	(15.2 - 42.4)
	APD	19.4 $\pm$ 2.8	(15 - 29.7)
<i>M. javanica</i> (n = 61)	VSL	28.2 $\pm$ 1.9	(23.5 - 31.8)
	AVD	21.3 $\pm$ 1.9	(18.8 - 26.6)
	IPD	24 $\pm$ 4.9	(14.4 - 38.7)
	APD	18.8 $\pm$ 2.4	(14.8 - 27.2)

¹VSL=Vulval-slit length, ²AVD=Anus to vulval distance, ³IPD=Inter-phasmidial distance, ⁴APD =Anus to phasmid distance

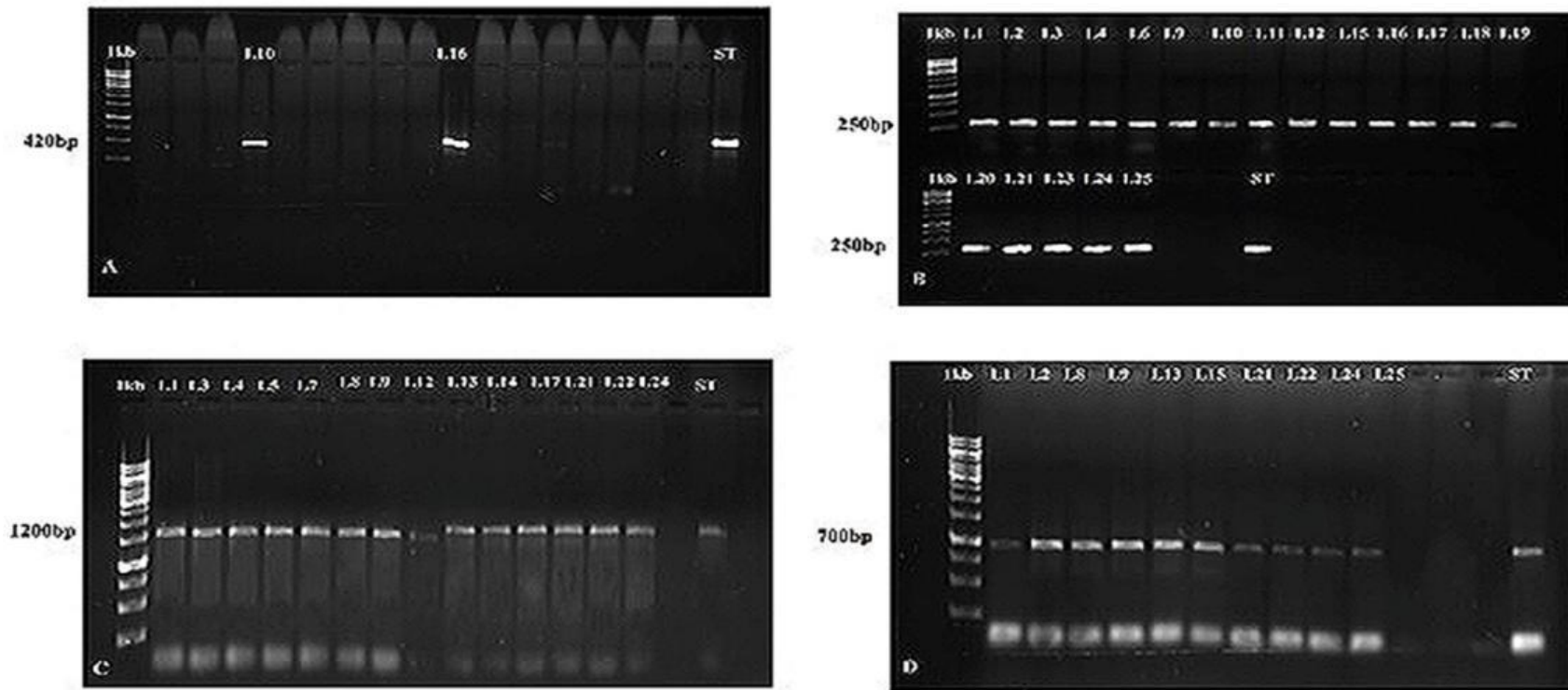


Figure 4.3: An ultraviolet illumination photograph showing patterns of amplification products of *Meloidogyne* spp. parasitizing watermelon roots from 25 communities in Nigeria using the sequence characterized amplified region- polymerase chain reaction (SCAR-PCR) 1kb DNA ladder; ST= DNA standard template for each species; A = *M. arenaria* (2 communities) (L10 and L16) B = *M. enterolobii* (19 communities) (L1, L2, L3, L4, L6, L9, L10, L11, L12, L15, L16, L17, L18, L19, L20, L21, L23, L24, L25), C = *M. incognita* (14 communities) (L1, L3, L4, L5, L7, L8, L9, L12, L13, L14, L17, L21, L22, L24) and D = *M. javanica*. (10 communities) (L1, L2, L8, L9, L13, L15, L21, L22, L24, L25). 1kb DNA ladder (1st well of each gel) was used for all samples; ST = DNA of standard (control) population used for each species.

Using the morphological perineal-pattern approach, three species were identified: *M. enterolobii*, *M. incognita* and *M. javanica* (Table 4.10) (Figure. 4.4A-C). The presence of *M. arenaria*, identified using the molecular SCAR-PCR technique as indicated above, was however not confirmed with this morphological approach. The perineal-pattern parameters of the three *Meloidogyne* spp. were generally within close range and overlapped (Table 4.10). For *M. enterolobii* the highest mean values were recorded for vulval-slit length, vulva to anus distance, inter-phasmid distance and anus to phasmid distance; differing substantially from data of *M. incognita* and *M. javanica* (Table 4.10). *Meloidogyne enterolobii* were present at 19 localities, *M. incognita* at 16 and *M. javanica* at nine.

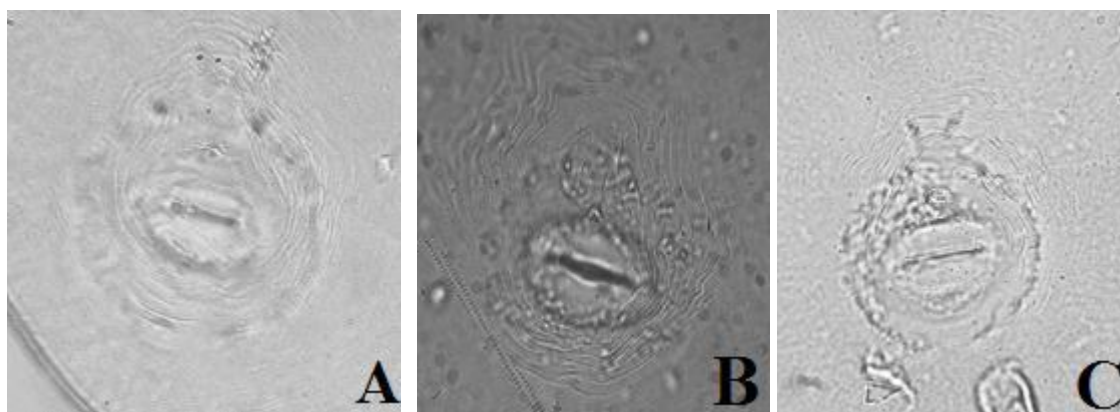


Figure 4.4: Perineal-pattern morphology of *Meloidogyne* spp. females: A) *M. enterolobii*; B) *M. incognita* and C) *M. javanica*.

4.5 Discussion and Conclusion

The 11 plant-parasitic species identified during this study have previously been reported from various other crops in Nigeria (Adegbite *et al.*, 2006; Orisajo, 2012; Daramola *et al.*, 2015; Coyne *et al.*, 2018). Several of them have also been reported from watermelon elsewhere in the world (Khair, 1982; Sikora & Fernandez, 2005; Abd-Elgawad *et al.*, 2007; Tan & Ökten, 2011; Marais, 2019), while to our knowledge *S. bradys* and *X. nigeriense* are here recorded for the first time from the rhizosphere of watermelon.

Meloidogyne spp. were most commonly found in association with watermelon during our study, as previously observed for the crop from eastern Nigeria (Mbawukwu *et al.*, 2016). *Meloidogyne* spp. are considered the economically most important nematode threats to crop

production worldwide, with an extensive host range (Moens *et al.*, 2009; Jones *et al.*, 2013), and most probably the major biotic factor threatening crop production in SSA (Coyne *et al.*, 2009, 2018). *Helicotylenchus dihystra* was second and *P. zae* the third most predominant species recovered from watermelon, both of which have been previously associated with watermelon elsewhere, namely Egypt (Abd-Elgawad *et al.*, 2007), Pakistan (Anwar & McKenry, 2012), South Africa (Marais, 2019) and Turkey (Tan & Okten, 2011). In Nigeria, *H. dihystra* was also reported as being widespread on upland rice (*Oryza sativa* L.) in the southwest of the country (Babatola, 1984). *Pratylenchus zae* is important on several major crops in Nigeria, such as maize (*Zea mays* L.), cowpea (*Vigna unguiculata* (L.) Walp) and upland rice (Egunjobi, 1973; Babatola, 1984; Coyne *et al.*, 2012). The occurrence of *S. bradys* as the fourth predominant species (PV of 77; FO of 12%) was unexpected and has not previously been associated with watermelon, but it is considered a major pest of yam (*Dioscorea* spp. L.) in West Africa (Bridge *et al.*, 2005; Baimey *et al.*, 2009), and more recently was associated with damage to potato (*Solanum tuberosum* L.) (Coyne *et al.*, 2011).

In the current study, four species of *Meloidogyne* were identified using SCAR PCR and three species using the perineal-pattern morphology technique, confirming the unreliability of using perineal patterns alone. The recovery of *M. enterolobii*, *M. incognita* and *M. javanica* from watermelon roots across south-west Nigeria agree with reports from elsewhere in the world (Montalvo & Esnard, 1994; Davis, 2007; Ramírez-Suárez *et al.*, 2014; Cohen *et al.*, 2014; Thies *et al.*, 2016) as well as other crops in Nigeria (Caveness, 1967; Nwauzor & Fawole, 1981; Mbawukwu *et al.*, 2016; Kolombia *et al.*, 2017; Coyne *et al.*, 2018; dos Santos *et al.*, 2019). The occurrence of *M. arenaria* on watermelon also provides the first report of this species on watermelon in Nigeria. However, it is possible that other *Meloidogyne* spp. could be present, since specific primers for the four most common and likely to be encountered tropical species (*M. arenaria*, *M. enterolobii*, *M. incognita* and *M. javanica*) were used for the current study.

Our findings agree with the report by dos Santos *et al.* (2019), who reported *M. enterolobii* as the most widely distributed species of *Meloidogyne* in Nigeria, followed by *M. incognita* and *M. javanica*. The occurrence of *M. incognita* and *M. javanica* from watermelon fields in our study further reflects results by Pagan *et al.* (2015) and dos Santos *et al.* (2019), who also recorded these species from numerous crops in Nigeria, and those who reported it from other SSA countries (Onkendi & Moleleki, 2013; Visagie *et al.*, 2018; Rashidifard *et al.*, 2019). The current distribution of *M. enterolobii* in Nigeria, which appears to be widespread and prevalent, emphasises the need for accurate identification of *Meloidogyne* spp., in order to effectively

implement appropriate management strategies against such pests. Also important is the detection *M. arenaria* from watermelon in two locations in the current study. This species has previously been reported from watermelon elsewhere (Sikora and Fernandez, 2005) but in Nigeria has been recovered from cowpea and yam in the northern and middle-belt regions, respectively (Olowe, 2006; Kolombia *et al.*, 2014). The difficulty to identify *M. arenaria* using perineal-pattern morphology can be explained since different females were selected and used for molecular and morphological identifications. This contributed to the dissimilarity in results between the two approaches, especially where mixed communities of *Meloidogyne* spp. were present, which were the case for the majority of the locations sampled. To further optimise consistency and the use of molecular methods towards accurate species identification, the posterior female area could for example be mounted and used for morphometric identification, while DNA can be extracted from the posterior part of the same female and used for molecular identification to prevent ambiguity. Detection of two and even three *Meloidogyne* spp. from the same watermelon root sample, indicates that multiple species infections occur, as has also been found for other crops (Moens *et al.*, 2009; Visagie *et al.*, 2018; Rashidifard *et al.*, 2019). Similarity between the use of the morphological and molecular approaches was, however, evident in *M. enterolobii* being recorded as the predominant species using both these approaches. This is an indication of the extent to which this highly injurious species is distributed in south-west Nigeria. Discrimination between *M. enterolobii* and *M. incognita* females using perineal-pattern morphology and morphometrics posed a challenge, compared with the relative ease experienced in identifying *M. javanica*. This further supports the difficulty that *M. enterolobii* poses to taxonomists, which is often misidentified due to the similarity in morphological characters with *M. incognita* as well as other species from the *Mi* group (Brito *et al.*, 2004; Landa *et al.*, 2008; Hunt & Handoo, 2009; Conceição *et al.*, 2012; Visagie *et al.*, 2018). Interestingly, however, is that the mean values for some morphometric characteristics (such as VSL, AVD and IPD) for Nigerian populations of *M. enterolobii* in the current study were generally greater than those recorded for its counterpart species *M. incognita* and *M. javanica*. For VSL in particular, our findings reflect those of Brito *et al.* (2004) who suggested its use as a useful parameter to separate *M. enterolobii* females from other species belonging to the *Mi* group.

The use of molecular techniques is more accurate and sensitive than traditional techniques (Hunt & Handoo, 2009; Onkendi *et al.*, 2014; Visagie *et al.*, 2018). This phenomenon was also confirmed by our study since using the SCAR-PCR method, four species (*M. arenaria*, *M. enterolobii*, *M. incognita* and *M. javanica*), were identified opposed to only the latter three

using morphological identification. A limitation to both approaches used is, however, the selection of only 20-21 adult female specimens for identification purposes. The true identity of *Meloidogyne* spp. present in a population may be masked compared to when more eggs or J2, for example, are used (Visagie *et al.*, 2018). This approach can provide better opportunities for detecting the full range of *Meloidogyne* spp. present within mixed communities.

Findings of the current study reveal that watermelon production in Nigeria is associated with economically important plant-parasitic nematodes. The widespread occurrence of, particularly *M. enterolobii*, but also the other root-knot nematode species identified, coupled with more intensified cropping of watermelon in south-western Nigerian will contribute to the rapid build-up of these damaging species and will aggravate the damage they are known to cause. Our study supports the observations of Coyne *et al.* (2018) that *Meloidogyne* spp. are the predominant problems facing crop production within SSA and the tropics. Therefore, we need a strong focus on mitigating these pests on crops within the region in order to improve crop production. Producers should thus be aware of the nematode problem and be advised about management options, such as the use of resistant crop cultivars in rotation with watermelon. Also, to complement changes in cropping practices the use of other nematode management strategies (e.g. biological control agents, nematicidal seed-coat products, and others) should be employed to enable sustainable crop production. However, accurate diagnosis is crucial to develop and employ appropriate and effective management strategies. Also, relatively little is known about the interactions or species-specific effects of the four identified *Meloidogyne* spp. Hence, interspecific diversity of such species parasitising watermelon in Nigeria requires broad range screening of both commercial and wild watermelon cultivars to enable identification of germplasm with suitable resistance. More investigations are therefore needed to establish the virulence as well as damage threshold of each *Meloidogyne* spp. as well as their combined effects on watermelon.

4.6 References

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CHAPTER 5: ARTICLE 4

Reproduction potential of Nigerian *Meloidogyne* spp. and the host status of six commercial watermelon cultivars to the predominant root-knot nematode species

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Tesleem T. BELLO^{1,2,3*}, Danny L. COYNE² and Hendrika FOURIE¹

¹*North-West University, Unit for Environmental Sciences and Management, Integrated Pest Management, Private Bag X6001, Potchefstroom, 2520, South Africa.*

²*International Institute of Tropical Agriculture, IITA, PMB 5320, Ibadan, Nigeria.*

³*Federal College of Education, Abeokuta, PMB 2096, Ogun State, Nigeria.*

*Corresponding author, e-mail: tesleembello@yahoo.com

5.1 Abstract

The reproduction potential of 25 *Meloidogyne* populations (single species) and/or communities (mixed species), associated with watermelon rhizospheres in south-west Nigeria, were determined in roots of tomato seedlings (cv. Tropimech). Also, the host response of six commercially available watermelon cultivars was assessed using the three predominant species (*M. incognita*, *M. javanica* and *M. enterolobii*). Greenhouse experiments with ± 5000 eggs and second-stage juveniles inoculated per seedling showed that the reproduction factors (Rfs) varied from four (*M. incognita*: population L14) to 46 (*M. enterolobii* & *M. javanica*: community L 15). All watermelon cultivars were susceptible ($Rfs > 1$) to the three *Meloidogyne* spp.: ‘Charleston Gray’ had the highest Rf (14.7) for *M. enterolobii*, while ‘Sugar Dragon’ had the lowest (2.1) for *M. incognita*. Cultivars with the lowest relative susceptibility values (%) for the respective *Meloidogyne* spp. should be the preferred choice of producers to enable sustainable cultivation of the crop in Nigeria.

Keywords - egg laying females, egg mass, population, reproduction factor, susceptible.

Production of watermelon (*Citrullus lanatus* (Thunb) Matsum & Nakai), one of the world's most important vegetable crops (Huh *et al.*, 2008), generates higher profits, provides higher income to farmers and represents an important source of employment compared to that of other exotic vegetable crops grown in Nigeria (Ajewole & Folayan, 2008). Although cultivation of the crop in Nigeria was initially confined to the north and middle belt regions, it is now being grown extensively in the south-western parts of the country. According to (Adeoye *et al.*, 2007), watermelon was rated as the most preferred among five other exotic vegetables in the south-western states of the country (Okunlola *et al.*, 2011). Due to the rapid expansion of watermelon production in this area, there is likelihood of the crop being adversely affected by diseases and pests, including *Meloidogyne* spp.

Meloidogyne spp. have been a major constraint to watermelon production worldwide with total crop losses reported under certain environmental conditions (Thies, 1996; Davis, 2007; Ngele & Kalu, 2015). The species identified in crop fields in south-western Nigeria where watermelon was grown in the 2016/17 growing season were *Meloidogyne arenaria* (Neal, 1889) Cobb, 1890, *Meloidogyne enterolobii* Yang & Eisenback, 1983, *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 and *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949 (see Article/Chapter 4, Paragraph 4.2.3). These species also cause damage to the crop worldwide: from the Mediterranean (Cohen *et al.*, 2014) to Puerto Rico (Montalvo & Esnard 1994); Mexico (Montalvo & Esnard, 1994; Moens *et al.*, 2009; Ramírez-Suárez *et al.*, 2014); and especially in the United States of America (USA) where extensive research has been conducted regarding *Meloidogyne*-watermelon associations (Thomason & McKinney, 1959; Winstead & Riggs, 1959; Sumner & Johnson, 1973; Davis, 2007; Thies, 1996; Thies & Levi, 2003, 2007; Thies *et al.*, 2010, 2015, 2016).

The reproduction potential of *Meloidogyne*, referring to the ability of a particular species, population (containing a single species) or community (containing mixed species) to reproduce

in roots of a susceptible host plant, is associated with injuriousness (Karssen *et al.*, 2013). Such a study has not been done for Nigerian *Meloidogyne* spp. Since it has been reported that the number of *Meloidogyne* spp. generations produced on crops per season differ among and within species, and the reproduction potential of populations and/or communities also differ substantially (Manzanilla-López & Starr, 2009), such information is crucial. Factors, such as the injuriousness of a particular species, population density and the availability of suitable host plants can furthermore impact on the reproduction potential of *Meloidogyne* spp. (Trudgill, 1995; Moens *et al.*, 2009). Soil temperature and texture, and other abiotic and biotic factors also influence the reproduction potential and pathogenicity of *Meloidogyne* spp. (Koenning *et al.*, 1996; Kiewnick *et al.*, 2009;).

Depending on the degree of injuriousness caused by *Meloidogyne* spp., host plants are classified as either non-, poor- or good hosts (Karssen *et al.*, 2013). The use of resistant germplasm has been adjudged as one of the most practical and cost-effective options available for managing *Meloidogyne* spp.. Depending on the level of resistance, nematode development and reproduction are substantially retarded in roots of a resistant compared to that of a susceptible cultivar (Kamunya *et al.*, 2008). The use of host plant resistance therefore plays an important role as part of an integrated, nematode pest management system (Giannakou & Anastasiadis, 2005; Wang *et al.*, 2006; Karssen *et al.*, 2013). Although the majority of studies to date reported that no resistance exist to root-knot nematode species in commercially available watermelon cultivars (Winstead & Riggs, 1959; Montalvo & Esnard, 1994; Boyhan *et al.*, 2003; Thies & Levi, 2007; Thies *et al.*, 2016), the trait has been detected against *M. incognita* (Thies & Levi, 2007; Thies *et al.*, 2016) and *M. arenaria* race 1 (Thies & Levi, 2003) in some wild accessions of *C. lanatus* var. *citroides* . Furthermore, variability has been reported among *Meloidogyne* spp. in terms of the penetration rates of second-stage juveniles (J2), the degree of root galling inflicted by feeding J2 and females, and final population densities (Pf)

(Winstead & Riggs, 1959; Edelstein *et al.*, 2010). *Meloidogyne javanica* has, for example, been reported to show lower root galling and egg production than *M. incognita* in roots of some watermelon genotypes (Cohen *et al.*, 2014; López-Gómez *et al.*, 2016). Three of the watermelon cultivars evaluated for their host status to *Meloidogyne* spp. in this study had also been reported by various authors as being susceptible to *M. incognita*. Montalvo & Esnard (1994) reported that both ‘Charleston Gray’ and ‘Sugar Baby’ were susceptible to a Puerto Rican population of *M. incognita*, with ‘Sugar Baby’ identified as being the least susceptible out of the 10 cultivars screened. From the USA, Thies & Levi, (2007) reported ‘Charleston Gray’ as being susceptible to both *M. incognita* race 3 and *M. arenaria* race 3. From south-west Nigeria, Aminu-Taiwo *et al.* (2015) reported the susceptibility of cultivar ‘Koloss F1’ to *M. incognita* (Aminu-Taiwo *et al.*, 2015). The latter report represents the only available information about the host status of cultivated watermelon cultivars to one of the commonly occurring *Meloidogyne* spp. in Nigeria. Since the use of resistant germplasm will be one of the preferred environmentally-friendly nematode control options for African countries such as Nigeria, the aims of this study were to i) assess the reproduction potential of different *Meloidogyne* populations and communities present in south-western Nigerian cropping systems and ii) screen commercially available watermelon cultivars for their host status to the three predominant *Meloidogyne* spp. identified in this part of the country.

5.2 Materials and Methods

5.2.1 REARING OF *MELOIDOGYNE* SPP. POPULATIONS AND COMMUNITIES

Two experiments were conducted in this study: one assessing the reproduction potential of 25 populations and communities of *Meloidogyne* spp. and the other assessing the host reaction of watermelon cultivars to the three predominant *Meloidogyne* spp. (*M. enterolobii*, *M. incognita* and *M. javanica*). The first experiment was conducted using 25 field populations

and/or communities of *Meloidogyne* spp. previously collected from different watermelon fields in south-west Nigeria and identified to species level (see Article 3/Chapter 4, Paragraphs 4.2.3 & 4.2.4) (Table 4.1). Single species of *M. enterolobii* and *M. incognita* represented the populations, while communities contained mixed species of *M. incognita* and *M. javanica*; *M. enterolobii* and *M. javanica*; *M. arenaria* and *M. enterolobii* (Table 4.1). These populations and communities were reared in roots of a susceptible tomato cultivar ('Tropimech') (Bello *et al.*, 2015) in a greenhouse using the same protocol described in Article 3/Chapter 4, Paragraph 4.2.2. The ambient temperature ranges were 22.1 °C – 30.4 °C for the initial experiment and 22.4 °C – 31.3 °C for the repeat experiment.

For the watermelon host status study, the single-species populations used were *M. enterolobii* (L18), *M. incognita* (L5) and *M. javanica* (L2). Single *Meloidogyne* sp. egg masses, for each of the respective species, were removed from infected watermelon roots obtained from the field survey, inoculated and reared *in vivo* in roots of tomato seedlings (cv. Tropimech). The identity of the species was confirmed using species specific primers (see Article 3/Chapter 4, Paragraph 4.2.3).

5.2.2 EXTRACTION OF *MELOIDOGYNE* SPP. EGGS AND J2 FOR INOCULATION PURPOSES

Inocula of all *Meloidogyne* spp. populations and communities were harvested after 56 days of *in vivo* rearing and maintenance in the glasshouse. The infected root systems of tomato seedlings containing each population and/or community were individually rinsed under tap water to remove excess soil and debris, chopped and eggs, and J2 extracted using the adapted NaOCl extraction method (Riekert, 1995). The inoculum suspension for each population was reduced to 40 ml and nematode density individually determined from 4 ml aliquots using a De Grisse counting dish (De Grisse, 1963) and a Leica MZ 12.5 dissection microscope (60x magnification). Nematode suspensions were then calibrated in order to inoculate

approximately 5 000 eggs and J2 of each population and/or community in 5-ml aliquots on roots of ten-day-old tomato seedlings (cv. Tropimech) for the reproductive potential study, while watermelon seedlings were used for the host suitability study. The roots of each seedling were re-covered with soil after inoculation. Seedlings of six commercially available watermelon cultivars that are commonly grown in Nigeria (Enujeke, 2013; Dantata, 2014) were used for this study, namely ‘Charleston Gray’, ‘Erato F1’, ‘Kaolak’, ‘Koloss F1’, ‘Sugar Baby’ and ‘Sugar Dragon’. Seeds of the cultivars were obtained from Agritropic Limited® and Greenfield Evergreen Resources® Ibadan (Nigeria). Two seeds of each watermelon cultivar were sown in 5-L pots containing steam sterilized sandy-loam soil (6.1% clay, 90.4% sand, 2.3% silt, 1.8% organic matter, pH (H₂O) 7.22). Two weeks after seedling emergence, seedlings were thinned to one seedling per pot before inoculation with the respective *Meloidogyne* sp. commenced. Tomato (reproduction potential study) and watermelon (host status study) plants were maintained in the greenhouse for 56 days before termination of the experiments and were manually irrigated four times per week. The plants also received Hoagland (nutrient) solution (Hoagland & Arnon, 1950) once per week.

For all experiments, a randomised complete block design (RCBD) layout were used; five replications for the reproductive potential study and seven replicates for the watermelon host suitability study. Experiments for each of these two studies were conducted twice to represent an initial and repeat experiment. Pots of all experiments were rotated at 26 days after nematode inoculation to help equalise exposure to light and temperature differences in the glasshouse.

5.2.3 DATA COLLECTION AND ANALYSIS

At termination of the experiments, each plant was carefully uprooted, rinsed under running tap water and egg masses stained by submerging the whole root system into a 1% phloxine B solution it for 45 min. Roots of each plant were then chopped into 1-cm pieces and egg masses

counted using a commercial magnifying glass. The egg-laying female index were next applied to score each root system on a scale of 0 to 5, where 0 = 0; 1 = 1 to 2; 2 = 3 to 10; 3 = 11 to 30; 4 = 31 to 100 and 5 = >100 egg masses/root system (Hussey & Boerma, 1981). Eggs and J2 were then extracted from each root system and counted by using a De Grisse counting dish (De Grisse, 1963) and a Leica MZ 12.5 dissection microscope (60-100x magnification). The reproduction factor (Rf) for each plant was determined, where $Rf = \text{final egg and J2 numbers (Pf)} / \text{initial egg and J2 numbers (Pi)}$ (Windham & Williams, 1987). Also the relative susceptibility (%) was calculated for each watermelon cultivar using the following equation: $\text{final egg and J2 densities of the most susceptible cultivar} / \text{final egg and J2 densities of each cultivar}$. A value <10 is indicative of resistance (Hussey and Janssen, 2002).

Nematode data for all experiments were $\log(x+1)$ transformed to conform data to normal distribution (Zuur *et al.*, 2010). Data were analysed using Main Effects Analysis of Variance (ANOVA) (Statistica, Version 13.2) and Factorial ANOVA to determine the interaction between factors (represented by the initial and repeat experiments). Subsequently, Tukey's HSD Test ($P \leq 0.05$) was performed for each data set to separate the means.

5.3 Results

5.3.1 REPRODUCTION POTENTIAL EXPERIMENT

Substantial variation existed among the 25 field *Meloidogyne* populations and/or communities in terms of their reproduction potential for the different nematode parameters measured (Table 5.1). Since no significant interaction existed between the initial and repeat experiments for all parameters, data from both experiments were pooled, analysed and are presented in Table 5.1.

The mean number of egg masses per root system ranged from 10 for L7 (single-species population of *M. incognita*) to 43 for L25 (mixed species community of *M. enterolobii* and *M.*

javanica) respectively (Table 5.1). The number of egg masses recorded for L7 was significantly ($P \leq 0.05$) lower only than those of L1, L2, L4, L22 and L25.

Mean ELF values ranged from 2 for L10 (mixed species community of *M. arenaria* and *M. enterolobii*) to 4 for L4 (mixed species community of *M. enterolobii* and *M. incognita*) and L25 (mixed species community of *M. enterolobii* and *M. javanica*), respectively (Table 5.1). The ELF index recorded for L10 was significantly ($P < 0.05$) lower than those of L1, L2, L4, L13, L15, L18, L21, L22, L24 and L25. Populations L4 and L25 with the highest ELF values were significantly different from all populations except L1, L2, L15, L18, L21, L22 and L24.

Egg and J2 numbers per root system, representing the Pf, ranged from 19 946 (L14; single species population of *M. incognita*) to 231 891 (L15; mixed community of *M. enterolobii* and *M. javanica*) (Table 5.1). The highest Pf recorded for L15 differed significantly ($P < 0.05$) from the other populations and or communities, except from L2, L4, L18, L22 and L24. The lowest Pf recorded for L14 was significantly ($P = < 0.05$) lower than those of the other populations and/or communities except for L3, L7, L20 and L21.

The Rf values differed substantially for the *Meloidogyne* spp. populations, ranging from 4 (L14; single species population of *M. incognita*) to 46 (L15; mixed community of *M. enterolobii* and *M. javanica*) (Table 5.1). Population L14 with the lowest Rf differed significantly ($P < 0.05$) from the others except for L3, L7, L11, L16, L20 and L21; community L15 with the highest Rf differed significantly from the others except from L2, L4, L18, L22 and L24.

Root masses of tomato plants infected with most of the *Meloidogyne* spp. populations were similar to each other, with only those of L7, L16, L17 and L19 being significantly ($P < 0.05$) lower than those of L22 and L24 (Table 5.1).

5.3.2 HOST STATUS EXPERIMENT

Significant interactions existed between the initial and repeat experiments for all nematode parameters: egg mass numbers (F ratio = 3.73; P = 0.0001); ELF (F ratio = 2.94; P = 0.0001); final eggs and J2 numbers (Pf) (F ratio = 8.51; P = 0.0001) and Rf (F ratio = 8.57; P = 0.001) (Table 5.2a). These significant interactions were generally ascribed to the substantial lower or higher values existing for such parameters between the two experiments.

The number of egg masses per root system for each experiment did not differ significantly ($P < 0.05$) among the six watermelon cultivars and the susceptible tomato standard for the three *Meloidogyne* spp. for the initial experiments as well as for the *M. enterolobii* repeat experiment (Table 5.2a). For the *M. incognita* repeat experiment, however, cv. Sugar Baby maintained significantly lower egg mass numbers per root system compared to that of cv. ‘Charleston Gray’ and the susceptible tomato standard, while for the *M. javanica* repeat experiment ‘Sugar Baby’ maintained significantly ($P < 0.05$) lower egg mass numbers compared to the susceptible tomato standard only.

For ELF indices no significant differences were evident among watermelon cultivars and the susceptible standard for the *M. enterolobii* and *M. javanica* initial experiments. However, for the *M. incognita* initial experiment the cultivars Sugar Baby and Koloss F1 had significantly ($P < 0.05$) lower ELF indices compared to the susceptible standard only (Table 5.2a). For the repeat experiments, no significant differences were recorded among the watermelon cultivars and/or the susceptible standard with regard to ELF indices for *M. enterolobii* and *M. javanica*. However, for *M. incognita* ‘Erato F1’ had significantly ($P < 0.05$) lower ELF compared to the susceptible tomato standard only.

Table 5.1: Nematode reproduction data for 25 *Meloidogyne* spp. populations (containing single species) and communities (containing mixed species) in roots of susceptible tomato plants (cv. Tropimech) in greenhouse experiments (data for an initial and repeat experiments pooled).

Code	Species	Egg mass	Egg-laying female (ELF) ¹	Eggs and J2 (Pf) ²	Reproduction factor (Rf ³)	Root mass (g)
L1	<i>Me^a, Mi^b, Mj^c</i>	35±8 ab	4±0.2 a	11.5 ⁴ (118 855 ⁵ ±23 595) bcdefg	24±5 bcdefg	23.3±1.7 ab
L2	<i>Me, Mj</i>	35±11 abc	4±0.4 ab	11.8 (165 787±34 384) abcd	33±7 abcd	23±1.7 ab
L3	<i>Me, Mi</i>	28±8 abcd	3±0.2 bcd	11.2 (105 788±34 081) cdefgh	21±7 cdefgh	19±2 ab
L4	<i>Me, Mi</i>	33±6 abc	5±0.2 a	11.9 (170 218±25 904) abc	34±5 ab	23.8±1.7 ab
L5	<i>Mi</i>	24±6 abcd	3±0.3 bcd	11.5 (126 056±229 83) bcdef	25±5 bcdef	19.1±2.3 ab
L6	<i>Me</i>	29±4 abcd	3±0.2 bcd	11.6 (138 057±25 542) bcdef	27±5 bcdef	19.7±2.6 ab
L7	<i>Mi</i>	10±2 d	3±0.4 d	9.5 (45 183±20 447) fgh	9±4 gh	17.8±1.1 b
L8	<i>Mi, Mj</i>	30±6 abcd	3±0.2 bcd	11.9 (154 143±18 044) bcde	31±4 bcde	21.4±1.2 ab
L9	<i>Me, Mi, Mj</i>	28±9 abcd	3±0.3 bcd	11.5 (118 672±27 667) bcdefg	24±6 bcdefg	20.7±1.6 ab
L10	<i>Ma^d, Me</i>	13±3 cd	2±0.3 d	10.5 (114 022±16 716) bcdefg	23±3 bcdefg	22.5±1.7 ab
L11	<i>Me</i>	21±4 bcd	3±0.3 bcd	10.4 (57 263±17 081) efgh	12±3 fgh	21.1±2.1 ab
L12	<i>Me, Mi</i>	26±5 abcd	3±0.3 bcd	11.6 (151 186±40 205) bcde	30±8 bcde	19.9±1.9 ab
L13	<i>Mi, Mj</i>	29±5 abcd	3±0.2 bc	11.6 (140 034±38 128) bcdef	28±8 bcdef	22.2±2.4 ab
L14	<i>Mi</i>	13±3 cd	3±0.2 cd	8.4 (19 946±5 666) h	4±1 h	20.9±2.2 ab
L15	<i>Me, Mj</i>	28±10 abcd	4±0.3 ab	12.2 (231 891±59 016) a	46±12 a	24±1.9 ab
L16	<i>Ma, Me</i>	22±5 abcd	3±0.3 bcd	8.9 (27 147±8 803) gh	5±2 h	17.3±0.9 b
L17	<i>Me, Mi</i>	22±3 abcd	3±0.2 bcd	11.7 (129 145±16 590) bcdef	26±3 bcdef	18.3±2.1 b
L18	<i>Me</i>	31±10 abcd	4±0.3 ab	11.8 (168 611±48 282) abcd	34±10 abcd	21.3±1.4 ab
L19	<i>Me</i>	26±3 abcd	3±0.2 bcd	11.6 (123 557±22 535) bcdef	25±5 bcdef	18.1±1.6 b
L20	<i>Me</i>	23±5 abcd	3±0.2 bcd	9.8 (70 809±21 235) defgh	14±4 efgh	18.7±3.3 ab
L21	<i>Me, Mi, Mj</i>	28±4 abcd	4±0.2 ab	11.4 (109 833±20 975) cdefgh	22±4 cdefgh	21.1±2.3 ab
L22	<i>Mi, Mj</i>	32±7 abc	4±0.2 ab	12 (176 680±17 855) abc	35±4 abc	24.5±2 a
L23	<i>Me</i>	20±6 bcd	3±0.3 cd	11.4 (131 110±27 374) bcdef	26±6 bcdef	21.9±1.7 ab
L24	<i>Me, Mi, Mj</i>	30±8 abcd	4±0.2 abc	12.1 (212 035±42 842) ab	42±9 a	24.2±2.1 a
L25	<i>Me, Mj</i>	43±8 a	5±0.2 a	11.8 (153 934±16 483) bcde	31±3 bcde	23.3±1.8 ab
<i>F</i>		1.63	2.21	3.45	3.45	1.25
<i>P</i>		0.03	0.001	< 0.0001	< 0.0001	0.20

^a*Meloidogyne enterolobii*; ^b*Meloidogyne incognita*; ^c*Meloidogyne javanica*; ^d*Meloidogyne arenaria*; ¹Number of egg laying females (according to Hussey and Boerma, 1981 where 0= zero; 1 = 1 to 2; 2 = 3 to 10; 3 = 11 to 30; 4 = 31 to 100 & 5 = >100 egg masses per root system; ²Pf = Final nematode population (total eggs and J2 per root system). ³RF = reproduction factor = Pf (final nematode population)/Pi (initial nematode population) (Windham & Williams, 1987). ⁴Log(x+1) transformed values; ⁵Real means ± standard error of real means (Tukey's HSD where P≤0.05). Means within the same column followed by the same letters are not significantly different at P≤0.05.

For the initial experiment, the *M. enterolobii* eggs and J2 numbers per root system were significantly ($P<0.05$) lower for ‘Super Dragon’ and ‘Koloss F1’ compared to that of the susceptible standard only (Table 5.2a). Similarly, for the initial *M. incognita* experiment cultivars Sugar Baby, Erato F1, Koloss F1 and Sugar Dragon maintained significantly ($P<0.05$) lower egg and J2 numbers per root system than those of the susceptible tomato standard. For the *M. javanica* initial experiment significantly ($P<0.05$) lower egg and J2 numbers were recorded per root system for cultivars Sugar Dragon, Charleston Gray, Koloss F1 and Kaolack compared to those of the susceptible standard. Concerning the repeat experiment, none of the cultivars differed significantly from one another or from the susceptible tomato standard for *M. enterolobii*, while for *M. incognita* and *M. javanica* all cultivars had significantly lower egg and J2 numbers per root system compared to the susceptible tomato standard.

The Rf values the initial experiment for *M. enterolobii* differed significantly among the cultivars, with those of cultivars Koloss 1 and Sugar Dragon being significantly ($P<0.05$) lower than those of the susceptible tomato standard (Table 5.2a). For *M. incognita*, cultivars Erato F1, Sugar Baby, Koloss F1 and Sugar Dragon also had significantly ($P<0.05$) lower Rf values than that of the susceptible tomato standard. The Rf values for *M. javanica* were also significantly lower for all cultivars compared to that of the susceptible tomato standard. For the repeat experiment, no significant differences existed for *M. enterolobii* among the six watermelon cultivars and the susceptible tomato cultivar in terms of Rf values, while for *M. incognita* and *M. javanica* all six cultivars had significantly lower Rf values compared to the susceptible tomato standard.

The relative susceptibility (%) of the watermelon cultivars ranged from 29% (‘Sugar Dragon for *M. javanica* for the initial experiment) to 99% (‘Kaolack for *M. incognita* for the initial experiment) when measured against the most susceptible cultivar for each particular species

and experiment. Therefore, none of the cultivars exhibited resistance (<10%) using this parameter.

A significant interaction ($F=1.83$; $P=0.021$) was observed for Cultivars x *Meloidogyne* spp. for root mass (Table 5.2b). However, this resulted from the significant differences between the masses of the watermelon cultivars vs. the masses of the susceptible tomato cultivar standard since the watermelon cultivar masses did not differ among the six cultivars. Therefore, due to the genetically inherent differences for the root mass trait for the watermelon and tomato crops, this data warrants no further discussion.

Table 5.2a Data for various nematode parameters used to determine the host status of six commercially available watermelon cultivars, grown in Nigeria, to three *Meloidogyne* spp. (*M. enterolobii*, *M. incognita* and *M. javanica*) in separate greenhouse experiments (initial and repeat).

Cultivar	Egg masses / root system		Egg-laying females (ELF) ¹		Egg and second-stage juvenile (J2) numbers (Pf) ²		Reproduction factor (Rf) ³		% Relative susceptibility ⁴	
	Initial experiment	Repeat Experiment	Initial experiment	Repeat experiment	Initial experiment	Repeat experiment	Initial experiment	Repeat experiment	Initial trial	Repeat trial
<i>Meloidogyne enterolobii</i>										
Charleston Gray	46 abAB	38 abcdABC	4.8±0.4 aA	4.2±0.4 aAB	11 ⁴ (73 522±40 182) abcABCDE	10.5 (42 101±18 947) bcdeBCDEFGH	14.7±8abcABCDE	10±3.1bcdeBCDEFGH	100	62
Erato F1	45 abAB	43 abcABCD	4.4±0.5 abcABC	4.4±0.5 abAB	10.8 (67 849±48 133) abcdeABCDEF	10.6 (41 449±12 107) bcdeABCDEF	13.6±9.6abcdeABCDEF	8.3±2.4bcdeABCDEF	92	62
Kaolack	39 abcABC	38 abcdABCD	4.6±0.5 abABC	4±0 abABC	10.2 (49 936±15 481) abcdeABCDEF	10.6 (49 026.5±34 056) bcdABCDEF	8.4±3.8abcdeABCDEF	9.8±6.8bcdABCDEF	68	72
Koloss F1	34 abcABCD	38 abcdAB	4.2±0.abABC	4.4±0.5 abAB	10.6 (30 712±13 482) cdeDEFGH	10.6 (40 002±6 568) bcdeABCDEF	6.1±2.7cdeDEFGH	8±1.3bcdeABCDEF	42	59
Sugar Baby	45 abABC	43 abcABCDE	4.4±0.5 abAB	4.8±0.4 aA	10.7 (60 088±42 662) abcdeABCDEF	11 (67 677 ±37 117) abcABCDE	12±8.5abcdeABCDEF	13.5±7.4abcABCDE	82	100
Sugar Dragon	41 abcABC	30 abcdeABCD	4.8±0.4 abA	4±1.2 abAB	10.1 (31 855±17 526) cdeDEFGH	10.6 (52 231.4±37 538) bcdABCDEF	6.4±3.5cdeDEFGH	10.4±7.5bcdABCDEF	43	78
Tomato*	48 aA	44 abABC	4.6±0 aA	4.6±0.5 aABC	11.7 (127 809±62 889) abABCD	11.8 (135 050±47 845) abABC	27.1±9.6abABCD	25.6±12.6abABC	-	-
<i>Meloidogyne incognita</i>										
Charleston Gray	32 abcABCD	33 abcdABCD	4.2±0. bcABC	4±0 abABC	10.9 (63 359±38 310) abcdeABCDEF	10.4 (31 233±14 078) cdefDEFGH	12.7±7.7abcdeABCDEF	6.2±2.8cdefDEFGH	100	100
Erato F1	27 abcABCD	17 deABCD	3.8±0.4 abcABC	3±0.7 bBC	9.8 (20 918±16 540) cdeEFGH	10.2 (15 233±6 077) cdeABCDEF	4.2±3.3cdeEFGH	3.2±0.8cdeABCDEF	33	49
Kaolack	29 abcABCD	23 abcdeABC	4±0 abcABC	3.2±0.9 abABC	10.2 (62 870±39 538) abcdeABCDEF	10.4 (20 903±12 596) cdefEFGH	12.6±7.9abcdeABCDEF	4.2±2.5cdefEFGH	99	67
Koloss F1	16 cCD	31 abcdeABCD	3.6±0.5 bcABC	4±0 abABC	9.8 (28 230±12 687) cdeEFGH	9.6 (16 982±9604) fGH	5.6±2.5cdeEFGH	2.2±1.2fH	45	54
Sugar Baby	19 bcBCD	7 eABCD	2.2±0.8 cC	3.6±0.9 abABC	9.6 (19 977±7 543) cdeEFGH	9.4 (11 139±4 054) efGH	4±1.5cdeEFGH	3.4±1.9efGH	32	36
Sugar Dragon	25 abcABCD	30 abcdeAB	3.8±0.4 abcABC	3.4±0 abABC	10.6 (52 086±39 231) deBCDEFGH	9.3 (10 670±6 631) fH	10.4±7.8deBCDEFGH	2.1±0.9f H	82	34
Tomato*	43 abcABC	35 abcdABCDE	4.6±0 aA	4.6±0.5 aAB	11.6 (118 568±29 861) abABCD	11.8 (138 098±47 849) abABC	27.6±9.6abABCD	23.7±6abABC	-	-
<i>Meloidogyne javanica</i>										
Charleston Gray	33 abcABCD	31 abcdeABCDE	4.2±0.8 abABC	4±0.7 abABC	9.6 (18 352±4 031) cdeEFGH	10.4 (33 602±14 807) cdefCDEFGH	3.7±0.8cdeEFGH	6.7±3cdefCDEFGH	38	79
Erato F1	24 abcABCD	25 abcdeABCD	4±1 abcABC	3.8±0.4 abABC	10.5 (47 684±33 227) abcdeABCDEF	10.3 (33 321±18 315) cdefDEFGH	9.5±6.6abcdeABCDEF	6.7±3.7cdefDEFGH	100	79
Kaolack	26 abcABCD	31 abcdeABCD	3.6±0.5 abcABC	4.2±0.4 abABC	9.4 (23 738±6 477) cdeEFGH	10.5 (42 307±23 419) bcdeBCDEFGH	4.7±1.3cdeEFGH	8.5±4.7bcdeBCDEFGH	50	100
Koloss F1	28 abcABCD	22 bcdeABCD	4±0 abcABC	3.8±0.4 abABC	9.8 (21 050±13 040) cdeEFGH	9.8 (23 151±9 504) cdefEFGH	4.2±2.6cdeEFGH	4.2±1.9cdefEFGH	44	55
Sugar Baby	35 abcABCD	18 cdeA	4.2±0.4 abcABC	3.6±0.9 abABC	10.5 (44 045±35 060.9) bcdeBCDEFGH	9.5 (16 366±10 066) defFGH	8.8±7bcdeBCDEFGH	3.3±2defFGH	92	39
Sugar Dragon	24 abcABCD	30 abcdeABC	3.4±0. abcaBC	3.2±0.4 abABC	9.4 (13 830±4 634) eFGH	9.3 (12 947±10 502) efGH	3.1±1.3efGH	2.6±2.efGH	29	31
Tomato	49 aA	48 aA	4.8±0.4 aA	4.8±0.4 aA	11.9(154 139±41 293) aAB	12 (175 679±54 953) aA	35.1±10.9aAB	30.8±8.3aA	-	-
P value	0.0001	0.0001	0.0001	0.002	0.0001	0.0001	0.0001	0.0001		
F ratio	3.32	4.23	3.36	2.50	8.44	10.24	7.61	10.38		
Interaction data: Cultivars x <i>Meloidogyne</i> spp.										
P value	0.0001		0.0001		0.0001			0.0001		
F ratio	3..73		2.94		8.51			8.57		

¹Pf = Final nematode population (total eggs and J2 per root system). ²Rf = Pf (final nematode population)/Pi (initial nematode population) (Windham & Williams, 1987). ³Number of egg laying females (according to Hussey & Boerma, 1981 where 0 = zero; 1 = 1 to 2; 2 = 3 to 10; 3 = 11 to 30; 4 = 31 to 100; 5 = >100 egg masses per root system). ⁴Log(x+1) transformed values; ⁵Real means ± standard error of real means (Tukey's HSD where P≤0.05). Lower case letters indicate differences in reproduction parameters among populations for each individual experiment, with means in each column followed by the same letter not differing significantly at P≤0.05; Upper case letters indicate differences in reproduction parameters among populations between the two experiments, with means in each line followed by the same letter not differing significantly at P≤0.05.

Table 5.2b Root mass data for six commercial watermelon cultivars following inoculation with *Meloidogyne enterolobii*, *M. incognita* and *M. javanica* in separate greenhouse experiments.

Cultivar	Root mass (g)	
	Initial experiment	Repeat experiment
<i>M. enterolobii</i> ^a		
Charleston Gray	9.6±0.4 ¹ bBCD	9.9±0.6 bBCD
Erato F1	10±0.3 bBCD	8.2±0.3 bcBCD
Kaolack	9.7±0.9 bBC	8.4±0.3 bcBCD
Koloss F1	9.9±0.1 bBCD	9.3±0.7 bcBCD
Sugar Baby	9.5±0.7 bBCD	7.5±0.4 cD
Sugar Dragon	8.4±1 bBCD	10.5±0.4 bBCD
Tomato (positive control)	18.7±2.9 aA	17±1.4 aA
<i>M. incognita</i> ^b		
Charleston Gray	9.3±0.7 bBCD	9.1±0.6 bcBCD
Erato F1	8±0.8 bBCD	8.1±0.6 bcBCD
Kaolack	8.5±0.4 bBC	7.2±0.7 cBCD
Koloss F1	10.1±0.5 bBCD	7.9±0.5 bcCD
Sugar Baby	9.4±1 bBCD	10.1±0.5 bBC
Sugar Dragon	9.2±0.7 bBCD	8.1±0.5 bcBCD
Tomato (positive control)	16.7±1.8 aA	19±1.6 aA
<i>M. javanica</i> ^c		
Charleston Gray	8.7±0.5 bBCD	9.7±0.6 bcBCD
Erato F1	9.6±0.5 bBCD	9.7±0.7 bcBCD
Kaolack	8.7±0.8 bBCD	9.7±0.4 bcBCD
Koloss F1	9.1±0.8 bBCD	8.7±1b cBCD
Sugar Baby	9.1±0.3 bBCD	9.8±0.2 bcBCD
Sugar Dragon	8.3±0.8 bBCD	8.6±0.7 bcBCD
Tomato (positive control)	20.8±2.4 aA	22±1.8 aA
<i>P value</i>	0.0001	0.0001
<i>F ratio</i>	13.8	19.7
<i>Interaction data: Treatments x Experiments</i>		
<i>P value</i>	0.001	
<i>F ratio</i>	6.18	

^a*Meloidogyne enterolobii*; ^b*Meloidogyne incognita*; ^c*Meloidogyne javanica*; Real means ± standard error of real means (Tukey's HSD where $P \leq 0.05$). Lower case letters indicate differences in reproduction parameters among populations for each individual experiment, with means in each column followed by the same letter not differing significantly at $P \leq 0.05$; Upper case letters indicate differences in reproduction parameters among populations between the two experiments, with means in each line followed by the same letter not differing significantly at $P \leq 0.05$.

5.4 Discussion

The findings of this study revealed relatively high reproduction potentials for 25 Nigerian *Meloidogyne* spp. populations and/or communities evaluated. These results are consistent with those by other authors that observed substantial variations among *Meloidogyne* spp. populations and/or communities (Agenbag, 2016; Djian-Caporalino *et al.*, 2011; Rashidifard *et al.*, 2019). Findings from our study also showed that the six commercially available watermelon cultivars screened for their host status to the predominant *Meloidogyne* spp., identified from south-west Nigerian production areas, were all susceptible. Such results also agree with those from other authors who reported that the watermelon cultivars screened in their studies all were susceptible to *Meloidogyne* spp. (Montalvo & Esnard, 1994; Thies & Levi, 2007). More specifically, as was reported for an earlier Nigerian study, the susceptibility of ‘Koloss F1’ to a Nigerian *M. incognita* population (Aminu-Taiwo *et al.*, 2015) also agreed with results of our study for this cultivar since it was susceptible not only for the *M. incognita*, but also the *M. enterolobii* and *M. javanica* populations used.

A particular interesting result from this current study is that five out of the seven most injurious (exhibiting the highest reproduction potentials) populations and/or communities had *M. javanica* as a common factor, with a mixed population (L15) of *M. javanica* and *M. enterolobii* being the most injurious. From Spain, Omat *et al.* (2001) identified a highly injurious population of *M. javanica* that rendered the *Mi* resistance gene ineffective in tomato plants indicating the highly injurious nature of certain populations of this species. Results from this study further agrees with findings of Nzeako *et al.* (2013) who identified *M. javanica* populations from southern Nigeria as being highly injurious on tomato. A similar phenomenon was reported by (Ogbuji, 1981) who identified five populations of *M. javanica* from south-eastern Nigeria as being highly injurious on pepper (*Capsicum* spp. L.) and tobacco (*Nicotiana tabacum* L.). This trend is also consistent with reports from South African studies where *M. javanica* populations were reported as being the most injurious on tomato in greenhouse and

microplot studies (Fourie *et al.*, 2012; Agenbag, 2016; Rashidifard *et al.*, 2019). Furthermore, studies from the USA reported *M. javanica* as the most injurious root-knot nematode species (compared to *M. arenaria* and *M. incognita*) causing damage to tobacco (Arens & Rich, 1981).

Concerning the reproduction potential of *M. enterolobii*, seven out of the 10 most injurious populations evaluated in our study had *M. enterolobii* as a common factor. Of these, four represented mixed communities with *M. javanica* (L2, L15, L24 and L25); two with *M. incognita* (L4 and L12); with only one containing a single species of *M. enterolobii* (L18). *Meloidogyne enterolobii* is known to be more destructive than its thermophilic counterparts since it display virulence, rendering resistant genes ineffective in different plant species (e.g. *Mi-1*, *N* and *Tabasco*) that confers resistance to species such as *M. arenaria*, *M. incognita* and *M. javanica* (Brito *et al.*, 2007; Kiewnick *et al.*, 2009). This virulence coupled with the wide distribution and host range contributed to the listing of *M. enterolobii* as an emerging threat and quarantine species (EPPO/PQR, 2017). Hence, the current study focused on identifying local *M. enterolobii* populations and more specifically their reproduction potentials since such information is crucial to designing appropriate management strategies to combat such a pest.

Another interesting outcome of this study is in terms of the number of egg masses produced per root system by the different *Meloidogyne* spp. populations and/or communities. While some populations produced high egg masses, they had low Rf values and *vice versa*. This scenario was, for example, evident for L15 which was the most injurious community, containing a mixture of *M. enterolobii* and *M. javanica*. This *Meloidogyne* spp. community produced fewer egg masses than some of the less injurious populations and/or communities which illustrates the different reproduction potentials exhibited by 25 populations and/or communities evaluated. This also implies that the egg masses produced in the case of this mixed *M. enterolobii* and *M. javanica* community contained more eggs per egg mass than those produced by some of the less injurious populations and/or communities which produced more egg masses but had significantly lower Rf values (e.g. L1, L2, L6, L8, L13, L18 and

L25). This phenomenon further accentuates that egg mass counts for *Meloidogyne* spp. are not necessarily an accurate indication of their reproductive potentials as has been previously demonstrated by several authors (Fourie *et al.*, 1999; Steyn *et al.*, 2014; Agenbag, 2016; Ntidi *et al.*, 2016;). Results from this study thus substantiate that information regarding the variable reproductive ability of different *Meloidogyne* spp. populations and/or communities that are geographically isolated from one another should be identified since it is crucial to enable the development of appropriate management strategies (Anwar *et al.*, 2000).

In terms of the host status study different interesting scenarios were evident concerning the reactions of watermelon cultivars to *Meloidogyne* spp. that are grown by Nigerian producers. For example, 'Sugar Dragon' and 'Koloss F1' had the lowest relative susceptibility values (%) and hence should be the preferred ones to be cultivated by producers to keep densities of particularly *M. incognita* ('Sugar Dragon') and *M. javanica* ('Koloss F1') at lower levels. It is foreseen that growing such cultivars population densities could be reduced by 58% for *M. enterolobii*; 68% for *M. incognita*; and 71% for *M. javanica* in fields where these species occur. However, although planting of these cultivars will keep densities of these three *Meloidogyne* spp. significantly lower compared to when growing any of the other cultivars tested, the ultimate aim should still be to develop watermelon cultivars resistant to the occurring *Meloidogyne* spp. to enable producers to grow the crop and other rotation crops sustainably. In the meantime, other management strategies (e.g. chemical control, use of poor- or resistant cultivars of rotation crops) could be combined with the use of the preferred cultivars 'Sugar Dragon' and Koloss F1' to enable producers to minimize *Meloidogyne* spp. infestation in their fields.

Another interesting scenario emanating from this study was that all six watermelon cultivars evaluated supported in most cases significantly lower populations of the three *Meloidogyne* spp. when compared to the susceptible tomato standard (cv. Tropimech) which had the highest, nematode reproductive parameters. This proved that although the watermelon

cultivars were susceptible, they were less susceptible when compared to the highly susceptible tomato standard used (an indication that the experiments were conducted successfully).

Additional, valuable information from this study is that lower values in terms of egg masses, number of eggs and J2 as well as Rf per root system were recorded for the six cultivars inoculated with *M. javanica* when compared to the other two *Meloidogyne* spp. This finding is in agreement with results of Cohen *et al.* (2014) and López-Gómez *et al.* (2016) that *M. javanica* is less injurious compared to *M. incognita* when it comes to causing damage on watermelon in particular. This further confirms the physiological variability that has been previously established among *Meloidogyne* spp. in terms of root penetration, gall indices and reproduction factor (Cohen *et al.*, 2014). Furthermore, some cultivars produced high egg mass numbers per root system but had low Rf and *vice versa* as is also reported earlier in this study for the different *Meloidogyne* spp. populations and/or communities evaluated for their reproduction potential experiments. This scenario was, for example, illustrated for cultivar Sugar Dragon that despite it having high egg mass numbers for all three *Meloidogyne* spp., substantially low Rf values were evident compared to most of the other cultivars. Furthermore, the higher values for nematode parameters recorded for the initial compared to the repeat experiment is suggested to be largely due to an increase of 3°C in the maximum mean temperature recorded. Temperature is known to play an important role in root-knot nematode reproduction (Roberts *et al.*, 1981; Giné *et al.*, 2014; Rashidifard *et al.*, 2019).

Another result that emanated from this study is that all six watermelon cultivars investigated generally supported higher populations of *M. enterolobii* compared to those of the other two species (*M. incognita* and *M. javanica*). This affirms previous reports that *M. enterolobii* is a highly injurious root-knot nematode species that possess the ability of causing damage to hitherto resistant cultivars (Kiewnick *et al.*, 2009; Kiewnick *et al.*, 2008; Karssen *et al.*, 2012; Castagnone-Sereno, 2012).

The importance of determining the reaction of watermelon cultivars to the three predominant *Meloidogyne* spp. in south-west Nigeria cannot be over-emphasised. To achieve optimal productivity for watermelon crops within the region, their susceptibility to major pests such as *Meloidogyne* spp. have to be determined. Most vegetable growers within SSA depend on chemical nematicides (Coyne *et al.*, 2018) which have both health and environmental consequences. Findings from this study provide an alternative in the sense that should watermelon farmers focus on planting the least susceptible cultivars identified instead of their highly susceptible counterparts, the build-up of population densities of the three predominant *Meloidogyne* spp. will be minimized. Could this trend be sustained over time, it will invariably cause a reduction in the amount and application intervals of nematicides and ultimately contribute to improve crop yields at no additional costs to the producers.

This study ultimately provides a first report on the variable reproduction potential of Nigerian. spp. populations and/or communities. Also, although the host response of tomato, pepper and other major crops to several root-knot nematode species occurring in Nigeria have been reported (Atungwu *et al.*, 2008; Ibiam *et al.*, 2014; Bello *et al.*, 2015; Adegbite, 2017), this study also provides a first report for watermelon cultivars grown in the country. Moreover, useful and valuable baseline information for *Meloidogyne*-watermelon associations have been generated not only for Nigeria, but for a wider sub-Saharan Africa context. Together with data generated on the reproduction potential of *Meloidogyne* spp. populations occurring in south-west Nigerian agricultural fields, it is recommended that the host status of wild watermelon accessions are evaluated to the predominant *Meloidogyne* spp. Should resistance against such species be present in wild accessions, breeders can together with nematologists introgress such resistance in watermelon hybrids that can perform optimally under Nigerian climatic conditions. This way growers as well as industries can exploit and develop appropriate integrated management strategies to diminish the effect of *Meloidogyne* spp. and ensure optimal yields of watermelon, and even other cucurbits and rotation crops.

5.5 References

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CHAPTER 6

CONCLUSIONS AND SUGGESTIONS FOR FUTURE RESEARCH

6.1 Aims and achievements

Plant-parasitic nematodes have been documented from various parts of the world as being economically important pests of watermelon (*Citrullus lanatus*) (Thunb) Matsum & Nakai (Maynard, 2001; Noling, 2015). *Meloidogyne* spp. is documented as the most prevalent, widespread and damaging nematode pests causing considerable yield losses on food crops within sub-Saharan Africa (SSA) (Coyne *et al.*, 2018). The ultimate motivation for this study is based on the dearth of information which exist about nematode pests associated with watermelon production within SSA. This is further substantiated by the fact that no previous study has focused on investigating the abundance and diversity of free-living nematode assemblages, and their implications on soil health within West African agroecological systems.

Therefore, the objectives of this study were to conduct a nematode study in south-western Nigerian cropping systems to i) record the abundance and diversity of free-living nematode assemblages obtained from watermelon fields, ii) identify new free-living nematode species found during the survey using selected morphological and molecular techniques, iii) record the abundance and diversity of plant-parasitic nematodes associated with the rhizospheres of watermelon fields with emphasis on *Meloidogyne* spp., and iv) determine the reproduction potential of single-species populations and mixed-species communities containing the three predominant *Meloidogyne* spp. identified, and v) assess the host status of six commercially available watermelon cultivars to single-species populations of the three predominant *Meloidogyne* spp. A summary of the achievements of these specific objectives are presented below for each chapter/article.

Findings of the first objective of this study as presented in Chapter 2: Article 1 (Bello *et al.*, 2019) listed 30 genera of free-living nematodes from watermelon fields in south-west Nigeria which is consistent with other similar studies. For example, concerning watermelon Porazinska and Coleman (1995) reported 17 free-living nematode genera from 21 families from cucurbit fields in Georgia. Furthermore, from other crop fields in Nigeria 43 free-living nematode genera were listed in association with the rhizospheres of 30 weeds species growing in mango, oil palm citrus, maize, cassava, and yam fields in north central region of Nigeria (Eche *et al.*, 2015). Bacterivores, mostly c-p2 category were found to be dominant in soils

from watermelon fields sampled during this study, agreeing with findings of previous studies. For example, Mary *et al.* (2013) also identified bacterivores, represented mainly by the family Rhabditidae, as the predominant trophic group present in soils of maize cropping systems in northern Nigeria. Also, Eche *et al.* (2015) identified bacterivores as the predominant trophic group in the rhizospheres of weed species sampled from the North Central agroecological zone of Nigeria. Regarding the faunal analysis, 52% of the watermelon fields sampled during this study plotted in Quadrat B, which refers to stable and enriched food webs in terms of terrestrial free-living nematode assemblages. Soils of such fields are maturing and characterised by low to moderate disturbance; such soils are also N-enriched with low C:N ratio's while their decomposition channels are balanced (Ferris *et al.*, 2001; Sieriebriennikov *et al.*, 2014). An important deduction from this suggests that watermelon farming seems to be fairly sustainable in the sampled fields located in the south-western part of Nigeria, hence enrichment could be reduced. Also, larger structure footprints recorded for the majority of the watermelon fields sampled during this study are indicative of a higher abundance of omnivores and predators, reflecting food web complexity and regulatory roles. Since significant associations were recorded between frequency of occurrence of free-living nematodes from the sampled watermelon fields and soil as well as environmental parameters, it was concluded that a more ecological interpretation of the distribution of these nematode genera, merged with potential driving factors (organic material, pH, rainfall, texture, temperature, previous crops), can help understand the biological state of soils in watermelon production areas. It can also provide some indication as to whether some agricultural practices might improve soil health in the region (*e.g.*, optimal level of organic fertilisation, liming, irrigation and crop rotation).

The second objective was directed towards identification of a new *Aporcelaimellus* sp. found in two localities (Ibarapa and Modakeke) during the survey of watermelon fields in south-west Nigeria. Identification was conducted using both morphological and molecular (D2-D3 28S-rDNA, 18r-DNA) characterisation. *Aporcelaimellus nigeriensis* sp. n. was distinguishable by its several distinct morphological features. Individuals of this species particularly have coarse ventral body pores and resembles *Aporcelaimus porosus* Álvarez-Ortega, Ahmad & Peña-Santiago, 2011, known to occur in West Africa too. However, *Aporcelaimellus nigeriensis* sp. n. distinctly differs from *A. porosus* due to its larger general body size, thicker cuticle with different layers (intermediate and inner layers with equal thickness vs inner layer much thicker than the others), wider lip region, longer odontostyle and much longer spicules. Regarding the molecular identification of the new species, one 769

bp long D2-D3 28S rDNA sequence and one 776 bp long partial 18S rDNA sequence (GenBank access codes MN685820 and MN505320, respectively) were obtained. Their analysis allowed the elucidation of evolutionary relationships of the new species with other species of this genus. These results agreed with those of previous studies (Álvarez-Ortega *et al.*, 2013a,b) concerning the non-monophyly of the family Aporcelaimidae Heyns, 1965 as sequences of the genera *Aporcella* Andrassy, 2002, *Metaporcelaimus* Lordello, 1965 and *Sectonema* Thorne, 1930 form part of respective, separate and highly supported clades.

The outcome of the third and fourth objectives of this study (Chapter 4: Article 3) showed that a total of 11 plant parasitic nematode species and 11 genera were identified with *Meloidogyne* spp. being predominant, followed by *Helicotylenchus dihystera* (Cobb, 1893) Sher, 1961, *Pratylenchus zae* Graham, 1951 and *Scutellonema bradys* (Steiner & Le Hew, 1933) Andrassy, 1958. However, the *H. dihystera* and *Pratylenchus* have earlier been associated with watermelon in other parts of the world (Abd-Elgawad *et al.*, 2007; Tan & Okten, 2011). *Helicotylenchus dihystera*, found in association with watermelon in this study is reported as being widespread in Nigeria but less often associated with any major damage on major crops. *Pratylenchus zae* also identified from watermelon fields sampled during this study, is considered an important pest of several major crops such as maize (*Zea mays* L.), cowpea (*Vigna unguiculata* (L) Walp), rice (*Oryza sativa* L.) within the Nigerian agroecological systems (Egunjobi, 1973; Babatola, 1984; Coyne *et al.*, 2012). The occurrence of *S. bradys* as the fourth predominant species on watermelon from this current study was unexpected but interesting since this species is considered the major pest of yam in West Africa (Bridge *et al.*, 2005; Baimey *et al.*, 2009) and especially in Nigeria where it has also been associated with potato (*Solanum tuberosum* L.) (Coyne *et al.*, 2011). Significant associations were observed between the frequency of occurrence of the predominant plant-parasitic nematode species and soil properties as well as rainfall. It was suggested that cognisance must be taken of the presence of these nematode pest species in watermelon fields in Nigeria. Moreover, their population densities should be monitored since planting of susceptible hosts can lead to build-up of damaging densities of such pests.

Meloidogyne spp. being the most prominent from the watermelon fields sampled was subjected to identification using a combination of perineal-pattern morphology and the molecular Sequence Characterised Amplified Region Polymerase Chain Reaction (SCAR-PCR) technique. *Meloidogyne* spp. communities, containing more than one species, were detected in 64% of the localities, while the remaining 36% was representative of single-

species populations. The morphological approach identified three species, viz. *Meloidogyne enterolobii* (Yang and Eisenback, 1983), *Meloidogyne incognita* (Kofoed and White, 1919) Chitwood, 1949, and *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949, with overlapping perineal-pattern parameters while the molecular technique identified four species, namely *Meloidogyne arenaria* (Neal 1889) Chitwood, 1949; *M. enterolobii*, *M. incognita* and *M. javanica*. In both cases however, *M. enterolobii* was found to be predominant (according to abundance and occurrence), followed by *M. incognita* and *M. javanica*. This agrees with a recent report by dos Santos *et al.* (2019) who reported *M. enterolobii* as the most widely distributed root-knot nematode species in Nigeria, followed by *M. incognita* and *M. javanica*. The occurrence of *M. incognita* and *M. javanica* from watermelon fields in our study further confirms the results by Pagan *et al.* (2015) and dos Santos *et al.* (2019), who also recorded these species from numerous crops in Nigeria and other SSA countries. The current widespread distribution of *M. enterolobii* in Nigeria emphasises the need for accurate identification of *Meloidogyne* spp., in order to effectively implement appropriate management strategies against such pests. *Meloidogyne arenaria* which was detected using SCAR-PCR technique from two watermelon fields during this study represents a first report for south-west Nigerian cropping systems since it has not been reported in roots/other below-ground parts of any crop within the zone. Furthermore, the inability to detect this species using perineal-pattern morphology further confirms that morphological identification should be used in combination with molecular techniques to ensure accurate characterization of *Meloidogyne* spp.

The fifth objective of this study (Chapter 5: Article 4) was achieved by conducting four separate greenhouse experiments. Two experiments, an initial followed by a repeat, were conducted to determine the reproduction potential of 25 *Meloidogyne* populations and communities in roots of a susceptible tomato (*Lycopersicon esculentum* Mill.) cultivar (Tropimech). The other two experiments aimed at determining the host status of six commercially available watermelon cultivars to the three *Meloidogyne* spp. identified as being predominant in south-west Nigerian crop fields (see Aim 2 above). The highest reproduction factor (Rf) was recorded for a mixed species community containing *M. enterolobii* and *M. javanica* (L 15), while that with the lowest Rf was evident for another mixed species community (L16) containing *M. arenaria* and *M. enterolobii*. An interesting finding of this study is that five out of the seven most injurious populations had *M. javanica* as a common factor. This affirms previous findings where a highly injurious population of *M. javanica*, that rendered the *Mi* resistance gene in tomato plants ineffective, was reported from Spain (Ornat

et al. 2001) indicating the aggressiveness of this species. This further agrees with findings of Nzeako *et al.* (2013) who identified a *M. javanica* populations from southern Nigeria as being highly injurious on tomato. A similar phenomenon was reported by (Ogbuji, 1981) who identified five populations of *M. javanica* from south-eastern Nigeria as being highly injurious on pepper. The results from this study further confirms the high injuriousness of *M. enterolobii* with seven out of the 10 most injurious communities used in this study having *M. enterolobii* as a common factor. Of these, four (L2, L15, L24 and L25) contained mixed species of *M. enterolobii* and *M. javanica*; two (L4 and L12) contained mixed species of *M. enterolobii* and *M. incognita*; and L18 contained a single species of *M. enterolobii*. *Meloidogyne enterolobii* which was present in 19 out of the 25 communities of this study is known to have a very extensive host range and is able to render existing resistance against *M. incognita* and other thermophilic species ineffective in various hitherto resistant cultivars of tomato, soybean (*Glycine max* L. Merrill) and sweet potato (*Ipomoea batatas* (L) Lam.) (Brito *et al.*, 2007; Blok *et al.*, 2002). Hence, it was suggested that continuous research should be done to identify more local *M. enterolobii* populations and communities and most specifically, to determine their reproduction potentials to enable researchers to find proper management strategies.

Concerning the host status of six commercially available watermelon cultivars (Erato F1, Charleston Gray, F1, Kaolack, Koloss F1, Sugar Baby and Sugar Dragon) that are grown in Nigeria to the three predominant *Meloidogyne* spp., findings of this study revealed that all of them are susceptible to single species populations of *M. incognita*, *M. enterolobii* and *M. javanica*. This is substantiated by $R_f > 1$ recorded for all six cultivars for both the initial and repeat trials. Furthermore, the current study established that cultivars Sugar Dragon and Koloss F1 were the least susceptible of the six cultivars evaluated, with the former supporting the least single-species populations of *M. incognita* and *M. javanica* while the latter supported the least populations of *M. enterolobii*. The significant variability that were established in the host responses of the cultivars to the single-species *Meloidogyne* populations will allow producers to choose the one which is the least susceptible. These for example include ‘Koloss F1’ for *M. enterolobii*, ‘Sugar Baby’ for *M. incognita* and ‘Sugar Dragon’ for *M. javanica*. It hence was suggested that growing these cultivars instead of the ones identified as being highly susceptible according to this study can reduce root-knot nematode densities by 58% for *M. enterolobii*; 68% for *M. incognita*; and 71% for *M. javanica*. However, the ultimate would be to investigate whether wild accessions of watermelon exhibit resistance to any of the three predominant *Meloidogyne* spp. identified. This will enable breeders to introgress such

resistance into cultivated watermelon germplasm and this way assist producers to reduce the root-knot nematode densities in their crop fields.

6.2 Suggestions for future research

Ultimately, this study has generated valuable baseline information for watermelon in Nigeria and in a wider context for SSA. Further studies should hence focus on the following:

- i) developing novel, environmentally-friendly and cost-effective nematode management strategies to control nematode pests that damage watermelon and other rotation crops in Nigeria, and
- ii) continuously update knowledge about both plant-parasitic and free-living nematode species that occur in Nigerian soils to enable pro-active actions in terms of their spread and management.

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APPENDIX A

Instructions for authors – European Journal of Plant Pathology (Springer)

Title page

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- The e-mail address, and telephone number(s) of the corresponding author
- If available, the 16-digit ORCID of the author(s)

Abstract

Please provide an abstract of 150 to 250 words. The abstract should not contain any undefined abbreviations or unspecified references.

Keywords

Please provide 4 to 6 keywords which can be used for indexing purposes.

Abbreviations

Abbreviations should be defined at first mention and used consistently thereafter.

Citation

Cite references in the text by name and year in parentheses. Some examples:

- Negotiation research spans many disciplines (Thompson 1990).
- This result was later contradicted by Becker and Seligman (1996).
- This effect has been widely studied (Abbott 1991; Barakat et al. 1995; Kelso and Smith 1998; Medvec et al. 1999).

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Reference list entries should be alphabetized by the last names of the first author of each work.

Journal article:

Harris, M., Karper, E., Stacks, G., Hoffman, D., DeNiro, R., Cruz, P., et al. (2001). Writing labs and the Hollywood connection. *Journal of Film Writing*, 44(3), 213–245.

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Slifka, M. K., & Whitton, J. L. (2000) Clinical implications of dysregulated cytokine production. *Journal of Molecular Medicine*, <https://doi.org/10.1007/s001090000086>

Book:

Calfee, R. C., & Valencia, R. R. (1991). *APA guide to preparing manuscripts for journal publication*. Washington, DC: American Psychological Association.

Book chapter:

O'Neil, J. M., & Egan, J. (1992). Men's and women's gender role journeys: Metaphor for healing, transition, and transformation. In B. R. Wainrib (Ed.), *Gender issues across the life cycle* (pp. 107–123). New York: Springer.

Online document:

Abou-Allaban, Y., Dell, M. L., Greenberg, W., Lomax, J., Peteet, J., Torres, M., & Cowell, V. (2006). Religious/spiritual commitments and psychiatric practice.

Resource document. American Psychiatric Association.

http://www.psych.org/edu/other_res/lib_archives/archives/200604.pdf. Accessed 25 June 2007.

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APPENDIX B

Nematology

International Journal of Fundamental and Applied Nematological Research

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Instructions for Authors

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Jones, J.T., Furlanetto, C. & Kikuchi, T. (2005). Horizontal gene transfer from bacteria and fungi as a driving force in the evolution of plant parasitism in nematodes. *Nematology* 7, 641-646. DOI: 10.1163/156854105775142919.

Give book titles in full, with place of publication, then name of publisher, *e.g.*:

Bird, A.F. & Bird, J. (1991). *The structure of nematodes*. New York & London, Academic Press.

Theses are cited in the same way as books.

For articles in books, give authors and article title, followed by In: editors' names, book title, place of publication then publisher, and page numbers of article; *e.g.*:

Decraemer, W. & Hunt, D.J. (2006). Structure and classification. In: Perry, R.N. & Moens, M. (Eds). *Plant nematology*. Wallingford, UK, CABI Publishing, pp. 3-32.

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Fwd: Submission Confirmation for Morphological and molecular characterisation of *Aporcelaimellus nigeriensis* sp. n. (Dorylaimida, Aporcelaimidae), a remarkable dorylaim from Nigeria

From: Milad Rashidifard (milad.rashidifard@nwu.ac.za)

To: tesleembello@yahoo.com

Date: Thursday, November 14, 2019, 08:24 AM GMT+1

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>>> "Nematology" <em@editorialmanager.com> 11/7/2019 10:15 PM >>>

Dear Dr Rashidifard,

Your submission entitled "Morphological and molecular characterisation of *Aporcelaimellus nigeriensis* sp. n. (Dorylaimida, Aporcelaimidae), a remarkable dorylaim from Nigeria" has been received by journal Nematology

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A manuscript number has been assigned to Abundance and diversity of plant-parasitic nematodes associated with watermelon in Nigeria, with focus on Meloidogyne spp.

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APPENDIX F

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APPENDIX G

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APPENDIX H

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