Characterization of wheat nematodes from cultivars in South Africa

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DEDICATION

To my grandmother Dlalisile Dlada, mother Nokuthula Bongi Dladla, aunt, Dumisile Dladla, and the rest of my family.

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To the almighty GOD for the strength, ability, knowledge, opportunity and perseverance you have given me from the beginning of this project till this day.

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ABSTRACT

Plant-parasitic nematodes (PPNs) naturally live in soil and attack small roots, but some species inhabit and feed in bulbs, buds, stems, leaves, or flowers. This leads to plant weakness and they often appear to suffer from drought, excessive soil moisture, sunburn or frost, as well as mineral deficiency or imbalance. According to the South African Plant-Parasitic Nematode Survey (SPPNS) and National Collection Nematodes (NCN) databases, 453 plant-feeding nematodes have been recorded in South Africa and species identified from wheat have not been included on the database as a result of not being georeferenced and the need to update information on NCN database. The aim of the current study is to characterize and document wheat nematodes that occur in South African wheat producing areas with an emphasis on morphological and molecular identification.

A total of 776 composite rhizosphere soil and root samples was collected from 56 localities over two seasons in 7 provinces; the Free State (FS), KwaZulu-Natal (KZN), Northern Cape (NC), Mpumalanga (MP), Limpopo (L), North West (NW) and Western Cape (WC) provinces. The majority of samples collected were in WC (452), FS (120) and NC (160), as these are the major producers of wheat in South Africa. Nematodes were extracted from soil and root samples by modified decanting and sieving baermann-funnel technique, followed by the adapted sugar centrifugal-floatation. Nematodes were extracted from kernels by means of soaking the samples in tap water for 24 hours and decanting the extract through a 20 µm sieve. Nematode species were identified basis on morphological features, while prominence values (PV), frequency of occurrences and abundances were calculated for each genus. Individuals from the following genera were identified from the following provinces: Free State: *Pratylenchus,* Spiral *(Rotylenchus, Scutellonema and Helicotylenchus), Criconema* and *Dolichodorus;* KwaZulu-Natal: *Melodogyne, Pratylenchus, Criconema, Helycotylenchus (*Spiral) and *Longidorus*; Northern Cape: *Pratylenchus, Criconema* and Spiral; Western Cape: *Pratylenchus, Rotylenchus, Scutellonema, Helicotylenchus, Coslenchus, Tylenchus and Xiphinema*. The predominant genera identified across the localities according to PV-value were *Pratylenchus* (PV=575), Spiral (PV=309)*, Tylenchus* (PV=348*), Criconema (*PV=377*), Aphelenchus* (PV=131) and *Xiphinema (*PV=32*)*. Cedara in KwaZulu-Natal had a high number of *Meloidogyne* (420 second-stage juveniles /200 g soil) compared to the other sampled localities. Western Cape (Malmesbury) showed a high number of *Pratylenchus* sp. (8750/200 g soil). In root samples, the PV of *Meloidogyne* in Cedara was 183 followed by De Vlei (PV=943), Tygerhoek (PV=490), Kopporfontein (PV=134) and Wellington (PV=57) for *Pratylenchus* sp*.* No plant-parasitic nematodes were found in kernel samples. However, a bacterivore species of *Panagrolaimus* was identified from wheat kernels from Clarens in the Free State, is first record both in South Africa and worldwide.

DNA was extracted from 320 nematodes using chilex method. PCR was used for amplification of 18S rRNA, ITS1, D2-D3 and CO1 genes and positive PCR products were sequenced. This study analysed 20 sequences of the D2–D3 expansion segments of the 28S rRNA gene, 80 sequences of the 18S rRNA and 91 sequences of ITS1 gene. Gene sequences of nematodes obtained from this study matched with their related species when subject to BLASTn on NCBI data. Phylogenetic trees constructed with 18S rRNA and ITS1 rDNA genes have shown that nematodes detected in this study including *Helicotylenchus dihystera, Amplimerlinius paraglobigerus, Bitylenchus maximus, Merlinius joctus, Paralongidorus bikanerensis, Hoplolaimus galeatus* and *Rotylenchus unisexus* each formed clades with strains of the same species obtained from the GenBank using the 18S rRNA gene. The *H, dihystera, H. pseudorobustus, R. brevicaudatus, B. ventrosignatus* and *Mesocriconema sphaerocephalum* also clustered with the strains of the same species obtained in the GenBank when using the ITS1 gene clusters with their related species from other countries.

Keywords: Nematodes, Wheat, morphology, molecular, PCR, South Africa

RESEARCH OUTPUTS

Lamula S.Q.N. Thekisoe O.M.M., Fourie H., Tsilo T.J. 2017. Nematodes Associated with nematodes associated with wheat crops in South Africa: A revision and the way forward. The 21^{st} symposium of the nematological society of Southern Africa: Fairmont Zimbali resort, Balito Kwazulu Natal 14th – 18th May 2017. (Poster)

Lamula S.Q.N. Thekisoe O.M.M., Fourie H., Tsilo T.J. 2018. Plant-parasitic nematodes associated with wheat crops in South Africa, UESM prestigious PhD conference, 30 August, 2018, Potchefstroom, South Africa. (Oral)

Lamula S.Q.N., Thekisoe O.M.M., Tsilo T.J. 2019. Characterization of nematodes associated with rhizosphere soil and roots of South African wheat cultivars. 3^{rd} new voices symposium. The grain building, Pretoria, 11 September 2019. (Oral)

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CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Plant-parasitic nematodes (PPNs) are mainly classified as active, slender, unsegmented roundworms (also referred to as nemas or eelworms) (Siddiqi, 2000; Ayanda et al. 2010; Hoorman, 2011). The great majority of these nematodes are microscopic and cannot be seen with the naked eye, because they are very small and translucent (Siddiqi, 2000). Full body size of a matured adult forms, fall within the range of 0.25 to 2 millimeters in length (Siddiqi, 2000; Bohlmann, 2015). It has been estimated that more than 1,200 species cause disease in plants. Furthermore, assumed that it is possible that each and every plant that exists, at least one species of nematode render it as a host and feed upon it for nutrients (Siddiqi, 2000; Bohlmann, 2015). Nematodes naturally live in soil and PPNs attach or feed on small roots, but some species (obligate or facultative), inhabit or feed-in bulbs, buds, stems, leaves and flowers (Sturhan and Brzeski, 1991; McPartland et al. 2000; Mai, 2018). They obtain food by sucking juices from plants. This lowers or interferes with the natural resistance of the plant, consequently reduces vigor and ultimately the yield of plants. This further allows easy entrance for wilt-producing and root rot-producing fungi and bacteria and other nematodes. These leads to the plants being weak and often appear to suffer from drought, excessive soil moisture, sunburn or frost, mineral deficiency or imbalance, insect injury to roots or stems, or disease (Manzanilla-López and Hunt, 2009; Lebot, 2009; McPartland et al. 2000; Siddiqi, 2000; Bohlmann, 2015).

Jordaan et al. 1992 recovered and morphologically identified nineteen plant-parasitic nematode species from 175 wheat fields in the seven major wheat-production areas of the republic of South Africa. Similar surveys of morphological identifications of PPNs that are found in South African wheat fields have been reported by Marais and Swart, (2003) and Fourie et al. (2017). Previous molecular studies of soil and plant nematodes have tended to work on DNA from either selected individual nematodes (Floyd et al. 2002; Jones et al. 2006), from a collection of extracted nematodes (Foucher et al. 2004; Hu¨ bschen et al. 2004) or extracted DNA from soil (Waite et al. 2003). Subbotin et al. (2008) used molecular techniques, 18S and D2–D3 expansion segments of 28S ribosomal RNA genes and morphological characters and phylogenetic framework to identify wheat nematodes, for example, *Pratylenchus* sp.

A significant and pertinent global challenge in the coming years will be to balance the supply and demand for food security and to sufficiently keep up with the exponential increase in human population. The sustainably increase in agricultural productivity that is in line with the increasing demand for food will be more pertinent in resource poor areas of the world, especially Africa, where there is a rapid exponential populations increase (Bohlmann, 2015; Janion-Scheepers et al. 2016). Resource use efficacy is consequently necessary to meet the demand and significant improvements of resource allocations, especial to poor or developing countries. To archive desired results in terms of crop yields globally, best control management of pest and disease will be essential, especially as the production of some commodities varies and steadily shifts (Ponge et al. 2013; Smith et al. 2015; Donatelli et al. 2017; Murrell, 2017).

Loses of cereals as a result of plant-parasitic nematodes (PPNs) are estimated up to 6.9 to 50% ($US 125 billion) worldwide and 3.4 million in profits are lost each year only in the U.S (Bernard et al. 2017). Furthermore, Okubara et al. (2019) estimates that, about $101 million yearly for wheat in Washington State. As soil pests, nematodes are either at the bottom or nowhere near the list of priorities of nematodes that are diagnosed with precision and if it happens, they are often misdiagnosed, particularly where proper expertise and knowledge are lacking and knowledge transfer systems are inadequately funded (Talwana et al. 2008). Notably, a survey of PPNs associated with wheat, listed according to different wheat producing areas to our knowledge has never been published. According to the South African Plant Parasitic Nematode (SPPNS) and National Collection Nematodes (NCN) databases, 453 plant-feeding nematodes have been recorded in South Africa and species identified for wheat have not been included to the database because they have not being georeferenced and the information available are outdated (Marais & Swart, 2003, 2014, 2015). Due to wheat or seeds importation, movements of goods (crops) and any other means of potential nematodes carry transportations from one geographical area to another, might results in emerging of new species being introduced. Monitoring of plant parasitic nematodes from the agricultural fields is very crucial, especial for control strategies. Therefore, there is a need to characterised and document the current status of wheat nematode occurrences in South Africa, distinguish between the rare and the common ones, the establishment of distribution maps, gathering of accurate wheat damage and yield loss data. Furthermore, we need to introduce accurate and efficient modern tools for definitive diagnosis of wheat nematodes in South Africa.

1.2 Problem statement

Plant-parasitic nematodes are described as the most widespread pests, most insidious and costly (Liebhold et al. 2012; Donatelli et al. 2017), but the data on their economic impact remain insufficient or limited, especially where crops are grown in resources scarce areas. It is estimated that nematodes losses to crop production accounted for 14.6% in tropical and sub-tropical climates compared with 8.8% in developed countries. Worthy to note is that only ~0.2% is allocated to fund nematological research to address the crop value lost due to nematodes (Siddiqui and Alam, 1997). One difficulty with assessing nematode impact is that damage resulting from nematode infection is often less obvious than that caused by many other pests or diseases (Rivoal and Cook, 1993).

Grain farming in Africa, especially in sub-Saharan Africa is compounded with combined effects of abiotic and biotic stresses. These include drought and inferior crop management technology, fungal diseases and pests. Among these, the cereal cyst nematodes (CCNs) (*Heterodera* spp.), root-knot nematodes (RKN) (*Meloidogyne* spp.) and root-lesion nematodes (RLN) (*Pratylenchus* spp.) are the main limiting factor for improved grain production and are considered as economically important for wheat worldwide (Rivoal and Cook 1993). Losses of cereals as a result of plant-parasitic nematodes (PPNs) are estimated up to 6.9 to 50% ($US 125 billion to $US157 billion per year) worldwide (Rivoal and Cook 1993; Coyne et al. 2018). According to Coyne et al. (2018), there are as yet no reliable estimates of wheat losses due to PPNS in sub-Saharan Africa. Invariably this supports the one of the objectives of this study. The behaviour and damage caused by these PPNs and their control strategies have received little attention in many African countries, mainly sub-Saharan African, in spite of indications by other countries that the productivity of grains can be severely reduced. Consequently, in future nematodes will continue to emerge as new or more aggressive pests of crops as farming practices adapt to fashion, as climate change occurs and cropping systems intensify in response to increasing global demand for food. To successfully formulate effective control strategies and monitoring programmes, it is crucial to accurately diagnose or identify the nematodes species recovered from the agricultural fields. Therefore, this study seeks to characterise (morphologically and molecularly) and to document the current occurrence of nematodes in wheat cultivars in South Africa.

1.3 Research aim

To characterise and document wheat nematodes in South Africa using morphological and molecular diagnostic assays

1.4 Objectives

-To identify wheat nematodes from cultivars in South Africa using morphological techniques

-To characterize wheat nematodes from cultivars in South Africa using molecular methods

1.5 Hypothesis

There is a high diversity of nematodes associated with wheat crops cultivated in South Africa.

1.6 Thesis outline

Chapter 1: Introduction:

Background on nematodes globally, statement of the problem, aim, objectives and hypothesis.

Chapter 2: Literature review:

Review the classification of nematodes, their morphology and anatomy, life cycle, host plants and the mechanism of feeding and parasitizing of the host plants. The damage it causes to the plants and the economic importance of their impacts. Furthermore, this chapter highlights different diseases and the effect of the association of nematodes and other pests.

Chapter 3: Materials and methods:

Gives a detailed description of the study approach including, description of the study areas, materials used and methodology, and data analyses.

Chapter 4: Results:

Representation of the data obtained in this study using a combination of text, tables and figures.

Chapter 5: Discussion, conclusion and recommendations:

Interpretation of data with conclusion and recommendations of further action and studies that need to be undertaken with reference to data obtained from this study.

CHAPTER TWO

LITERATURE REVIEW

2.1 Wheat around the globe

Wheat (*Triticum aestivum*) is considered to be the main staple crop in most countries, especially, in the Sub-Saharan Africa. (Knox et al. 2012). According to FAO (2013), the average wheat yield in the world has been estimated to be approximately 3.3 t/ha. It has been reported that countries with highest average yield in the world were Ireland, Belgium, the Netherlands, Germany, the United Kingdom, Denmark, France, and Namibia, with 8.9, 8.9, 8.7, 7.9, 7.4, 7.3, 7.3, and 7.0 t/ha, respectively (FAO, 2013; Jabran et al. 2017). In addition to being a main source of food in most countries, it is an important grain crop and a prime component of human diet around the world, contributing significant amounts of nutritional levels of starch (about 60–70%), protein (about 10–18%) and fat (about 10–17%) to the human body (Singh et al. 2017). In developing countries, it is often used to make wheat-based food, such as pasta, bread and other products which are commonly known and mostly consumed (Świeca et al. 2017). Furthermore, these products also contain some pro-health components such as phenolics, phytic acid, and dietary fiber. On the other hand, the production of wheat per capita is slightly declining and wheat yield remains very low (Dababat et al. 2015; Husenov et al. 2015; Muminjanov, 2015).

2.2 Wheat production in African continent and South Africa

In countries such as Egypt and Algeria, trends have shown an increase in research of cereals, such as wheat, rye and oat, wheat being the most important of these crops (Ahmadi et al. 2015; Maafi et al. 2009; Mokrini et al. 2015; Haddadi and Mokabli, 2015). According to Haddadi and Mokabli, (2015), national production reached 49.12 million quintals in 2013, representing a yield of 18.11 t/ha. The report shows there is little improvement in wheat production and this contributed to importation from other countries. Furthermore, grain farming in Algeria encountered a similar situation associated with abiotic and biotic stress caused by drought, pest infestation and poor management practices. According to FAO (2010), statistics has shown that is wheat is cultivated in 128 countries and it is the second most important food crop in the developing world after rice. In sub-Saharan Africa, 13 countries (Algeria, Nigeria, Egypt, South Africa, Eritrea, Sudan, Ethiopia, Tanzania, Kenya, Tunisia, Libya, Zimbabwe and Morocco) produce wheat. Furthermore, FAO, estimates that South Africa and Ethiopia produce larger quantities compared to other African countries and despite that fact that yield from wheat production is lesser when compared to other crops (FAO, 2010).

According to FAO (2015), it is estimated that the human population will reach or exceed 2 billion by 2050 in Africa. This means that more that 50% of food will have to be produced for the next 50 years in order to meet the nutritional requirements of its growing population. According to the report by Grain, (2015), Southern Africa is no different from other African countries in terms of food production. Wheat (*T. aestivum*) ranks second after maize in terms of the area used for planting and its production. It is cultivated in an area that ranges from 417 500 to 757 700 ha on the total average area of 533 000 ha during production seasons of 2004 to 2015, which produces an average annual production of 1.3 to 2 million tons (Grain, 2015). In addition, major production areas or provinces, according to the yield per hectare of wheat in South Africa were reported to be; Free State, Western Cape, Northern Cape, North West, Mpumalanga, Limpopo and Eastern Cape. Furthermore, small quantities of wheat production have been reported in KwaZulu-Natal and Gauteng provinces over the years (Grain, 2015). However, the overall wheat production in sub-Saharan Africa, including South Africa is currently static or probably declining (FAO, 2015).

2.3 Current productivity and economic status of wheat in South Africa

According to the statistics by Nicol and Rivoal, (2008), the demand for wheat is expected to surpass the supply of global production by 2.5 times in the next 30 years, especially in the developing countries, where the population increase is expected to reach about 84%. Additionally, in recent years, these trends of the growing deficit and the high demand have been growing steadily contributing to a large amount of crops and food aid being imported from other countries. However, Nicol and Rivoal, (2008), believe that many sub-Saharan African countries, especially Eastern and Central Africa, are biophysically suitable for wheat production. The deficit in wheat production was reported by Agriculture South Africa (Agric.SA 2015; Thierfelder et al. 2015) which showed that the expected commercial production of wheat to be 1 457 million tons, and which also registered a decrease of 2.94% from the previous forecast. To support previous forecast, during the 2015/16 production season, most of the country's wheat crop was produced in the Western Cape (48%), followed by the Northern Cape (18%) and Free State (13%) provinces (Agric. SA, 2015). It further showed that the overall wheat production decreased in South Africa during 2014/15 planting season, with a decrease in production of wheat in Free State resulting in overall drop of 50%. Furthermore, the department of Agriculture, Forestry and Fisheries (RSA, 2015), reported t a decrease in wheat production, from various areas or provinces which significantly contribute to the national cultivation of wheat. Several tons produced during the 2015 season were from Western Cape (Swartland and Ruens) (697 000), Northern Cape (262 800), Free State about (224 000), Northwest (91 500), Mpumalanga (20 300), Limpopo (151 200), KwaZulu-Natal (41 610), Gauteng (1 500) and Eastern Cape about (14 880) (RSA, 2015). According to RSA (2015), South Africa is importing about 300 000 tons per annum from other countries to meet production deficiencies.

2.4 Limiting factors of wheat production

In addition to environmental stress conditions (drought, fluctuating temperatures, soil nutrients and etc.) that cause constraints in crop yields, the emergence of a serious infestation of quarantine and transboundary pests and diseases severely damage crop yields. Each year the farmers observe damage caused by nematodes, rusts, locusts, American whitefly, gypsy moth and other dangerous pests and diseases (Oerke, 1999; Mujeeb-Kazi et al. 2013; Smith et al. 2015; Dettori et al. 2017). Nematodes are now considered as one of the most important groups of plant-parasitic pests on cereals worldwide, with an estimate of 80 to 100 billion $ lost every year, as a consequence of parasitism (Nicol et al. 2011; Bohlmann, 2015; Escobar et al. 2015). In recent years, researchers have documented organizations such as International Maize and Wheat Improvement Centre (CIMMYT), have dedicated more resources in developing new wheat varieties that are well adapted to African environments and this has given a rise to the introductions of these varieties which are considered resistant to diseases. Harvest Choice is another organization working with CIMMYT to assess wheat production potential in smallholder farming systems and their economic profitability in sub-Saharan Africa (FAO, 2010). To compensate for the additional demand for wheat, new methods must be employed to minimize yield production constraints.

2.5 Diseases and pest associated with wheat

Pests and diseases survey have been conducted for decades since 1970s in United Kingdom (UK) till now and continued to be carried out in most American, European and Asian countries, including India, Indonesia, Malaysia and the Philippines (Donatelli et al. 2017). According to Trematerra and Throne (2012), the prevalence and severity of pests depend on the genotype of the host, climatic conditions and many other factors". Furthermore, the ever changing status of global and climate changes resulting to natural disturbances or disasters have already caused severe co-epidemic of pests and diseases in winter wheat, such as aphids, *fusarium*, yellow rust, and powdery mildew. As a consequence these threats, cultivation may result in serious deterioration of grain yield and quality (Shi et al. 2017; Solà et al. 2018). According to Yuan et al. (2017), "Dramatic changes in temperature, precipitation, humidity and other climatic factors due to climate change have increased the potential occurrence and severity of crop diseases and pests, thereby threatening crop production". Furthermore, the traditional way of crop and pest detections and discriminations on agricultural fields involve manual scouting, but these are expensive and time-consuming (Shi et al. 2017).

There are two main groups of pests and insects, which feed on various plant hosts (Oerke, 1999; Pener and Dhadialla, 2012; Trematerra and Throne, 2012; Colloff et al. 2013; Murrell, 2017). These two groups include general pests, which feed on several plant hosts. The other group are special pests, which feed on either a single host or a few hosts. Some plant pests are believed to be specialized insects affecting mainly specific crops such as wheat and barley in the Middle East especially in Iran (Saadatia, 2015). Furthermore, in Australia, the loss of grains due to disease and pests are estimated to be $77 (AUD) per hectare annually, representing 19.5% of the average annual value of the crop production over the last decade (Hatfield et al. 2018). In addition, the biological pest control in the United States (US) was estimated with an annual value of 4.5 billion US dollars (Pimentel and Burgess, 2014). Adding to environmental stress conditions, pests and diseases that severely damage crop yields and PPNs have been considered as the new emerging serious infestation of quarantine and transboundary pest that causes significant constrain in crop yields around the world (Gardner et al. 2009; Bohlmann, 2015; Escobar et al. 2015). However, the overall information on the detection and survey of disease and pest monitoring on the large-scale is still insufficient (Donatelli et al. 2017). Therefore, more efforts are geared towards reducing challenges associated with PPNs in most countries including South Africa.

2.6 Plant-parasitic nematodes (PPNs)

The occurrence and economic importance of the cereal nematodes (CNs) has been documented by many countries over the past two decades and this has increased the awareness worldwide on the impact that they have caused on small grain cereals (Brown, 1985; McSorley and Duncan, 1995; Oerke, 1999; Cui et al. 2015). Plant-parasitic nematodes often become a grain yield production limiting factor, if agricultural practices employed favours their population`s build up or for easy reproduction (Bohlmann, 2015; Lins et al. 2015; Murrell, 2017). Nematodes are cosmopolitan parasites of plants and they can also act as facilitators for fungi and bacterial penetration or infections. They exhibit negative impact on the quality of the grain yields (Trematerra and Throne, 2012; Fernandez et al. 2015). Nematodes are now considered as one of the most important groups of plant-parasitic soil-borne pests of cereals worldwide (Grillo et al. 2016). In addition to the damage on agricultural crop production by environmental stresses, these are further compounded by the damage caused by biotic stresses including attack by bacteria, fungi, nematodes, or viruses, each of which may cause a serious economic loss for the farming industry worldwide (Bird et al. 2003: Kirby et al. 2014; Grillo et al. 2016).

The symptoms or injuries caused by nematodes are not immediately visible for most crops, this includes stunting, loss of green colour and yellowing; dieback of twigs and shoots; slow general decline; wilting on hot, bright days; and lack of response to water and fertilizer (Melakeberhan and Webster, 1993; Stirling and Stanton, 1997; Holgado et al. 2009). Furthermore, feeder root systems are reduced and they may be stubby or excessively branched, often discoloured, and decayed (Mai and Abawi, 1987; Hillocks and Wydra, 2002). Winterkill of orchard trees, raspberries, strawberries, ornamentals, and other perennials is commonly associated with nematode infestations (Bridge, 1975; Jelliffe and Stanfield, 1978; El-Borai and Duncan, 2005). Root injury develops partly as a result of the PPNs feeding on root cells and partially from toxic salivary excretions of the parasite (Schmitt and Sipes, 1998). In response to infections, tissues often become either enlarged or cells degenerate; sometimes both occur (Schmitt and Sipes, 1998).

Many nematodes, including PPNs are native and attack cultivated plants when their natural hosts are removed (Duncan and Cohn, 1990; Schmitt and Sipes, 1998). Further introduction through the seedling plants, bulbs, tubers and particularly in soil balled around roots of infested nursery stock (Ruehle, 1967; Hominick et al. 1996; Nicol and Rivoal, 2008; Nicol et al. 2011).

In South Africa, the status of PPNs as a limiting factor of wheat production has only recently been the subject of detailed study. The recent dramatic decrease in small grain crops, especially wheat in South Africa, has become a concern to wheat farmers. As much progress is being made on the research of other PPNs, such as root-lesion (*Pratylenchus* species) and root-knot (*Meloidogyne* species) nematodes (Rivoal and Cook, 1993), there is still a big information gap existing on the *Heterodera* (cyst species) genus or and other nematodes of wheat in South Africa.

2.7 Classification, morphology, biology and feeding habits of plant nematodes

2.7.1 Classification, morphology and biology

Nematodes belong to the animal kingdom Animalia; phylum Nematoda that includes plants, animals, humans and other free-living species (Mitreva et al. 2007). Two known classes are Adenophora and Secernentea. Plant-parasitic nematodes are highly specialised soil-borne (mostly wormlike organisms), pests which have the ability to feed on each and every part (roots, stems, leaves, flowers and seed) of the plant (Bohlmann, 2015). These nematodes are classified in terms of their mode of feeding on plants in terms of their stylet size, length and shape and those are the key parasitic features or characteristics adapted by nematodes for feeding on the different parts of the plants, hence classification or identification can be made by their mode of feeding (Bird et al. 2003). A clear example can be seen between the *Belonolaimus* species and *Longidorus* species, which are ectoparasites but feed deep within the roots using their long stylets, while *Helicotylenchus* species feeds on the exterior of the root or partially burrows into the root using its short stout stylet for feeding (Sebastiano et al. 2017). Using their mode of feeding, seven plant-parasitic nematodes can be classically grouped as follows;

2.7.2 Feeding habits of plant parasitic nematodes

2.7.2.1 Ectoparasites

These nematodes remain outside of the plant and use their stylet to feed on the cells of the plant. They may pose extremely long stylet that allows them to feed deep within the plant root on nutrient-rich plant cells while remaining outside the plant. This style of feeding makes it easier for them to move around and switch plants for feeding from time to time (Taylor, 1971; 1972).

2.7.2.2 Semi-endoparasites

At a certain point in their life cycle, these nematodes are able to partially penetrate the plant roots for feeding. They usually manage to penetrate the roots using the insect head to establish feeding site. Once the head is inserted, the nematodes enter to an endoparasitic phase; establish a feeding site, which later becomes permanent. The nematode’s head permanently remains inserted within the roots to induce the feeding site which can be beneficial to the nematodes but increases the risk dying when the plant dies as a result of immobility. e.g *Rotylenchulus reniformis* (Bert et al. 2008).

2.7.2.3 Migratory endoparasites

These groups are considered disruptive nematodes associated with massive plant tissue necrosis because of their migration and feeding stile. They have a significant negative impact because they don’t form any permanent feeding site, but they simply suck out the plant cell cytoplasm using their stylet, killing the plant cell and moving ahead of the lesion. Examples of migratory endoparasitic nematodes are *Pratylenchus* (lesion nematode), *Radopholus similis* (burrowing nematodes) and *Hirschmanniella oryzae* (rice root nematode) (Haegeman et al. 2012).

2.7.2.4 Sedentary endoparasites

These nematodes are considered as the most damaging plant-parasitic soil-borne pathogens in the world. The two mainly recognised and documented nematodes are (*Heterodera* and *Globodera*) and the root-knot nematodes (*Meloidogyne*). At a certain stage, these nematodes invade the plant near the tip of a root and migrate through the tissue to the developing vascular cells. They are completely embedded in the root during their initial stages of development, but later the cyst nematodes protrude from the roots. These nematodes then inject secretions into and around the plant cells to stimulate the formation of large feeder cell(s), which they non-destructively feed on throughout their life cycle, but interfere with the development of the host plant in terms of water and nutrients uptake from the soil. Both types of nematodes have the same basic feeding strategy (Amir and Sinclair, 1996: Brown, 1985: Akar et al. 2009: Al-Hazmi and Dawabah, 2014; Bohlmann, 2015).

2.7.2.5 Stem and bulb nematodes

As their name suggests, these are nematodes that attack the upper and lower parts of plants. Some researchers have suggested that water induce damage of plants by plant-parasitic nematodes (Kirby et al. 2014). This statement might be correct in the case of stem and bulb nematodes since their migration and reaching the host depends on the soil (dry or wet) condition (Kirby et al., 2014). Under wet conditions, these nematodes often enter emerging plant tissues below ground but can crawl up stems in a film of water and enter shoots via buds, petioles, or stomata. In the host plant, these feed as migratory endoparasites, molt into adults and reproduce, extensively macerating and distorting the plant tissue until the host dies (Taylor, 1971).

2.7.2.6 Foliar nematodes

These nematodes migrate from the stems to the leaves of their host plants in the presence of water. They penetrate the leaves through natural openings (stomata), disruptively feed, molt and lay eggs within the leaves. In the presence of water or under wet environmental conditions, these nematodes can move from leaf to leaf extending the damage to the whole plan and ultimately killing it. These nematodes are associated with interveinal chlorosis and necrosis of the leaf. Foliar nematodes are able to withstand harsh weather conditions of winter until the favourable spring conditions arise (Taylor, 1971).

2.8 Diagnostic measures

2.8.1 Morphological identifications

The long-standing method used for identifying PPNs has rely on the recognition of morphological characteristics to discriminate between the species (Mirmajlessi et al. 2015). This methodology requires one to be experienced in order to accurately identify these organisms to the genus and to the species level (Mirmajlessi et al. 2015). In addition, often requires the nematode to be at a certain life stages. Furthermore, classical extensive taxonomical knowledge is required and one drawback is that, it is a time consuming (Mirmajlessi et al. 2015). Morphological diagnostics are primarily based on morphological characters and morphometric features of nematodes such as the body length, stylet length, tail length and length of the hyaline part of the tail as well as in case of cysts, fenestral length, semifenestral width, vulval bridge width, and vulval slit length are measured (Cui et al. 2017). Morphological studies have been a base or focus of identifying species for centuries, but the emerging of new or similar species have made it difficult or challenging to rely on morphological studies (Cantalapiedra-Navarrete et al. 2013). Handoo, (2002) published character keys for morphological identification of some *H. avenae* species and the study addressed the small differences in morphological and morphometrical characters of this complex group. According to Handoo (2002), with increasing number of species in this group, reliable identification based on morphology is becoming more difficult. Further, states that studies using molecular and biochemical techniques have revealed the presence of additional species. For example, the *H. avenae* complex which was not derived using the morphological study. However, morphological identifications are still used and said to be reliable for most species and also serve as pre-requisites for molecular studies (Subbotin et al. 2008; Kumari and Subbotin, 2012; Kumari, 2017). Molecular phylogenies are evaluated for implications on evolution of morphological characters (Alvani et al. 2015).

2.8.2 Molecular identification

(a) Polymerase Chain Reaction (PCR) assays

The recent advances in the developments and use of molecular-based techniques for diagnosing PPNs have been extensively documented, the most worthy to note being those using Polymerase Chain Reaction (PCR) (Al-Banna et al. 2004). This methodology gives an ideal understanding of complex genetic traces between nematode genera and species (Mirmajlessi et al. 2015). It is a rapid and sensitive technique which has shown much improvement over the traditional diagnosis methods. Worthy of note, is that micro-organisms do not need to be cultured; it can detect a single target molecule in a complex mixture and also considerably reduce the time compared to other traditional diagnostic methods. However, more work is required for the identification of the PCR products when southern blot or sequencing are needed (Mackay, 2004). The method is primarily based either on the size of the specific PCR product or on presence or absence of PCR products influenced by specific primers involved. However, a major challenging part arising from the PCR-base method on PPNs is that samples consisting of several genera from a single original sample, the PCR is unable to accurately discriminate individual genera (Singh et al. 2014; 2017).

(b) Real-time Polymerase Chain Reaction (qPCR) assays

The development of the conventional qPCR-base method is able to address the short comings of the conventional PCR and improve the challenge of multiplex approaches (Seesao et al. 2017). It is has been concluded by most researchers that the challenge of quantification of nematode DNA a promising technique to overcome this problem is the quantitative qPCR (Mackay, 2004; Fillaux et al. 2008). It is a fast, highly sensitive and specific method that allows accurate detection and or quantification of pathogens that cannot be extracted or cultured easily from host tissue or are presented at low inoculum load in samples (Mirmajlessi et al. 2015). It can discriminate between closely related organisms and is, therefore, a versatile method for the accurate, reliable, and high throughput quantification of target DNA in various biological fields such as botany and genetics (Mirmajlessi et al. 2015). This method (Real-time PCR), has become widely used in many research applications such as the quantitative analyses of mRNA expression and single nucleotide polymorphisms (Mori et al. 2011). In addition, an important technique for routine detection and/or quantification of plant pathogens including viruses, bacteria, fungi and other viruses (Mackay, 2004; Mason et al. 2008; Singh et al. 2016; Seesao et al. 2017).

2.9 Nematode control strategies

In order to successfully maintain the population densities of these species of nematodes below damaging levels, appropriate management measures are necessary, such as rotational schemes and the use of resistant varieties (Timsina and Connor, 2001). The good news is that the damage caused by PPNs, mainly, cereal nematodes, in addition to population density, depends on several factors (Fosu-Nyarko and Jones, 2015), i.e. availability of water and nutrition (Amir and Sinclair, 1996; Kirby et al. 2014; Dettori et al. 2017), genotypical factors (Akhatou et al. 2016) and tolerance and resistance of cultivars to the environment (Oerke, 1999; Mujeeb-Kazi et al. 2013; Murrell, 2017). Chemical, cultural and biological control measures of nematode`s population have been practices for decades, but over the year they have proved to be difficult to maintain or apply with effect (Smith et al. 2015; Fusser et al. 2017).

2.9.1 Chemical controls

The use of chemicals fumigants (halogenated and aliphatic hydrocarbons) and non-fumigants (organophosphates, carbamates and others) as a control strategy for nematodes, including pest (Brown, 1985; Hong-xing et al. 2017), fungi (Abawi and Widmer, 2000), bacteria (Crawford, 1997), viruses (Taylor, 1971) and diseases (Shi et al. 2017), has been documented by various authors (Bird et al. 2003). The use of agrochemicals (nematicides) have been more effective for a long time, however, environmentalist believes that the continuous use risk exposure of harmful effect to humans and could be a source of air pollution (Brown, 1985). In addition, chemical control could be the best and effective method for some pathogens but could be poor for other pathogens like bacteria, and may have minor effects on viruses (Siddiqui and Mahmood, 1996; Zhang et al. 2016). Furthermore, they are expensive and toxic and most are being phased out because of their environmental impact and health hazards (Hong-xing et al. 2017; Wright et al. 2018). In other countries, the use of pesticides has primarily focused on the production systems for `high-end` product such as the flower industry (Wright et al. 1995; Wright et al. 2018). One of the main challenge in subsistence agriculture is the lack of sufficient knowledge for correct pest and diseases diagnosis, applications and correct or appropriate choice of management option (Yuan et al. 2017; Shi et al. 2017). In addition, most cases reported over certain period of times, suggests that these products for sales can be of poor quality, thereby reducing their reliability and effectiveness leading to mistrust by farmers (Shi et al. 2017). Hence, there has been a clear decrease in the over counter purchases of such chemicals by famers (Colloff et al. 2013; Lahm et al. 2017; Hong-xing et al. 2017).

2.9.2 Biological control

Recently, new biological control methods have been reported to be effective and still gaining ground (among others, the use of microorganisms, such as fungi, bacteria, and actinomycetes have been successfully used as biocontrol agents of nematodes on different crops (Lax et al. 2013; Boyer et al. 2013; Zhang et al. 2016; Chinheya et al. 2017; Karuri et al. 2017). Furthermore, bacterial species such as *Bacillus thuringiensis*, *Pasteuria penetrans* and *Pseudomonas fluorescens* have been reported as potential biological control agents of cereal cyst nematodes (Siddiqui and Mahmood, 1996; Siddiqui et al. 2014). The study by Zhang et al. (2016), contributed to immensely to understanding the roles of *Achromobacter xylosoxidans* and *Bacillus cereus* isolates as potential biological control agents of PPNs, along with the success of selecting local strains. Meanwhile, the use of marigolds (Tagetes species) has been studied for decades and found to be effective on suppressing PPNs population such as *Meloidogyne* and *Pratylenchus* species and there is still increasing interest to fully understand this method and its implications on PPNs. However, although well known among nematologists for its ability to produce compounds such as terthienyl that are allelopathic to many species of PPNs, this may not be feasible for extensive agricultural farming and may tend to have negative effect on other plants (Hooks et al. 2010). PPN`s biological control based methods have proven effective and a number of commercial products are available (Oerke, 1999). However, biological control strains maybe less effective due to different environmental conditions and diversity of nematodes species (El-Fakharany et al. 2012; Colloff et al. 2013). Therefore, it is important to isolate and identify local strains that are well adapted to local environmental conditions (Thacker, 2003).

2.9.3 Cultural practices

Trap crops, no-host crops and fallowing have been documented to reduce the nematodes populations in agricultural fields (Brown, 1985; Donatelli et al. 2017). Solarisation is another strategy which expose nematodes to extreme environments (high temperature and or water), reducing the population numbers are considered to be a good practises (Kirby et al. 2014). Uprooting harvest crops, burning infected roots and exposing the roots to sunlight are among strategies which have been endorsed to be effective in reducing the population of PPNs in agricultural fields (El-Fakharany et al. 2012; Hazir et al. 2016; Janion-Scheepers et al. 2016; Franco-Navarro and Godinez-Vidal, 2017). However, control strategies such as trap cropping with known effectiveness to PPNs populations, usually tend to not be the methods of choice to famers as a results of their labour intensiveness, water consumptions and costly applications (Ponge et al. 2013; Colloff et al. 2013; Lacey et al. 2015; Rivera et al. 2015; Mashele and Auerbach, 2016).

Natural resistance and crop rotation strategies are based on the notion that many PPNs do not reproduce equally well on all crops or even on different cultivars of the same crops (Oerke, 1999). This is evident on the case of root-knot nematodes (RKN) which are regarded as polyphagous, which do not reproduce equally well on different plants or even on same crops (Berry et al. 2008). Some studies (Cadet and Floret, 1999; El-Fakharany et al. 2012; Colloff et al. 2013; Rivera et al. 2015) have suggested that the uneven reproduction was attributed to crop rotation which is a strategy to reduce or control pest population from building up. However, only few plants or crops have been documented to possess such natural traits and fewer crops have been recoded to be totally resistant to one or more PPNs (Hazir et al. 2016). Therefore, there is need for sufficient information on these strategies.

Genetically modified crop or organisms are mostly employed to target the yield with less expenses and also to some extent help protect against abiotic factors (drought and salinity) (Murrell, 2017). In most cases, nematicides as PPNs control and its applications will increase in order to enhanced or increase the yield, e.g. banana and musa species (Song et al. 2017), however, these tend to have negative effect overtime. Soil organic matter and green manure crops which usually consist of various waste materials, rotational and cover crops that are ploughed back into the soil while they are green and allowed to decomposed have been documented to suppress RKN populations (El-Fakharany et al. 2012; Yuan et al. 2017). *Tithania disersifolia, Desmodium uncinatum, Tagetes minuta, leucaene leucocephala* and *Crotalaria juncia* are among the few examples of green manure plants, together with the soil amendments to have known effects on PPNs suppression (Talwana et al. 2016).

2.9.4 Emerging nematode control strategies

Research and development of disease resistant cultivars is currently a centre of interest and one of the most cost-effective and environmentally friendly methods for disease control in crops (Akar et al. 2009; Yuan et al. 2011; Mujeeb-Kazi et al. 2013). However, this has had some complications whereby a particular disease resistant targeted gene will be resistant to certain species, while being susceptible to other species in a mixed PPN population (Timsina and Connor, 2001; Yuan et al. 2011; Ahmed et al. 2012; Janion-Scheepers et al. 2016). However, progress has been made towards minimising these challenges. This has culminated in the development of varieties that are resistant to one or more diseases that are now available in most agricultural crops that have economic importance (Siddiqui and Mahmood, 1996; Yuan et al. 2011). One major drawback is that this is not feasible to extensive farming practices because it is time consuming to select parents, make crosses and back crosses and select desired progeny, thereby making it difficult to react adequately for pathogens (Yuan et al. 2011). Cui et al. (2015), suggested that using resistant and tolerant wheat varieties is the most effective, economic and environmentally friendly option for controlling this nematode. However, this might affect the quality of production in tolerant wheat cultivars. Furthermore, emphasise in understanding PPNs population`s pathotype has proven to be essential tool when developing resistance gene cultivars in breeding and PPNs management programs (Cui et al. 2015).

Integrated Pest Management (IPM): this strategy involves the combination of chemical, biological, cultural and genetic host-plant resistance as control measure for PPNs (Colloff et al. 2013; Lacey et al. 2015; Hazir et al. 2016; Chinheya et al. 2017; Wright et al. 2018). It is a holistic approach that involves several strategies or overall plans for pathogens (PPNs) management and tactics (e.g. specific tools) to carry out the plans that limit pests damage to tolerable or below threshold levels (El-Fakharany et al. 2012; Chavez et al. 2014; Fernandez et al. 2015; Björsell et al. 2017). Most researchers (McSorley and Duncan, 1995; Stenberg, 2017), believe that IPM is the way to go because it is considered as eco-friendly, durable and cost-effective to protect crops against pests and pathogens. According to Talwana et al (2016), for the successful application of this strategy, IPM should act as the overarching basis for PPNs management and must be based on good information of prevailing conditions, PPN biology and local capacity for proper implementation..

2.10 Plant-parasitic nematodes associated with wheat in South Africa

In South Africa, the status of PPNs as a limiting factor in wheat production has only recently been the subject of detailed study (Kleynhans et al. 1996; McDonald et al. 2001; Perry, 2013; Sikora et al. 2018). PPNs and their damage are potential intertwined with intensified systems and can be an indicator of unsustainable agricultural practices (Trivedi and Barker, 1986; Luc et al. 2005; El-Fakharany et al. 2012). Optimal pest management will be essential because infestation of any system create more intense selection pressures for pest build ups (Murrell, 2017). At higher densities, the potential of infection could constitute a real threat to subsequent crops, particularly if no control measures are taken for proper management of these nematodes in time and in space (McSorley and Duncan, 1995).

Till date, Africa is still lacking behind in terms of PPN surveys, with an exception of South Africa. In 1987, the South African Plant-Parasitic Nematode Survey (SAPPNS) was launched by the Nematology Unit, Biosystematics Division, Agricultural Research Council-Plant Protection Research, with the main objective of making assessments of nematodes biodiversity of South Africa. This was deemed as an ideal tool to determine the extent of nematodes associated with different crops in South Africa. The SAPPNS includes a checklist which contains a data from PPNs collected in South Africa since 1950s and some published data. Additional data were obtained from systematic surveys undertaken by personnel's of the National Collection Nematodes (NCN) (Van den Gerg, 1996; Marais and Swart, 1998; 2007) and via diagnostic services rendered by the Nematology Unit (ARC-PPR). Information contained in both SAPPNS and NCN databases is published periodically as a part of the “plant nematodes in South Africa” series of publications (Marais & Swart, 2003; 2014; 2015). However, CCNs (*Heterodera* spp., RKN (*Meloidogyne* spp.) and RLN (*Pratylenchus* spp.) are among the PPNs that have been documented extensively to be the main limiting factor for grain production and are considered as economically important for wheat worldwide (Rivoal and Cook, 1993).

Cereal cyst nematodes (CCN); *Heterodera* spp. are cosmopolitan and constitute one of the best documented and most economically important PPN species (Swarup and Sosa-Moss, 1990; McDonald and Nicol, 2005). CCNs have been found in many countries and are likely to be responsible for estimated wheat yield loses of 40-78%, especially under sub-adequate moisture conditions (Nicol et al. 2003). The most frequently reported pathogenic species are *Heterodera avenae, H. filipjevi* and *H. latipons*. Furthermore, it was estimated that the genus *Heterodera* contains slightly over 70 species, including a complex of 12 species known as the *H. avenae* group. Species in this group invade and reproduce only in living roots of cereals and grasses (Rivoal and Cook, 1993; McDonald and Nicol, 2005). CCNs are identified by cysts forming on root systems of host plants, excluding broadleaf plant. Recent studies have estimated up to 25% and 50% yield loss caused by *H. avenae* and *H. filipjevi* to commonly cultivated spring and winter wheat varieties in the Eastern Mediterranean region (Ortiz-Monasterio and Nicol, 2004; Imren and Elekcioğlu, 2014; Dababat et al. 2015). Few reports, suggested that *H. filipjevi* is the most widespread species between the two and generally occurs alone, but mixed populations with *H. latipons* have also been reported (Abidou et al. 2005; Nicol et al. 2010). Despite not being widespread, *H. latipons* has been reported to be among the important nematode pest that prevail in wheat growing areas throughout the Mediterranean region, including Asia and Europe (Peng et al. 2009). These species can cause yield losses up to 90% in severely infested fields (Rivoal and Cook, 1993). In Africa, *H. avanea* has only been reported from some semi-arid regions (parts of Morocco, Libya and Tunisia) of North Africa (Sikora, 1988), while in South Africa it was reported in late 1990s from subtropical and tropical regions (McDonald and Nicol, 2005). *Heterodera mani, H. hordecalis* and *H. schachtii* are among the *Heterodera* species that ranked high according to their impact and distribution on small grain crops worldwide (Rivoal and Cook, 1993; McDonald and Nicol, 2005). Damage caused by cereal nematodes depends on several factors, i.e. availability of water and nutrition, genotypical factors, and tolerance and resistance of cultivars which needs to be determined under different environmental conditions. In addition, the intensity of the incidence and impact of CCN depends on the type of host and soil, pathotype and/or ecotype and climatic conditions (Rivoal and Cook, 1993). The recent dramatic decrease in small grain crops, especially wheat in South Africa is a concern to wheat farmers. PPNs, especially *Heterodera* species have not been given much attention until recently and the information about their incidence, impacts and distribution in South African wheat production areas is limited and fragmented.

Root-lesion nematodes (RLN, *Pratylenchus* spp.) also constitute one of the most economically important plant-parasitic nematode species which invade roots of wheat, causing extensive necrosis with consequent crop losses with yield losses up to 70% (Murray and Brennan 2009; Vanstone et al. 2008). *Pratylenchus* species are of the most widespread plant-parasitic nematodes and densely populated where they occur, causing significantly yield losses of small grain cereals worldwide. Plants with heavily damaged roots may exhibit stunting, poor vigour, reduced tillering, and premature wilt at the onset of moisture stress (Van Gundy et al. 1974; Doyle and Doyle, 1987; Amir et al. 1991). *Pratylenchus neglectus* and *P. thornei*, have been subjected to intense investigations for the past decades (Filipjev and Schuurmanns, 1941; Sher and Allen, 1953; Taheri et al. 1994) and currently are reported to be associated with significant yield losses where ever they occur (Maafi et al. 2009). Murray and Brennan (2009), through surveys of plant pathologists nationally, estimated that the two primary RLN affecting wheat in Australia were *P. neglectus* and *P. thornei*, and caused annual yield losses exceeding $ 73 million and $ 50 million, respectively. These were followed by *P. quasitereoidesa* and *P. penetrans* with annual yield losses estimated at $ 9 million and $ 2 million in Western Australia, respectively. Ahmadi, (2015), through a survey of cereal fields in South-Western Iran, reported that *P. thornei* as the dominant species in both wheat and barley fields. According to Smiley and Nicol, (2009), *P. thornei* is more damaging than *P. neglectus* in the world. Another study listed *P. ritteri, P. alkani, P. crenatus, P. pseudopratensis, P. brachyurus, P. coffeae* and *P. zeae* as the major pests reported from maize, wheat, barley and rice (Ahmadi et al. 2010). In South Africa, the two predominant RLN species, *P. neglectus* and *P. thornei* have not been given much attention; while significance reported losses of crop yields due to these species worldwide suggest otherwise (Keetch and Buckley, 1984).

Root-knot nematodes (RKN) are economically important polyphagous pest belonging to the *Meloidogyne* genus. *Meloidogyne* species are highly adapted obligate plant parasites, distributed worldwide and parasitize nearly every species of higher plant (Moens et al. 2009). The infective second stage female juveniles (J2) migrates within the roots where they reproduce and feed on modified living plant cells and induce small to large galls or root-knots, hence their vernacular name (Moens et al. 2009). Karssen, (2002), was the first to use the name *Meloidogyne,* which is of Greek origin and means `apple-shaped female`. *Heterodera marioni* was the name commonly used to describe RKN, until 1949, when Chitwood removed them from the genus *Heterodera* because they differed from cyst nematodes and reinstated the genus *Meloidogyne.* Chitwood further re-described *Meloidogyne arenaria, M. exigua, M. incognita, M. javanica* and *M. hapla*. The species were separated from each other on the basis of perineal pattern morphology, stylet knob shape, length of the stylet and dorsal gland orifice (Moens et al. 2009). Currently, the economically most important RKN species worldwide (including South Africa) are *M. arenaria, M. hapla, M. incognita* and *M. javanica* (Jones et al. 2013). Heavily infected crops or plants may not immediately show any visible symptoms. Despite this, the disruption of plant physiology may interfere with the plant`s water and nutrients uptake and ultimately reducing not only the crop yield but also the product quality (e.g. of potatoes and carrots) and therefore are of great economic and social importance (Perry et al. 2013). In spite of South Africa discovering and documenting RKN species such as *M. arenaria, M. hapla, M. incognita* and. *M. Javanica* in some provinces, the status of their prevailing rate of infestation on small grain cereals has not been reported (Onkendi et al. 2014; Agenbag, 2016). Therefore, this study was conducted to i) survey and generate data on the distribution of the three economically important nematode pest species CCN, RLN and RKN in local wheat-producing areas, ii) conduct morphometric and molecular identification and phylogenetic analyses of local *Heterodera, Pratylenchus* and *Meloidogyne* spp.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study sites

Soil and root samples were collected South African provinces (Figure 3.1) which are generally wheat producing areas including Northern Cape (NC), Western Cape (WC), North West (NW), Free State (FS), KwaZulu-Natal (KZN), Mpumalanga (MP) provinces, and Limpopo (LP) Provinces . WC province is situated in the southern extremity of the African continent. It is also the provincial country’s legislative capital. It is found along the front line of the Atlantic and Indian oceans, which forms the WC’s western and southern boundaries, respectively. It further forms the borders within the NC province to the north and the EC province to the east. The WC province experiences Mediterranean climate characterised by cool, wet winters and hot dry summers. Mean annual rainfall varies from 200 to 450 mm and more than 80% of the rainfall is received in winter between April and September, making the WC predominantly a winter rainfall area (Grain, 2015). The arable land in this area covers approximately 1.5 million ha. Winter cereals are the major crop in the WC and at present, the WC accounts for nearly half of the wheat produced in South Africa. The Swartland (on the west coast) and the Rûens (Southern Cape) are the main distinguishable geographic regions of the winter rainfall area. The Swartland region is divided into four sub regions, based on soil and climatic characteristics, while the Rûens, in three sub regions (Grain, 2015). The localities sampled during this project in the sub regions in the Swartland and Rûens are given in the tables (3.3; 3.4), respectively. The Free State province is situated in the east-central Republic of South Africa. The province forms the borders on the north by NW, Gauteng (GP), MP, EC, NC, KZN province and the independent state of Lesotho. FS province experiences varying climatic conditions from warm and temperate, with an annual rainfall of 40 inches (1,020 mm) in the east to semiarid regions, with rainfall of only 15 inches (380 mm) in the far west. Mean annual surface temperatures gradually increase from about 14°C in the east to 17°C in the west. Frost is common over the entire province from May to September and because rainfall is unreliable, long periods of drought are frequent and can persist for considerable times. FS province is the second major province that produces winter cereals and samples were collected from the east region of the province. Northern Cape province it is bordered to the east by NW, FS, EC, southwest by WC province, to the west by the Atlantic Ocean and north by the independent states of Namibia and Botswana (Grain, 2015). The province produces at least more than a quota of wheat in the Cooler Central irrigation region. KwaZulu-Natal province is situated in the south eastern part of the country and forms the borders in the south by EC province, to the west by Lesotho and FS province and is bounded to the north by Swaziland and Mozambique, further to the east by the Indian Ocean and to the northwest by MP province. The climate varies from subtropical to temperate. Precipitation decreases from more than 50 inches (1,270 mm) annually along the coast to 30 to 40 inches (760 to 1,020 mm) inland. Temperatures decrease from the frost-free coastal area but still remain warm. This climate is considered to be suitable for *Zea mays* L. then the wheat. However, Highveld regions have also planted wheat in irrigation areas. Limpopo Province is situated in the North-Eastern corner of South Africa and forms borders with NW, MP, GP and the independent states of Botswana, Zimbabwe and Mozambique. Temperatures range from warm (23ºC in January) to cooler (13ºC in June) and rainfall of about 100 mm in November (www.weather.com/limpopo/averages). The samples were collected in Highveld regions, where wheat was planted. Mpumalanga province is characterized by warm and temperate weather. MP is a city with significant rainfall and even in the driest month, there is a lot of rain. The temperature averages 18.3°C and about 913 mm of precipitation falls annually (Engelbrecht and Engelbrecht, 2016). The samples were also collected from the Highveld regions where wheat was planted (Table 3.6).

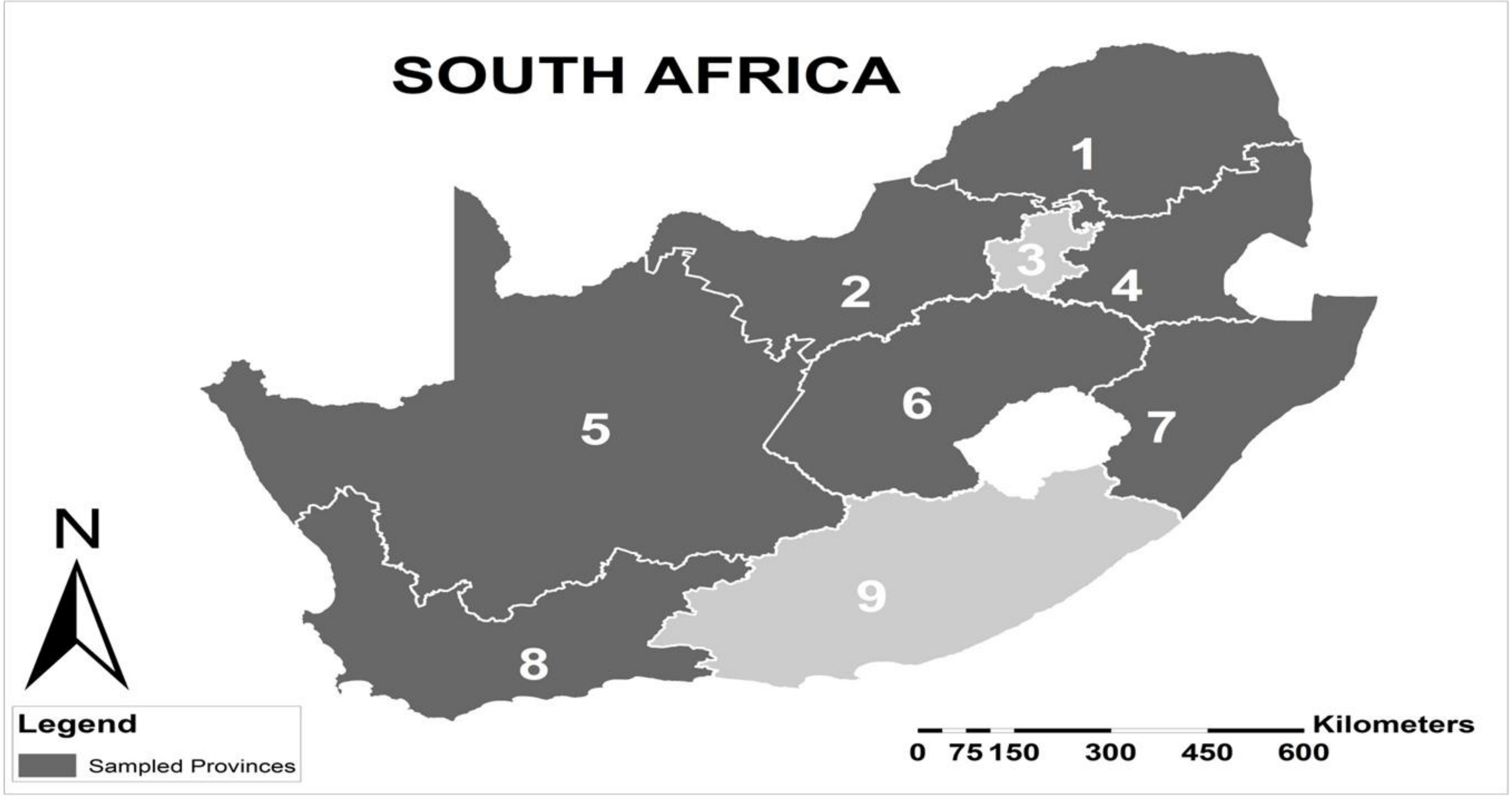

Figure 3-1(A): Map of South Africa showing nine provinces. (1) Limpopo, (2) North West, (3) Gauteng, (4) Mpumalanga, (5) Northern Cape, (6) Free State, (7) KwaZulu-Natal, (8) Western Cape, (9) Eastern Cape. Sampled provinces are highlighted in black colour.

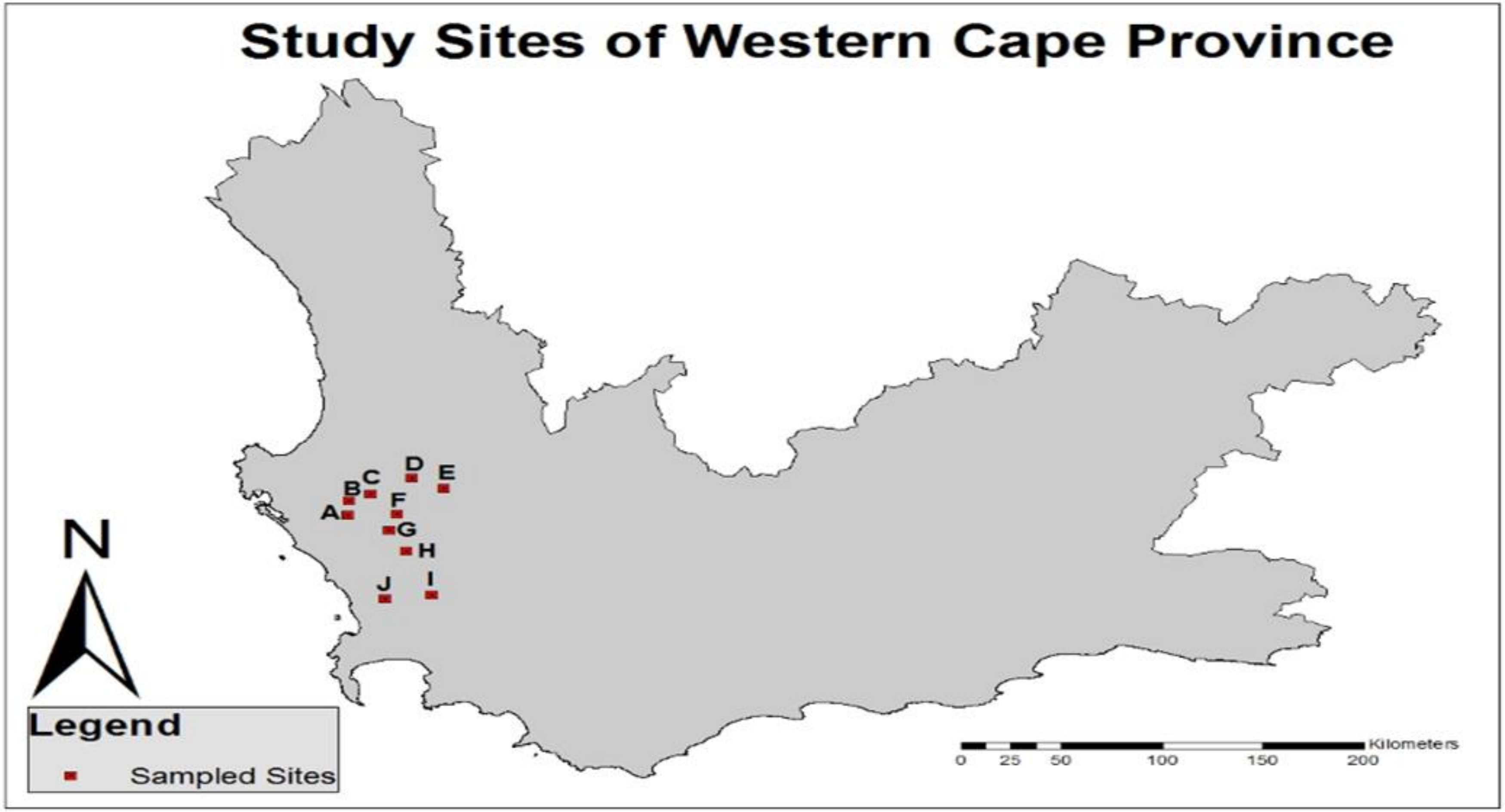

Figure 3-1(B): Map of Western Cape Province showing localities sampled; (A); Enkelvlvei. (B); Watersboerkraal, (C); Langkloof, (D); Kolsvlei, (E); Eikenhof, (F); Koringplaas, (G); Klein Swartfontein, (H); Papkuilfontein, (I); Boland Farm, (J); Altona

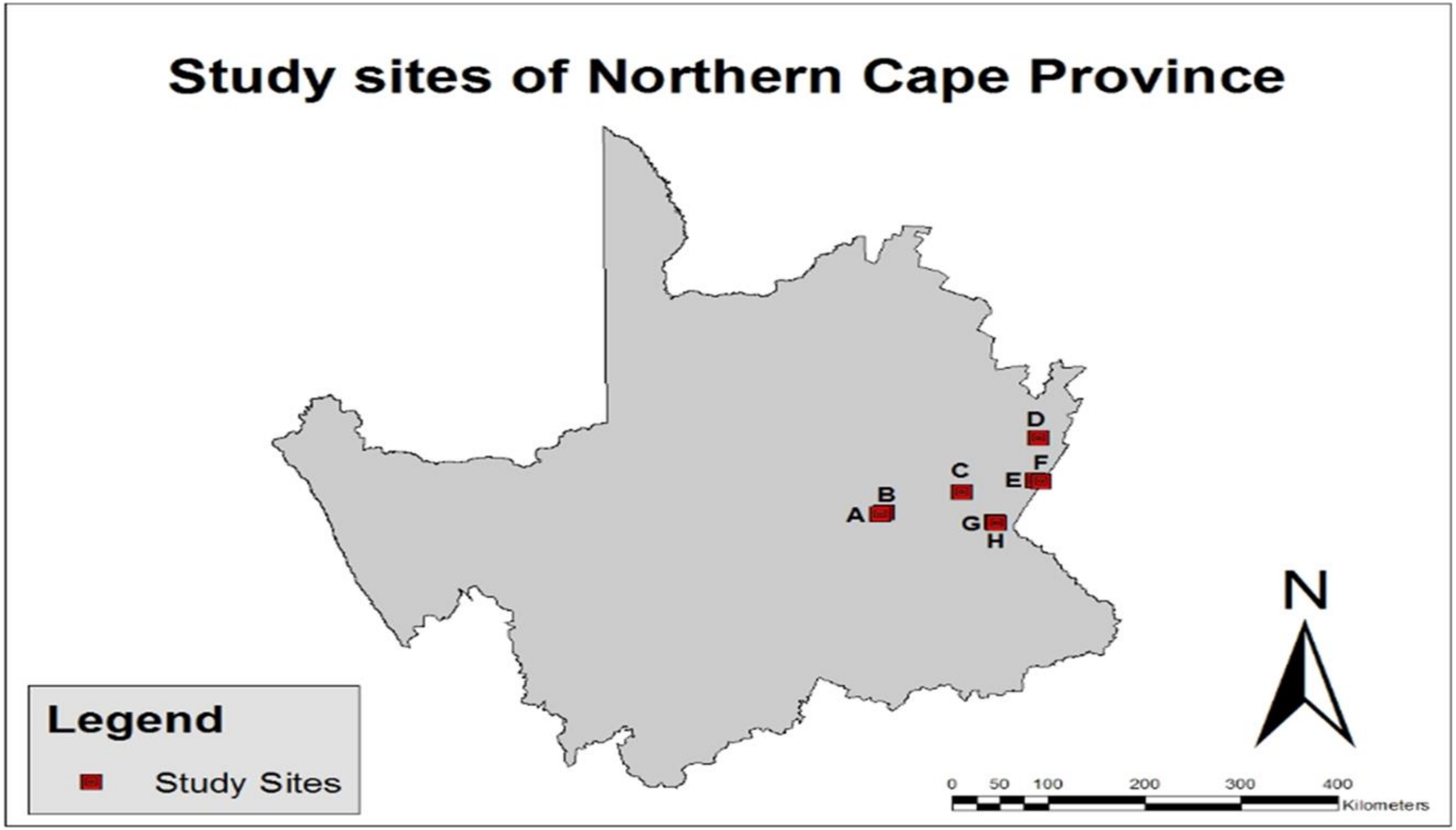

Figure 3-1(C): Map of Northern Cape Province showing localities sampled; (A); Prieska, (B) Prieska 2, (C); Douglas, (D); Barkley west, (E); Modderrivier 1, (F); Modderrevier 2, (G); Hopetown 1, (H); Hopetown 2

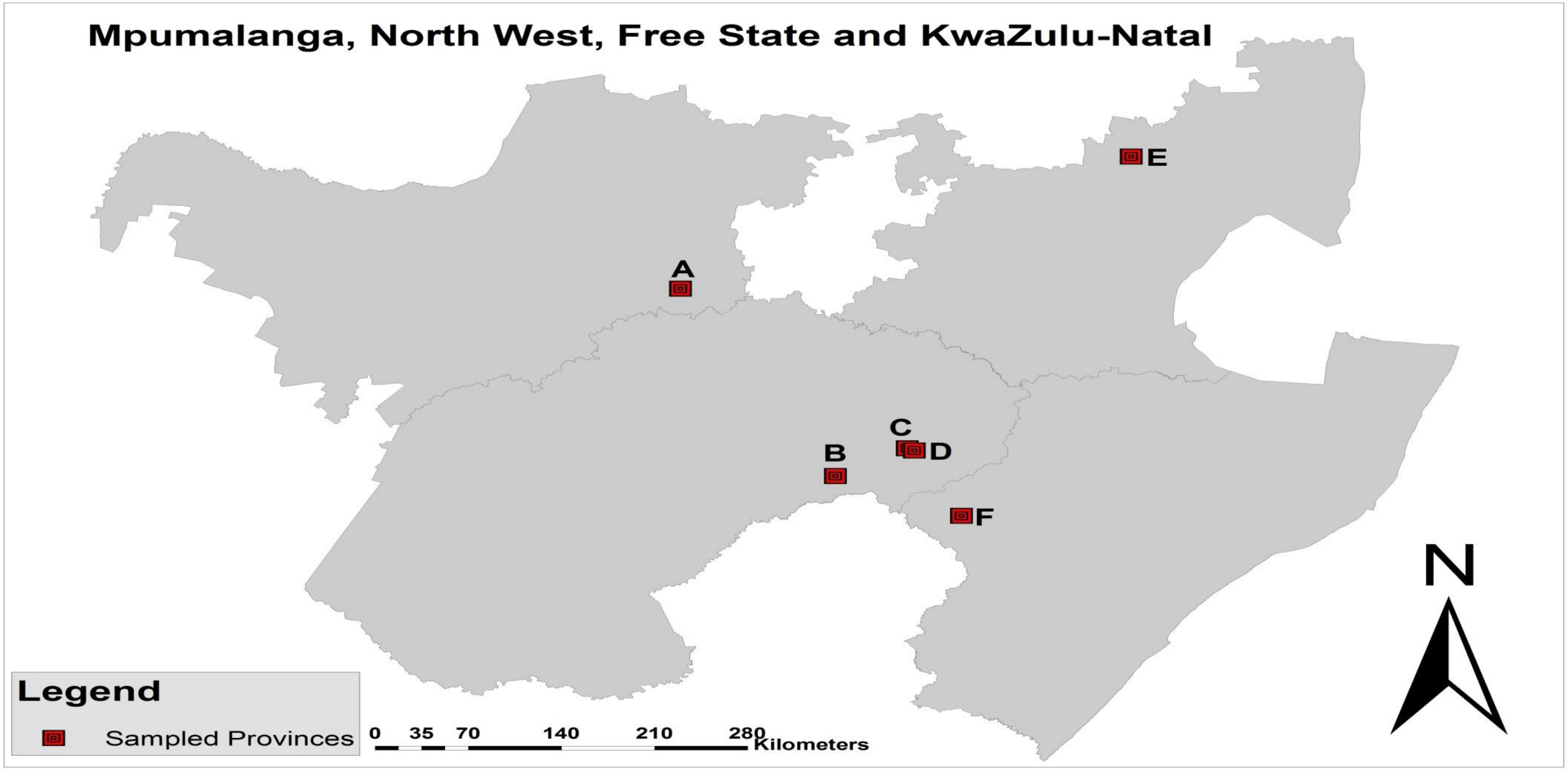

Figure 3-1(D): Map of Free State, KwaZulu Natal, North West and Mpumalanga Provinces showing localities sampled; (A); Potchefstroom), (B); Clarens, (C); Harrismith(JL), (D); Harrismith (TF), (E); Coromandel (MP), (F); Cedara

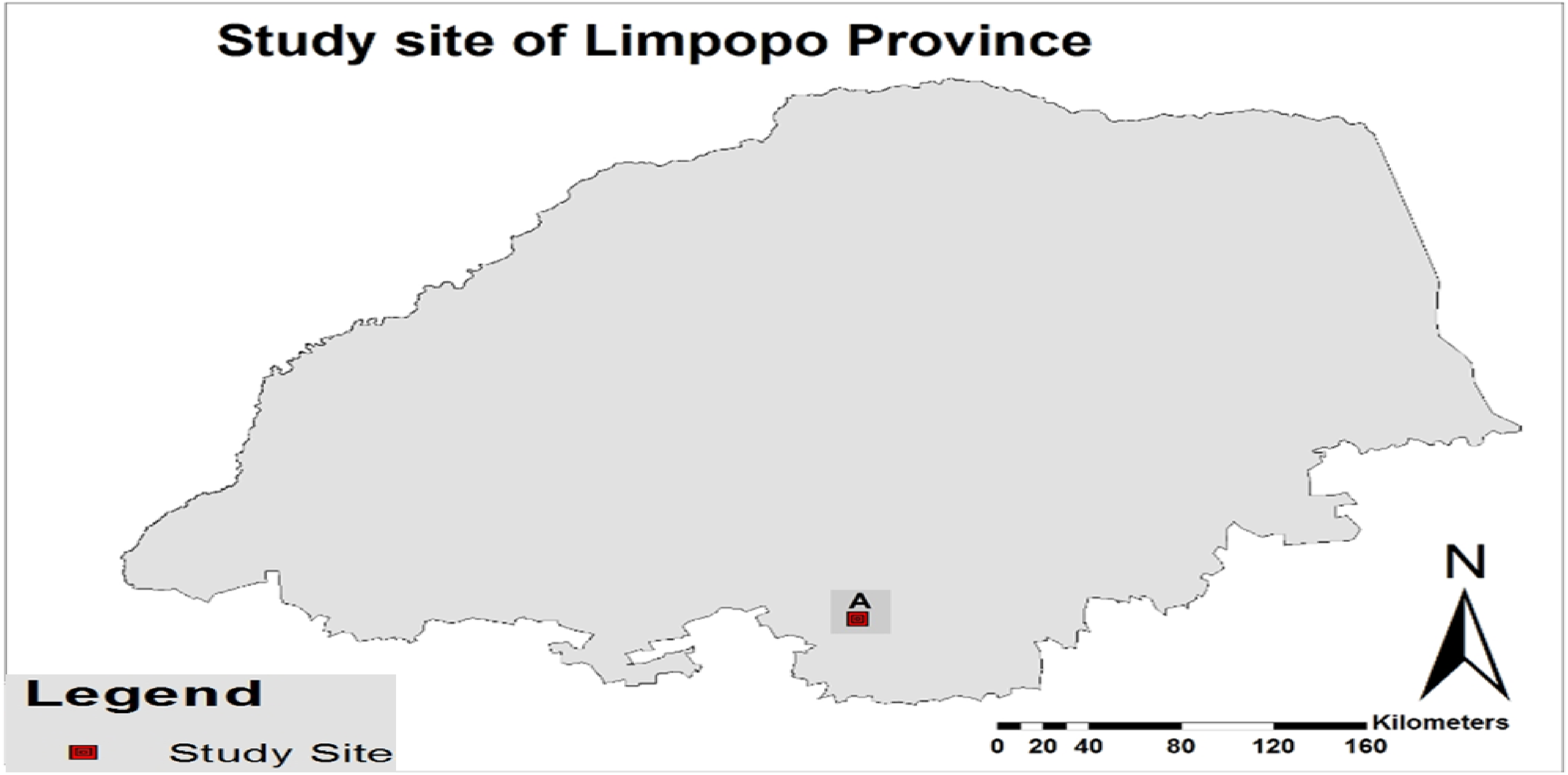

Figure 3-1(E): Map of Limpopo Province showing sampled locality. (A); Rietvlei.

3.2 Collection of samples from wheat growing fields around South Africa

Samples were collected from the National Cultivar Trials conducted annually by the Agricultural Research Council-Small Grain Institute (ARC-SGI) in Bethlehem (FS) and Stellenbosch (WC), two months before wheat harvesting time using a spade and at the depth of 15-35 cm depth. Wheat (*Triticum aestivum* spp.) was planted in all the localities sampled during the current study. A total of 776 composite rhizosphere soil and root samples were been collected from 56 localities over two seasons in the Western Cape (452), Northern Cape (160), Free State (120), KwaZulu-Natal (8), Mpumalanga (12), North West (12) and Limpopo (12) provinces. At least 4 random samples were collected from each of the fields to form a composite sample. The numbers of samples collected in each province were representative of the number of localities sampled during the survey (Table 3-1—3-7). Limpopo, North West and Mpumalanga provinces were sampled for only one season and from a single site each. This was due to wheat not being planted during the sampling season and the number of samples collected depended on the size of the farms. Table 3-8, shows the types of cultivars used in trial programmes during the survey. All trials plans were row and column Latinised designs with four replications. The row spacing was 30 cm and seven rows of seven meters were planted. The plots were then reduced to 5 m post-emergence, by spraying out a path of 2 m between the plots. Only five of the seven rows were harvested (Plate 3.1). A subtotal of 2 kg of soil was taken from capillary roots and rhizosphere of wheat plants, including the wheat kernels. The collection of samples was conducted during both winter and summer periods (Plate 3.1). Each sample was labelled with the following information: collection date, area, the crop rotation in the previous year, growing season, the phonological stage of the plant, etc., and was transported to the laboratory for nematode extractions.

Plate 3.1: Shows the collection of samples from the field trials. (A); shows the bock layout of the field trials: (B); Shows the display of different cultivars found on each bock: (C); Collection of the soil and plant samples: (D); Samples packaged and ready to be transferred to the laboratory for extraction

Table 3-1: Indicates the localities in the sub regions in the Rûens (ARC-GI, 2017)

Swartland

Sub region	**Locality**
High rainfall	Philadelphia (Altona)
	Malmesbury (Koringplaas)
	Malmesbury (Papkuilsfontein)
	Darling (Klipvlei)
	Wellington (Boland Landbou)
Middle Swartland	Moorreesburg (Langrug)
	Moorreesburg (Klein Swartfontein)
	Piketberg (Kolsvlei)
	Halfmanshof (Uitkoms)
Koringberg	Eendekuil (The Rest)
	Koringberg (Langkloof)
	Pools (Panorama)
	Porterville (Eikenhof)
Sandveld	Hopefield (Dankbaar)
	Hopefield (Enkelvlei)
	Velddrift (Volstruiskuil)
	Koperfontein (Waterboerskraal)
	Vredenburg (Holvlei)

Table 3-2: Indicates the localities in the sub regions in the Swartland ARC-GI, 2017)

Rûens

Sub region	**Locality**
Western Rûens	Roodebloem (Caledon)
	Protem (Kleinfontein)
	Riviersonderend (Tygerhoek)
	De Vlei (Caledon)
	Uitvlug (Caledon)
Southern Rûens	Klipdale (Alpha)
	Protem (Volmoud)
	Bredasdorp (Karsrivier)
	Napier (Bo- Schietpad)
	Klipdale (Panorama)
Eastern Rûens	Riversdale (Uitkyk)
	Witsand (Uitkyk)
	Heidelberg (Voorstekop)
	Swellendam (Klippenrivier)
	Buffelsjag (Volmoed)

Table 3-3: Indicates the list of localities, GPS coordinates, farm names and previous crops on the sites

Swartland sites planted by the Agricultural Research Council

Locality	**GPS Coordinates**	**Farm Name**	**Previous crop**
Moorreesburg	S33°15.770` E018°40.100`	Koringplaas	Medics
Wellington	S33°40.246` E018°52.842`	Boland Landbou	Fallow
Piketberg	S32°56.362` E018°47.831`	Kolsvlei	Medics
Porterville	S33°00.450` E018°57.880`	Eikenhof	Oats
Koringberg	S33°02.002` E018°34.486`	Langkloof	Medics
Malmesbury	S33°23.726` E018°45.451`	Papkuilsfontein	Medics
Moorreesburg	S33°09.586` E018°42.497`	Klein Swartfontein	Medics
Philadelphia	S33°41.434` E018°38.075`	Altona	Medics
Hopefield	S33°09.667` E018°27.044`	Enkelvlei	Fallow
Koperfontein	S33°04.516` E018°27.583`	Watersboerkraal	Canola

Table 3-4: Indicates the list of localities, GPS coordinates, farm names and previous crops on the sites

Rûens sites planted by the Agricultural Research Council

Locality	**GPS Coordinates**	**Farm Name**	**Previous crop**
Riviersonderend	S34°09.330` E019°54.439`	Tygerhoek	Lupines/ Fallow
Caledon	S34°18.328` E019°33.544`	Uitvlug	Lucerne
Caledon	S34°14.296` E019°31.955`	Roodebloem	Medics
Klipdale	S34°17.699` E019°50.470`	Alpha	Oats
Klipdale	S34°20.960` E019°54.104`	Panorama	Canola
Protem	S34°08.782` E020°15.513`	Kleinfontein	Fallow
Bredasdorp	S34°28.224` E020°07.857`	Karsrivier	Fallow
Heidelberg	S34°07.872` E020°44 257	Voorstekop	Coriander
Riversdal	S34°09.624` E021°09.277`	Uitkyk	Canola Cover crops

Table 3-5: Indicates the list of localities in KwaZulu Natal, GPS coordinates, farm names and previous crops on the sites

Locality	**GPS Coordinates**	**Previous crop on trial site**
Cedara	S28º46.106’/EO29º21.636	Soybean

Table 3-6: Indicates the list of localities in the Highveld Free State, North West, Mpumalanga and Limpopo, GPS coordinates and previous crops on the sites

Locality	**GPS Coordinates**	**Previous crop on trial site**
Clarens	S28º26.488’/EO28º22.252’	Potatoes
Harrismith (JL)	S28º08.486’/EO28º53.803’	Potatoes
Harrismith TF)	S28º09.657’/EO28º57.242’	Maize
Potchefstroom	S26º55.38.5`/E27º04,13.3`	Sunflower
Coromandel (MP)	S25°14.05.9`/E30°21.41.6`	Beans
Rietvlei (L)	S24°54.56.11`/E30°69.48.6`	Maize

Table 3-7: Indicates the list of localities in Northern Cape Cooler Central irrigation area, GPS coordinates and previous crops on the sites

Locality	**GPS Coordinates**	**Previous crop on trial site**
Prieska 1	S29'32.4 E0 22'58.1	Maize
Modderrivier 1	S29’06.5 E024’35.1	Onion
Hopetown 1	S29’38.0 E024’10.4	Soybean
Modderrivier2	S29’07.0 E024’38.4	Maize
Douglas	S29’16.8 E023’48.0	Maize
Barkley West	S28’35.0 E024’35.4	Potatoes
Prieska 2	S29’33.1 E022’56.3	Maize
Hopetown 2	S29’38.4 E024’10.8	Maize

Table 3-8: Shows cultivars/entries used for the 2017 program

Origin	Entry	Type	Released
Small Grain	Ratel	Pure Line	2011
Small Grain	Tankwa	Pure Line	2007
Sensako	SST 88	Pure Line	1998
Sensako	SST 015	Pure Line	2001
Sensako	SST 027	Pure Line	2002
Sensako	SST 056	Pure Line	2005
Sensako	SST 087	Pure Line	2008
Sensako	SST 096	Pure Line	2009
Sensako	SST 0127	Pure Line	2012
Sensako	SST 0117	Pure Line	2011
Sensako	SST 0147	Pure Line	2014
Sensako	SST 0116 *	Pure Line	2011
Pannar	PAN 3471	Pure Line	2004
Pannar	PAN 3408	Pure Line	2001

3.3 Soil analyses

Soil samples were analyses with standard methods (The Non-affiliated Soil Analysis Work Committee, 1990) at the ARC-Grain Crops Institute included, except for extractable P (Ambic 1 and Bray 1), also particle size distribution (pipette; Schroeder et al. 1984; Brady and Weil, 1999), electrical conductivity (saturation extract), pH (distilled H_20 and M KCl), exchangeable acidity (M KCl), organic C (Walkley-Black) and exchangeable Ca, Mg, K and Na (Ambic 1), From the above mentioned analyses the base and acid saturation, as well as ECEC (effective cation exchange capacity calculated as the sum of cations) value of soils were calculated. In addition, the mineralogical composition of the samples was also determined with X-ray diffractometry as well as the dithionite-citrate-bicarbonate (DCB) extractable free oxides of Al, Fe and Mn (Velde, 1992; Schmidt et al. 2004) at the ARC-Institute for Soil, Climate and Water (data no provided). Soil texture was determined by a rapid hydrometer method based on Day's, (1965) modification of Bouyoucos' (1951) technique. Soil type was determined according to the triangular textural diagram (Hodgson, 1974).

3.4 Extraction and detection of nematodes from soil and plant samples

The soil and root nematodes were extracted by a modified decanting and sieving baermann-funnel technique, followed by the adapted sugar centrifugal-floatation (Flegg, 1967; Jenkins, 1964). Unfortunately, there is no single method suitable for all purposes. Wheat kernels nematodes were extracted using the soaking method (Coolen and D'Herde, 1972; Augustin and Sikora, (1989); Modified-tray technique by Whitehead and Hemming, (1965) from Tenente and Evans, (1995) and EPPO, (2013). The first principle is motility of the nematodes, meaning that the nematodes actively move from the sample to water. The second principle is size and shape of the nematodes, meaning that nematodes are collected on sieves by passing samples. The third and last principle is the densities of the nematodes, meaning that nematodes are separated from samples by centrifuging or floating (EPPO, 2013). For comparison of methods and extraction efficacy see Böhmer and Weil, (1978), McSorley et al. (1984), Oostenbrink, (1960), Penas et al. (2002), Tarjan, (1960, 1972) and Viaene et al. (2007)

3.4.1 Extraction of nematodes from soil:

Soil samples (250 g) were each washed through a coarse-meshed sieve (2 mm apertures) into a 5-litre bucket. Water was added to the bucket to increase suspension to 5 l. Stirred suspension, then allowed settling for 30 seconds. The suspension was poured through a 45 µm-aperture sieve. The procedure was repeated with soil in bucket two more times, but shortening of settling times to 20 and 10 seconds. The residue was then transferred on 45 µm sieve to four 50 ml centrifuge tubes and then, centrifuged for 7 minutes at 1750 rpm. The supernatant was then decanted and discarded. The sugar solution (450 g/l water) was added to the tubes. The tubes were shaken, centrifuged for 3 minutes at 1750 rpm and the suspension poured through the 45 µm sieve. Afterwards or thereafter, the residue was rinsed from the 25 µm sieve for examination.

3.4.2 Extraction of nematodes from plant materials:

The root plant samples (50 g and 20 g) were each shredded in a food blender or cut into 1 mm pieces, then covered with water. The material was then left for 24 hours. The materials were then added to the centrifuge tubes together with 50 g of kaolin powder, centrifuged and decanted as described above.

3.4.3 Extraction of nematodes from wheat kernels:

The wheat kernels (20 g) were cut into 1 mm pieces and then covered with water. The material was then left for 24 hours. The suspension was poured through the 45 μm sieve. Afterwards, the residues were rinsed from the 25 μm sieve for examination.

3.4.4 Cyst nematodes extractions

The cysts were extracted using the Seinhorst cyst-elutriator. With this apparatus, *Heteroderid* cysts were extracted from both wet and dry soil. The cysts were kept in suspension by a controlled upward current of water. The clamp was closed on lower downpipe and the cylinder closed with a rubber bung. The speed of upward current of water in cylinder is then adjusted to about 4 cm per second by manipulating feeder–tank stopcock. The water was then let out of apparatus and both downpipes were placed in 150 μm-aperture bucket sieve. The cylinder was closed with rubber bung and the feeder-tank stop cock was then open to the desired setting. As water rosined in cylinder, soil mixture (100 ml) was washed through a coarse-meshed sieve (2 mm apertures) into top bowl. As water spilled over collar and into bucket sieve, the residues were washed on the sieve with a strong fan-shaped spray of water. After about 20 seconds the clamp was open on lower downpipe, continued spraying residue on sieve to prevent clogging of sieve meshes. The top bowl and the cylinder were then rinsed until water in cylinder was clear. The water was let out of apparatus and rinsed. Then the residue was washed on sieve onto a 120 mm square of fine gauze supported on a coarse-meshed plastic screen clamped to a retort stand. The fold gauze containing residue into a small packet and was then left to dry (residues with cysts other than *Globodera* cysts were not be allowed to dry completely). A large glass trough (19 cm diameter) was lined with a 7 x 60 cm strip of blotting paper. Then, water added through until lower half of paper was submerged and placed dried residue into water. A large conical flask, half-filled with water, was then slowly pushed down into trough. After 15 seconds the flask had to be lifted out of water and adhering debris was rinsed into trough with a wash-bottle. The water was then siphoned off in trough, and then removed paper strip was placed flat on a strip of perspex. Cyst were separated from debris under a dissecting microscope with a dissecting needle and collected with a wet camel-hair brush for identification.

3.5 Nematode identification

3.5.1 Morphological assays

Nematode population levels were determined in a counting dish under a stereo microscope and expressed either as the number of nematodes per 20 g roots, 5 g roots or 200 ml soil. Root-knot nematodes eggs and J2 from 20 g root samples were counted without fixation, while plant-parasitic nematodes per 5 g roots and 200 ml soil were fixed after counting for morphological species identification. Morphological data was used for diagnosis of Juveniles (J2), males and females of CCN, while the same was done for all vermiform life stages of RLN as well as J2, males and swollen females of RKNs. For this purpose, the nematodes specimens were killed and fixed according to the procedure described by Hooper, (1986).

Nematodes suspended in water after extraction from root (5 g) and soil (200 g) samples, were individually picked with a needle and transferred to an eye glass (Syracuse dish) containing 5ml distilled water. Five millilitre of a heated (±60-70°C) formalin, propionic acid and glycerine solution (F.P.G solution: 100ml of a 40 % formalin solution, 10ml propionic acid, 890ml distilled water, 10ml glycerine and 1mg of picric acid for staining) were added to the nematode suspension. The eye glass was transferred to a dessiccator filled with F.P.G solution and kept in an incubator at 40°C for 72 hours. Nematodes were transferred from the F.P.G solution to an eye glass containing a glycerin I solution (200 ml of a 95 % ethanol solution, 10 ml glycerin and 790ml distilled water). The eye glass with the nematodes suspended in the glycerin I solution was then placed in a dessicator containing a 95-% ethanol solution to saturate the atmosphere inside the dessicator. The dessicator containing the eye glass with nematodes was subsequently placed in an oven at 38-40°C for 12 hours. The eye glass containing the nematodes was then removed from the dessicator, the nematodes were transferred to an eye glass containing a glycerin II solution (950 ml of a 95-% ethanol solution and 5 ml glycerin) and placed back in an oven at 38-40°C. The alcohol slowly evaporated so that the nematodes remained in pure glycerin after 12 to 24 hours. The nematodes were then mounted on slides in anhydrous glycerin and send to ARC-PPRI for morphological species identification.

To study finer morphological structures of plant-parasitic nematode species under a light microscope, nematodes were mounted in glycerin. Glycerin has a refraction index of nearly the same as that of glass. However, when living or fixed nematodes are suddenly placed in pure glycerin they will plasmolyse. Therefore, transferal of nematodes to glycerin must be done gradually, as described by De Grisse (1965). The following parameters were used for morphological identification of adult CCN females: type of fenestration, fenestral length, semi-fenestral width, bridge width of the vulva, underbridge present or not and vulva slit length (Subbotin et al. 2010). Identification of RLN species was based on morphological characters described by Castillo et al. (2007), Siddiqi, (2000) and Handoo, (2002) such as body shape and size, stylet shape and tail shape. For RLN, the protocol of Castillo et al. (2007) was followed, which described the diagnostics and biology of RLN. For the identifications of PPN, mainly families Belonolaimidae, Criconematidae and Tylenchidae, the method by Geraert, (2008) was used Figures (4-1—4-7), provides summary examples of the identifications protocol.

Plate 3.2: Shows extraction methods of nematodes. (A); 200 g soil, 5 g and 50 g roots and 20 g wheat sample: (B); modified decanting and sieving baermann-funnel technique: (C); washing and removing soil debris form the root samples: (D); soaking method of wheat kernels in water: (E); adapted sugar centrifugal-floatation method: (E); different nematodes (free living and plant-parasitic) under the microscope

3.5.2 DNA extraction

DNA extraction from nematode specimens was done as follows: For molecular identification of RLN, CCN and RKN, only one each cyst, a single nematode of RLN and second stage (J2) RKN was transferred into 25 µl of double distilled water (ddH2O) in an 1.5 Eppendorf tube and crushed using a micro homogeniser and a tissue lyser (qiagen tissuelyser lt bead mill (tissue disruption/ homogenization) DNA, RNA). Thereafter, 30 µl of chelax and 5 µl proteinase K were added to the homogenate, vortexed, followed by a brief centrifugation to collect contents and then incubated at 56°C for 2 h and then at 96°C for 10 min after which the mixture was stored at −20°C for further use (Subbotin et al. 2003).

3.5.3 Polymerase chain reaction

(a) PCR with Cytochrome oxidase 1 (CO1) primers

PCR targeting the CO1 gene was conducted to identify nematodes to species level. The forward and reverse primers, NEM_COI_F (5`-GGWSMAMMAAATCATAAAGATATTGG-3`) and NEM_COI_R (5`-GTAATAGCMMCHGCYAAHACMG-3`) were used to amplified approximately 600 base pairs (bp) of the target gene (Malysheva et al. 2016). The PCR reaction contained the final volume of 25 µl which was composed of 12.5 µl GoTaq® Colorless Master Mix, 2X (GoTaq® DNA Polymerase is supplied in 2X Colorless GoTaq® Reaction Buffer (pH 8.5), 400 µM dATP, 400 µM dGTP, 400 µM dCTP, 400 µM dTTP and 3 mM MgCl2) (Thermo Scientific, Belgium). Then 0.5 µm of each primer, 2 µl of DNA template and double distilled water added (ddH_2O) was added to make the final volume. PCR cycling parameters with NEM_COI_F/R primers included primary denaturation at 94°C for 3 min followed by 34 cycles 94°C for 30 seconds, 52°C for 30 seconds and 72°C for 1 min, followed by post-amplification extension at 72°C for 7 min. Following PCR amplification, 5 µl of each PCR product was mixed with 1 µl of 6× loading buffer (Fermentas Life Sciences, Germany) and loaded on a 1% agarose gel. And then electrophoresed at 100 V for 30 min, thereafter visualized under UV-light. The remaining PCR product was stored at -20°C.

(b) PCR with 18S rRNA primers

PCR targeting the 18S rRNA gene was also conducted to identify nematodes to species level. The forward primer (Nem_18S_F; 5`-CGCGAATRGCTCATTACAACAGC-3`) and the reverse primer (Nem_18S_R; 5`-GGGCGGTATCTGATCGCC-3`) were used to amplified approximately 900 base pairs (bp) of the target gene (Floyd et al. 2005). A standard reaction volume was 25µl, comprising of 12.5 µl GoTaq® Colorless Master Mix, (GoTaq® DNA Polymerase is supplied in 2X Colorless GoTaq® Reaction Buffer (pH 8.5), 400 µM dATP, 400 µM dGTP, 400 µM dCTP, 400µM dTTP and 3 mM MgCl2) (Thermo Scientific, Belgium) Then 0.5 µm of each primer, 2 µl of DNA template and double distilled water (ddH2O) was added to make the final volume. The PCR conditions were an initial denaturation at 94°C for 5 minutes; 35 cycles of amplification (94°C for 30 seconds; 54°C for 30 seconds; 72°C for 1 min); followed by a final extension at 72°C for 10 min. The PCR products were eletrophoresed as described above.

(c) PCR with ITS1 primers

For molecular identification, the ITS1-rDNA region was amplified. 2 µl of DNA was added to the PCR reaction mixture containing 23 µl ddH2O, 25 µl 2× DreamTaq PCR Master Mix (Thermo Scientific, Belgium) and 1 µM of each forward primer (5’-CG TAACAAGGTAGCTGTAG-3’) and reverse primer (5’-TCCTCCGCTAAATGATATG-3’) (Imren et al. 2015). The PCR thermal cycler program condition stet were 95°C for 5 min; 48 cycles of amplification (94°C for 30s; 48°C for 45 s; 72°C for 45 s); followed by a final extension at 72°C for 8 min, followed by a holding temperature of 4°C. The PCR products were eletrophoresed as described above.

(d) PCR with D2–D3 expansion fragments of 28S rRNA gene

PCR targeting the D2–D3 expansion fragments of 28S rRNA gene was conducted to identify certain nematodes to species level. The forward primer 28S rRNA D2A (5`-ACA AGT ACC GTG AGG GAA AGT-3`) and 28S rRNA D3B reverse primer (5`-TCG GAA GGA ACC AGC TAC TA-3`) were used to amplified approximately 900 base pairs (bp) of the target gene (Nunn, 1992; Kumari and Subbotin, 2012). A standard reaction volume was 25µl, comprising: of 12.5 µl GoTaq® Colorless Master Mix, 2X (GoTaq® DNA Polymerase is supplied in 2X Colorless GoTaq® Reaction Buffer (pH 8.5), 400µM dATP, 400µM dGTP, 400µM dCTP, 400µM dTTP and 3mM MgCl2) (Thermo Scientific, Belgium) Then 0.5 µm of each primer, 2 µl of DNA template and double distilled water added (ddH2O) was added to make the final volume. The optimal thermocycling conditions were found to be: an initial denaturation at 95°C for 3 min; 35 cycles of amplification (95°C for 1 minute; 56°C for 30 seconds; 72°C for 1 minute); followed by a final extension at 72°C for 5 minutes. The PCR products were eletrophoresed as described above.

3.6 Phylogenetic analysis

The newly obtained gene sequences of plant-parasitic nematodes were aligned with a selected set of other gene sequences obtained from the GenBank. Gene sequences obtained from all positively tested amplicons were edited using BioEdit to remove any degenerate base pairs (Hall, 1999) and saved as FASTA format. To confirm sequences obtained from 18S rRNA and ITS1 rDNA analysis, the nucleotide basic local alignment search tool (BLASTn) was used. Only gene sequences with or more than 50% to 100% similarity match score were considered as significant. Gene sequences of species closely related to newly generated gene sequences in the current study from the BLASTn search results were downloaded from NCBI database. The nucleotide sequences were aligned by Clustal W using multiple alignment fast Fourier transform (MAFFT) program to conduct multiple and pair-wise sequence alignment (Katoh and Standley, 2013). Thereafter, the aligned sequences were trimmed using TrimAl version 1.4 to remove the uneven ends from the aligned sequences. The trimmed alignment was subsequently transferred to MEGA 7 for Maximum likelihood (ML). For ML analysis 10 000 bootstrap replicates were used. Sequences were analysed by using the Maximum Likelihood method based on the Tamura 3-parameter model (Tamura, 1992).

3.7 Statistical analysis

Results were analysed using prominence value; population density and percentage of occurrence

Population density for each nematode species on the sites sampled was calculated as follows:

$$\frac{\text{total number of nematodes present per genus/species}}{\text{number of sites on which the nematode species occurred}}$$

Population density of each nematode species for each region was calculated using the following formula:

$$\frac{\text{total number of a nematode genus/species present at each site for each region}}{\text{number of sites on which the nematode genus/species occurred/region}}$$

Population density of each nematode genus/species on soil and each wheat species per was calculated using the formula:

$$\frac{\text{total number of each nematode genus present on soil/wheat species}}{\text{number of sites on which the species occurred on each wheat species/region}}$$

Frequency of occurrence for each nematode genus/species from the sites is expressed as a percentage and was calculated using the formula:

$$\frac{\text{number of sites on which the nematode genus/species occurred x 100}}{\text{number of sites sampled}}$$

Frequency of occurrence of each nematode genus/species on soil and each wheat species is expressed as a percentage and was calculated using the formula:

$$\frac{\text{number of times the nematode genus/species occurred on soil/wheat}}{\text{number of sites sampled/region}}$$

Prominence values were calculated for plant-parasitic, free-living and predatory nematode populations in root (20 g and 5 g) and soil (200 g) samples (De Waele & Jordaan, 1988) according to the following formula:

$$\text{Prominence value (PV)} = \text{population density x } \sqrt{\text{frequency of occurrence}}/10$$

Statistical software: associations with different provinces, significant differences and nematode populations were evaluated by regression analysis using R studio software package. The one-way analysis of variance (ANOVA) was used to determine whether there are any statistically significant between mean population densities of nematode genus and the geographical areas, while a t-test statistical analyses the densities of each genus in each sample were analysed by both cluster and principal co-ordinate was used to determine if there was significant difference at 5% level in population densities of nematodes between the soil and root samples. Differences at a $P < 0.05$ level were considered statistically significant using the LSD (least significant difference) test. All statistical analyses were performed using an R-studio software package (Hodda, 1990; Hu et al. 2010).

CHAPTER FOUR

RESULTS

4.1 Soil, root and kernel samples

A total of 776 composite rhizosphere soil and root samples were collected from 56 localities over two seasons in the Western Cape (452), Northern Cape (160), Free State (120), KwaZulu-Natal (8), Mpumalanga (12), North West (12) and Limpopo (12) provinces. The properties of the soil and the agronomic practices of the selected wheat fields are representative for the prevailing production conditions used by the farmers in the different areas. In all areas, except in Northern Cape and some fields in the Orange Free State, wheat was grown under dryland conditions. Conservation agriculture was practices in all the trials sampled.

4.2 Soil analyses

The soil pH was slightly acidic to neutral and differed between the sub-counties; EC, which was within the normal range, was not different across sub-provinces (Table 4.1). The P level was higher in all provinces except, which was within normal range (Table 4.1 and 4.2). The Mg level was higher relative in all the provinces and above the normal range (Table 4.1 and Table 4.2). Soil analysis results in WC showed the soil acidity (pH) ranging from 5.1 in Tygerhoek and Roodebloom to 6.7 in Hopefield and Piketberg. The phosphorus (P) ranged from 15 mg/kg in Kleinfortein to 89 mg/kg in Protem. Potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), sulphur (S), acid saturation (AS) and exchangeable acidity (Exch Acid) were also analysed (Table 4.1). On the irrigation fields, the pH ranged from 4.0 in Douglas (NC) to 7.7 in Prieska (NC). P ranged from 21.6 mg/kg in Harrismith (FS) to 100.3 mg/kg in Barkley West (NC). K ranged from 51.1 mg/kg in Potchefstroom (NW) to 428.8 mg/kg in Hopetown (NC). The exchangeable acidity recorded to be 0.27 cmol/kg in Douglas (NC) and 0.38 cmol/kg in Modderriver (NC). Ca, Mg, Na and S were also analysed (Table 4.2).

There were mainly weak correlations between nematode trophic groups and soil chemical characteristics. However, a few strong correlations between irrigation soil and non-irrigated soil were found: fungal feeders and bacterial feeders and PPNs had a similar negative correlation with N, P and Ca, respectively.

Table 4-1: Soil analyses results obtained from non-irrigation areas

Locality	**pH (KCl)**	**P (mg/kg)**	**K (mg/kg)**	**Ca (mg/kg)**	**Mg (mg/kg)**	**Na (mg/kg)**	**S (mg/kg)**	**Exch.acid (cmol/kg)**	**AS (%)**
Bredasdorp	6.0	53	587	1616	201	65	18	0	0
Alpha	5.9	24	524	1629	234	10	29	0	0
Panorama	6.4	78	406	1768	420	35	25	0	0
Kleinfontein	6.3	15	246	1720	354	247	27	0	0
Koringplaas	5.5	34	370	762	133	104	20	0	0
Koperfontein	5.4	20	105	160	41	22	10	0	0
Malmesbury	5.5	34	370	762	133	104	20	0	0
Philadelphia	6.5	43	160	1556	129	165	25	0	0
Piketberg	6.7	38	348	1032	70	32	52	0	0
Hopefield	6.7	67	178	705	97	95	11	0	0
Langkloof	5.4	52	340	743	130	45	29	0	0
Roodebloem	5.1	22	356	1021	141	63	10	0	0
Tygerhoek	5.1	21	293	731	793	66	7	0	0
Hopefield	5.9	61	104	278	132	16	6	0	0
Langrug	6.0	57	50	938	202	41	5	0	0
Napier	6.4	19	531	2888	518	97	23	0	0
Protem	5.9	89	159	1785	436	75	8	0	0
De Vlei	6.0	57	325	1388	316	71	6	0	0

Table 4-2: Soil analyses results from various irrigation localities

Locality	pH (KCl)	P (mg/kg)	K (mg/kg)	Ca (mg/kg)	Mg (mg/kg)	Na (mg/kg)	S (mg/kg)	Exch. acid (cmol/kg)
Harrismith (JL)	5.7	24	155.7	762	121	258.8	19	0.00
Harrismith (TF)	5.7	21.6	201	1037	226	4.0	5.51	0.00
Clarens	5.5	61.7	192.1	873	146.3	9	89.92	0.00
Prieska 1	7.7	46.5	369.9	2013	447.3	65.0	39.87	0.0
Modderrivier 1	5.7	59.1	266.1	559	113.2	6.5	9.31	0.0
Hopetown	6.1	41.7	241.6	2243	404.2	36.5	8.17	0.0
Modderrivier2	4.1	54.0	152.4	438	103.9	5.5	3.22	0.38
Douglas Late	4.0	77.3	249.9	2620	292.2	24.6	157.90	0.27
Barkley west	7.2	100.3	267.6	936	202.1	42.9	28.31	0.00
Prieska2	5.6	41.0	75.3	1248	363.4	36.7	1.86	0.00
Hopetown 2	6.8	80.1	428.8	3264	419.9	38.8	15.68	0.00
Potchefstroom	7.0	21.9	51.1	768	255.7	35.1	56.89	0.00

4.3 Nematode survey

Twenty four genera of plant-parasitic nematodes distributed over 56 localities in irrigated, non-irrigated, and high and low altitudes under different climates, were found to be associated with wheat crops in this study. Nematode prevalence, relative abundance, frequency of occurrences (FO%) and prominence values (PV) for these nematodes are presented as follows:

4.4 Morphological analysis

The most prevalent nematode genera across most agricultural fields in all sampled provinces sampled were: *Pratylenchus, Scutellonema, Helicotylenchus, Rotylenchus, Criconema, Tylenchus, Hemicyclophora and Ditylenchus.* Other genera with lower prevalence and relative abundances, but found occasionally at high densities, were *Dolichodorus, Tylenchorhynchus, Hoplolaimus, Longidorus, Xiphinema, Coslenchus*, *Panagrolaimus, Trichodorus* and *Paratrichodorus* (Table 4.3 4.4; 4.5; 4.6). Free nematodes species identified during this study were; *Panagrolaimus, Acrobeles nanus, Zeldia punctata, Aporcelaimellus, Dorylamida* and. *Rhysocolpus pararoxus.*

The most dominant species recovered across the sampled field were, *Pratylenchus neglectus, Rotylenchus unisexus, Scutellonema brachyurus, Pratylenchus* sp. *Meloidogyne* sp *Aphelenchus* sp. *Ditylenchus* sp. and *Dolichodorus* sp. These were identified morphological and those identified to genus level were further identified using molecular techniques.

Common diagnostic characters used to identify the species are found in the stylet and pharynx. Length of the stylet and the relative length of its conus are useful, as are the size and shape of the stylet knobs Siddiqi (1971; 1986) (Plate 4.1). These include the tail length and structure (Plate 4.2), the cuticle and the vulva/caudal position (Plate 4.3; 4.4) and more often than not, the male of the species. The identification using the metacorpus (median bulb) and the length of the head are only used to identify few species (Plate 4.5). If the head region (A), the vulva structure or position (B) and the tail and annuli (C), are not clear under the dissecting microscope (Plate 4.6). The scanning electron microscope is useful for identifying the internal diagnostic features of the species (Plate 4.7). Identifications of the species were conducted by the taxonomists at the Agricultural Research Council-Plant Protection Institute, Pretoria, South Africa. Plates displayed in this study, demonstrates the examples of identifications processes of species displaying obvious feature. Some species such as, *Pratylenchus thornei, P. bolivianus, Paralongidorus bikanerensis, Pararotylenchus brevicaudatus, Bitylenchus maximus, Bitylenchus ventrosignatus, Merlinius brevidens, Hoplolaimus galeatus, Paramerlinius hexagrammus, Amplimerlinius paraglobigerus, Ecumenicus monohystera, Pararhyssocolpus paradoxus, Acrobeles complexus, Aporcelaimellus obtusicaudatus, Aporcella simplex* and *Alaimus sp.* were only identify using the molecular diagnostics (Table 4—7 and Table 4—8).

Pratylenchus neglectus **Filipjev & Schuurmans Stekhoven, 1941)**

Female

Female Body 437–568 μm long, slightly curved after fixation, cuticle finely annulated with 1.0–1.5 μm wide at midbody. Maximum body diameter 16–21 μm. Lip region flat, with slight depression, continuous with the body, bearing two annuli, the second anuuli wider than the first one. Lateral field with four lines, two outer lines crenated, occupying about 22–27% of midbody diameter. Stylet length 15–17 μm; basal knobs usually rounded and flatted, upward. Dorsal pharyngeal gland opening (DGO) at 1.8–3.6 μm posterior to stylet base. Median bulb oval, with conspicuous valve at the middle, nerve ring located just after median bulb, at 49–56%of the neck. Excretory pore at 61–85 μm from anterior body, at 66–73% of the neck. Hemizonid two annuli anterior to excretory pore. Glands overlapped with intestine about 25–42 μm, pharynx 58–89 μm long, body length about 5.5–7.9 times pharynx length. Ovary not reached to pharyngeal glands. Oocytes in one or two rows. Vulva at 71–82% of body length. Spermatheca round, without sperm. Post-vulval uterine sac 16–22 μm long, 0.8– 1.3 of corresponding body diameter, vulva with a transverse slit. Tail terminus shows variation, round, smooth and in some specimens with fine groove. Tail 19–23 μmlong, about 2.0–2.8 times anal body diameter. Phasmid located at posterior half of the tail, 53–60% of tail length.

Pratylenchns tlrornei **Sher & Allen, 1953.**

Female

Body large and slender, assuming an open “C” shape when killed by genfle heat. Cuticle with transverse striae about 1 p apart, not conspicuous. Lateral field with 4 incisures, the outer onesl paight or weakly crenate. In one specimen, oblique striae were observed by Loof (1960) in the central zone. Lip region with 3 annules, not set off from body. Outer margin of sclerotized labial framework extends conspicuously about 2 annules into body and about 1 annule into 1ip.region. Spear guiding apparatus extends posteriorly from basal plate for about 4 annules. Spear medium sized (17-19 µ long) with broadly rounded to almost anteriorly flattened basal knobs. Orifice of dorsal oesophageal gland about 3 µ behind spear base. Nerve ring directly behind oesophageal bulb; hemizonid about 2 annules long, I annule anterior to excretory pore. Ovary not extending up to oesophagus; Ovary not extending up to oesophagus; oocytes in the single row, row, except for the anterior zone of multiplication; oviduct indistinct, uterus short, Spermatheca difficult to see, not containing spermatozoa (males very rare); post uterine sac a little more than one and one half times body width a vuIva. Phasmids slightly posterior to middle of tait; the 4 incisures extend past the phasmids. Tail dorsally convexconoid, terminus bluntly rounded to truncate, unstriated.

Meloidogyne **sp.**

Female

Species of *Meloidogyne* were identified based on female adult morphology, including head structures, perineal patterns, and stylet (Eisenback et al. 1980). The perineal pattern is a valuable morphological feature used in the species identification of the genus *Meloidogyne* (Chitwood, 1951; Eisenback et al. 1980; Hirschmann, 1985). However, significant perineal pattern variability observed in females from a single-egg mass population undermines the value of this character for comparing *Meloidogyne* species (Chitwood, 1951; Netscher, 1978; Karssen and van Aelst, 2001; Carneiro et al. 2004). No adult females were recovered. Only second-stage juvenile were counted to the genus level.

Scutellonema brachyurus **(Steiner, 1938) Andrássy, 1958 South African populations, type B (Spiral nematode)**

Female

Body form ranging from an open to closed spiral. Lip region rounded, slightly set off with distinct labial disc with mostly three but also four lip annuli when seen laterally, labial disc rounded with small amphid openings laterally. SEM photographs showing mainly three, rarely 4-5 lip annuli, great variation in number and arrangement of longitudinal and oblique lines on lip annuli but mostly confined to basal lip annulus. Circa 8-20 regular and irregular blocks seen on basal annulus. Lip region slightly set off from body. Labial framework moderate. Anterior and posterior cephalids not seen. Stylet robust with stylet knobs rounded posteriorly and slightly hollow anteriorly. Metenchium shorter than telenchium, rarely of equal length. Median bulb slightly longer than wide, not filling body cavity. Pharyngeal gland overlapping dorsally with three gland nuclei. Excretory pore situated from rarely opposite middle of isthmus to mostly opposite basal pharyngeal lobe, as far posterior as opposite posterior part of pharyngeal lobe. Hemizonid two annuli long, situated from opposite excretory pore to five annuli anterior to excretory pore. Hemizonion indistinct in most specimens, in one specimen it was one annulus long and situated 13 annuli posterior to hemizonid. Annuli on body distinct. Spermatheca visible in most specimens but small, round and empty. Vaginal glands small, oblong, not very distinct. Epiptygma appearing double and folded into vagina or over vulval opening. Intestine mostly not overlapping rectum. Scutellum size moderate, situated from three annuli anterior to three annuli posterior to anus. Lateral fields with four lines, areolated anteriorly opposite without any areolation around scutellum. Tail rounded, more so on dorsal side with 9-17 annuli, those on posterior tip slightly larger than on rest of body.

Female (n =3): L=739 (744-826) µm; a= 27.7 (25.9-29.8); b = 5.1 (4.8-5.4); c = 56.6 (50-63.3); c= 0.75(0.7-0.8); o = 20.2 (15-25.7); V = 57 (56.5-57.5); G1 =23 (n = 1); G2 = 23 (n = 1); stylet = 28 (25.5-29.5) µm;stylet knob height = 3 µm; stylet knob width = 4.5 µm; metenchium = 13.5 (13-14) µm; telenchium = 16 (15.5-16) µm; m = 46.2 (44.9-47.6); lip region diam. = 9 (8-9.5) µm; lip region height = 5 µm; DGO = 5.5 (4.5-6.5) µm; median bulb length = 13 µm; median bulbdiam. = 12 µm; excretory pore from anterior = 118 (114-134) µm; pharynx length = 155 (151.5-157.5) µm; diam.at mid-body=28.5 (27-30) µm; diam. at anus=19 (17.5-21) µm; lateral fields width = 6 (5-7) µm; scutellum length = 4.5 (3.5-5) µm; scutellum width = 4 (3.5-5) µm; tail length = 14 (12-16.5) µm.

Rotylenchus unisexus **Sher, 1965 (Spiral nematode)**

Females (n=309): L=0,7 mm (0,5-0,9); a=25,9(19,0-33,5); b=6,6 (5,3-9,0); b'=5,6 (4,4-7,0);c=51,9 (30,3-105,6); c'=0,7 (0,3-1,3); 0=23,4(10,1-33,0); V=56 (47-59); OV I =23 (16-37);OV2=22 (15-34); spear=24,3p (19,5-28,7) Female: Body posture varying from a complete circle to a C. Lip region rounded, varying from not set off to slightly set off, with mostly 4 annules, though in some specimens 3 and 5 were noticed. Outer margins of labial framework reaching back It to 3 annules posterior from basal plate. Spear knobs rounded posteriorly and flattened to slightly hollow anteriorly, 4,7j1 broad (3,7 to 6,6) and 2,6j1 high (1,8 to 3,7). Metenchium mostly shorter than telenchium, but a few specimens (11%) were found where the metenchium was slightly longer than the telenchium (m=40 to 51 %). Median bulb 12,9j1 long (11,0 to 15,0) and 10,2/1 broad (7,7 to 13,6). Posterior margin of median bulb 76, 2j1 (64, ° to 90,1) from front end of body. Position of excretory pore varying from opposite basal part of oesophageal lobe to middle of isthmus, 101, 5j1 (77,9 to 136,0) from anterior end of body. Hemizonid ito 3 annules long, its position varying from just posterior to 1 annule anterior to excretory pore. Hemizonion 5 to 13 annules posterior to hemizonid, -!- to 1 annule long. Anterior cephalid 2 to 5 annules and posterior cephalid 8 to 12 annules from base of lip region.

Width of annules on body I, 5j1 (0,9 to 2,1). Spermatheca not seen, but in a few specimens a small empty spermatheca was noticed. Epiptygma mostly double and appearing to be bent into the vagina. Intestine overlaps rectum slightly in most specimens, but some were noticed with no overlap. Lateral field aerolated only anteriorly from about middle of spear to oesophageal region. Phasmids 4 to 17 annules anterior to anus. Caudalid 3 to 7 annules anterior to anus, I to 2 annules long. Tail mostly rounded, but variations do occur, such as slightly pointed and indented; 6 to 15 annules, 14,0j1long (6,6 to 21,7).

***Helicotylenchus dihystera* (Spiral nematode)**

Female

Identification of spiral nematodes up to species level was done by satisfying the characteristic features described by Siddiqi (1972). Body of adult female is spiral, length is 630-810 µ, a=30, b = 6. The body shape becomes coiled after death due to heat. Lip region hemispherical, cephalic framework is conspicuous, head slightly set off and conoid. Stylet is well developed. Dorsal esophageal gland orifice (DGO) is situated at less than half of spear length behind the knob. Esophagus is tylenchoid type. Basal bulb overlaps anterior part of intestine from ventral side. Ovary is didelphic, amphidelphic and outstretched. Vulva is transverse slit like at 65-70% of the body from anterior. Tail is short, conoid with a narrow terminus called mucron.

Ditylenchus dipsaci

Female

Lip region flattened, 2Æ7 ± 0Æ4 (2Æ0–3Æ3) lm high and 7Æ8 ± 0Æ5 (6Æ7–8Æ7) lm wide, separated from the rest of the body by a slight constriction. Under the light microscope, the lip region contour appears smooth in two-thirds anterior and with a basal annulus in the third posterior (Fig. 2h). In SEM view, labial area with quadrangular outline, showing incisures like incomplete annuli in submedial and subdorsal sectors of the first lip annulus (Fig. 4b), giving the appearance of a lip region composed of three to four annuli. Stoma opening porelike, in the middle of a slightly raised, small and circular oral disc. Amphidial apertures often partially covered by debris, and therefore difficult to detect. Stylet delicate, conus 5Æ5 ± 0Æ2 (5–6) lm long, knobs distinctly sloping backwards, 2Æ8 ± 0Æ3 (2Æ3–3Æ3) lm across. Dorsal gland orifice (DGO) 1Æ9 ± 0Æ5 (1Æ3–2Æ7) lm, 15Æ4 ± 4Æ1 (11– 23) % of stylet length. Median bulb oval, measuring 20Æ4 ± 1Æ7 (17–24) lm and 12Æ5 ± 0Æ7 (11Æ3–14) lm in longitudinal and cross diameter, respectively. Isthmus elongate, slender, 61 ± 6Æ9 (51–72) lm long, slightly swelling in the medial third. Basal pharyngeal bulb ovate to quadrangular with rounded margins, shortly overlapping intestine 1Æ5–10 lm. Lateral field with four plain lines (Figs 2e and 3h), the inner two appearing sometimes faint and indistinct (Fig. 4f). Ovary mono-prodelphic, outstretched, 1084 ± 120 (868–1265) lmlong, 63 ± 6Æ6 (50–72) % of body length, with the apex of germinal zone sometimes reaching to middle of pharyngeal bulb. Spermatheca strongly elongated, 127 ± 22Æ9 (98–176) µm long, with its posterior end distant 174 ± 35Æ6 (131–223) lm from vagina, usually filled with round sperm. Uterus with a quadricolumella, comprised of four rows of four cells each (Fig. 4e), followed by a proper uterus which swells near the vagina. Post-vulval uterine sac well developed, 2Æ0 ± 0Æ3 (1Æ7–2Æ5) times the vulvaanus distance. Tail elongate conoid, tapering posteriorly to a finely rounded terminus. Phasmids were seen in two specimens only, located at 63 and 76 lm from the tail terminus (Vovlas et al. 2011).

Criconemoides sp. (Ring nematode)

Female

The nematode was identified using the descriptions of Loof (1974). Body of the adult female is short and fusiform, both terminus spherical to truncate under light microscope. Body consists of 92-112 annules. Annules course, retrose, margin irregularly serrated. Average length of adult female is 506.5 µm, a=12.65, b=4.22 and c=16.87. Stylet strong, with anchor shaped base. Esophagus criconematoid type. Excretory pore is just behind the esophageal base. Vulva is a transverse slit. Ovary single, reaches beyond the esophagous base. Anus is very indistinct. Tail conoid, terminus truncate to broadly rounded.

***Hoplolaimus* sp. (Lance nematode)**

Female

Body of adult female is elongate-cylindrical, tapering towards both ends. Length of the body is 1104-1202 µ, a, b and c values are 28.78, 8.2 and 53.82, respectively. Cephalic framework strongly sclerotized. Head is distinctly set off, 10.1 µm in height. Stylet massive, 35 µ long with anteriorly projected knobs. Esophagus tylenchoid, 131 µm long. Esophageal gland overlaps the intestine dorsally and laterally. Ovary two, outstretched. Vulva is conspicuous, transverse slit, 10.0-12.0 µm wide with two distinct lips. In female, tail short, 22.1-22.4 µm long, 25.5-26.5 µm wide at anal body, with round terminus. In male, tail conoid, enveloped by bursa. Anus is circular, 2.0-2.2 µm diameter. All morphological characters described above are similar as described by Handoo and Golden (1992).

Dolichodorus sp.

Female

Body long and cylindrical, slightly arcuate ventrally. Cuticle 1.5-2.0 μm thick, clearly annulated; annuli 1.8 ± 0.4 (1.5-2.5) μm wide at mid-body. Lateral fields areolated, with three equidistant incisures, 8.6±1.0 (7-11) μm wide; beginning at ca stylet knob level and ending posterior to anus. Lip region rounded, prominent, clearly offset from body, 12.9±1.4 (11-16) μm diam. and 6.9±0.6 (6-8) μm high, bearing 4-6 very fine annuli. Oral disc clearly separated from lip region, distinguishable under light microscope, 3.9 ± 0.6 (3-5) μm wide. Anterior portion of lip region with strong labial framework and massive basal plate. SEM micrographs revealing presence of cavities or clefts under oral disc, and a four-lobed lip region with two large subdorsal and two large subventral lobes; two 2.5-3.0 μm long, longitudinally oval, amphidial openings. Stylet long and flexible. Stylet conus 55.5 ± 3.9 (48- 64.5) μm long; stylet shaft 37.0 ± 3.1 (28-43) μm long, with a short constricted area immediately anterior to stylet knobs. Stylet knobs rounded posteriorly, anterior surfaces inclined, 7.2 ± 0.7 (6-8) μm wide. Dorsal pharyngeal gland orifice 6.3±3.0 (4.5-11) μm from stylet base. Procorpus of pharynx cylindrical with slight depression just anterior to median bulb, 121.6±11.1 (100-146) μm long. Median pharyngeal bulb well developed, oval, 27.5 ± 3.0 (23-35) × 16.2 ± 2.0 (13-20) μm; with valvular apparatus 8.1 ± 0.7 (7-9.5) μm long. Isthmus 34 ± 6.0 (24-48) (Landa, et al. 2007).

Trichodorus magnus n. sp.

Female

Body straight or slightly curved ventrad upon fixation. No dorsal intestinal overlap or subventral ta ventral overlap of the pharynx. Excretory pore at 1.82.0 rimes the onchiostyle length from anterior end i.e. at level isthmus, from slightly posterior to the nerve ring to opposite the anterior end of pharyngeal bulb. Reproductive system didelphic-amphidelphic with reflexed OVâries, each branch with a large spermatheca, obvious when filled with Sperm. Vulva a pore in ventral view. Vagina shape more or less cylindrical, 22-24.5 fLm long, i.e. 41-47 %of corresponding body width. Vaginal sclerotizations weil developed, rounded triangular to oval shaped, about paralJel with vaginal lumen in lateral view. One pair of advulvar sublateral body pores, \vith corr€sponding pores not always at the same level. (up to13 fLm apart), 24-41 fLm posterior of the vulva i.e. 0.4~0.8 body widths. Tail rounded. Anus and one pair of caudal pores ventro-subterminal (Decraemer and Marais, 1993).

***Nanidorus minor* (Colbran, 1956) Siddiqi, 1974**

Female

body straight or slightly curved upon fixation. Cuticle smooth about 4.6 µm separated from subcuticle on median body, gradually tapering towards both ends. Head rounded, continuous with body contour. Pharynx expands into pyriform basal bulb that overlaps intestine ventrally and subventrally. Onchiostyle curved, guiding ring 5.8 µm from oral aperture. Secretory-excretory pore situated 12.7 µm posteriorly pharynx. Reproductive system amphididelphic, reflexed. Vulva situated 27.3 µm posterior from mid body. Sclerotizations within vagina rod-like, parallel to the longitudinal body axis, 6.4-9.6 µm from cuticle. Rectum 14.4 µm long. Tail 7-11 µm long and bluntly rounded. Lateral body pores absent. Male: not found Measurements Female (n = 8): L = 656 ± 52.1 (567-699), body width = 30.5 ± 3.7 (24.0-34.6), a = 21.9 ± 4.0 (18.4-28.9), b = 5.1 ± 0.6 (4.5-5.9), onchiostyle length = 36.2 ± 2.6 (32.8-41.0), pharynx length = 87.7 ± 10.8 (80.0-113.4), distance oral aperture to guiding ring = 5.8 ± 0.5 (5.2-6.4), secretory excretory pore = 100 ± 5.2 (94.2-109), pharynx-intestine overlap = 8.3 ± 0.8 (7.1-9.6), V = 54.1 ± 1.5 (51.3-55.6). Morphology of the present specimens were within the range of those described by Colbran[30], Siddiqi[39], Allen[40], Vermeulen & Heyns[36] and Park et al.[41], but few exceptions occurred. Total length of the Ethiopian specimens was similar to the type description from Australia (567-699 µm vs 550-700 µm) but was longer than specimens from India (480-550 µm) and Korea (428-506 µm). Onchiostyle length of the Ethiopian specimens was close to those from South Africa (32.8–41.0 µm vs 26–39 µm)[30, 36, 39, 41] but was longer than for specimens from India (26-30 µm), Japan (18-28 µm) and Korea (24.9-31.4 µm).

***Paratrichodorus* sp.**

Males

Habitus straight. Lip region with indistinct labial sensilla. Cuticle strongly swollen upon fixation, consisting of two layers of approximately the same thickness, 17 ± 5.6 (13-20) 11 µm thick at midbody. Nerve ring situated just posterior to onchiostyle base.

Secretory-excretory pore at 1.5- 1.8 times onchiostyle length from anterior end of body, opposite posterior part of pharyngeal isthmus. No ventromedian cervical papillae or lateral cervical pores were discernible. All specimens with both a ventral pharyngeal gland overlap and a dorsal intestinal overlap. Pharyngeal gland overlap more pronounced, 19 ± 7.9 (11-28) 11m long. Intestinal overlap 8 ± 3.3 (4-13) 11m long. Five pharyngeal gland nuclei present. Dorsal gland nuclei usually at midbulb. Two, exceptionally three, precloacal ventromedian supplements present. Posterior (SP1) and median supplements (SP2) situated at 21-22 % and 45-50 %, respectively, of spiculum length anterior to cloacal opening. Anterior supplement (SP3) at 156 %, i.e. out of reach of retracted spicules. Spicules finely striated without bristles. Distal half of gubernaculum thickened. Anterior lip of cloaca with two finger-like projections. Tail 18 ± 2.4 (15-22) 11m long, with one pair of postcloacal subventral papillae and one pair of subterminal subventral pores; caudal alae present (Baujard, 1980).

***Tylenchorhynchus* sp.**

Female

Body arcuate to open C shape after fixation, no longitudinal striae or ridges. Body annules distinct 1.6–2.4 μm wide around midbody, 0.8–2.4 μm at oesophageal end and 1.6–2.4 μm at tail regions. Lateral fields originating at the conus of the stylet and extending up to hyaline region of tail, with four incisures, outer two incisures crenate. Lip region low, rounded, almost continuous to body contour; with three to four annules. Stylet moderately strong, shaft and conus equally long 8–8.6 μm; knobs anteriorly directed. Dorsal gland orifice (DGO) about 2.4 μm long behind stylet base. Median oesophageal bulb oblong, basal bulb pyriform. Cardia well developed. Hemizonid usually just anterior to excretory pore, 1–1.5 annules wide. Vulva a transverse slit slightly posterior to the middle of the body and distinctly protruding. Epiptygma absent. Reproductive system amphidelphic, didelphic; anterior and posterior ovaries equally developed. Spermatheca rounded, filled with rounded spermatozoa. Tail conoid to bluntly rounded hemispherical, tail terminus smooth. Phasmids located slightly anterior to middle of the tail, 12–16 annules posterior to anus. Post anal extension of intestine absent (Castillo et al.1989).

Tylenchus sp.

J2 female

Body slender, curved slightly ventrally. Body transverse striae very faint, almost not recognisable. Lip region rounded, not set off from body. Cephalic framework weak. Stylet well developed with large rounded knobs. Median bulb oval, basal bulb pyriform. Nerve ring posterior to median bulb. Excretory pore situated at 49.8-58.4% from anterior end. Genital primordium with 2-4 cells, 61-70% from anterior end. Anus and rectum not visible in fixed specimens. Hyaline posterior body portion ending in a finely pointed or mucronate terminus. J2 male Not examined (Van Gundy, 1958).

Coslenchus rhombus **(Andrassy, 1982).**

Female (n=1.5): under compound microscope fourteen longitudinal striae + lateral field. Lateral fields one fifth to one quarter body width, with four incisures at midbody, inner incisures closely spaced . Annules 2.5-3.0 µm wide near midbody. Eight specimens show some annule divided into secondary small ones, irregularly distributed on the intestinal region and either dorsal or ventral, but in the seven other specimens such a characteristic was not observed. Head region rounded and slightly truncate anteriorly, slightly offset by constriction, bearing four annule. Vulva slightly sunk into body. Vulva membranes not observed, but the blocks adjacent to vulva are modified, sometimes giving the appearence of vulval membranes of one to one and a half annules wide, when the nematode is seen laterally. Vagina at a right angle to the body axis or directed slightly forward and with thickened walls. The ventromedial ridge of the cuticle diverges anteriorly o the vulva. Joining it posteriorly, giving a rhomboid-like appearance to the area surrounding the vulva. Post vulval uterine sac lenght one quarter to one third vulval body width. Spermatheca ppoorly developed. Tail terminus finely pointed.

Quinisulcius capitatus Siddiqi, 1971, Quinisulcius capitatus (Allen, 1955) Siddiqi, 1971. Syn.: Tylenchorhynchus capitatus Allen, 1955, Siddigi 2000

Female

Body strongly arcuate to C- or spiral shaped, being typically depressed (concave) mid-ventrally for about 30 µm anterior and posterior to vulva. Annuli fine, particularly along ventral side, width usually 1 µm or less (0.6-1.5 µm). Lateral field 8-10 µm wide, 33-42% of body width, marked by 5 incisures and scattered areolations; outer incisures crenate, inner ones irregularly linear, middle incisure terminating at the phasmid. Head rounded, with 6-8 annuli, set off from body by constriction well posterior to base of head framework; framework weak. Stylet medium-built, knobs rounded, sloping posteriorly, 3-

4 µm across. DGO 1.5-4 µm. Median bulb 15-20 x 10-12 µm. Basal bulb elongate-pyriform, 30-35 x 10-14 µm. Cardia spherical. Intestinal densely globular, with “thick” fasciculi throughout its length; post-rectal intestinal sac absent. Spermatheca small, axial, never observed with sperms. vulva depressed, always observed cntered 51-58% of the body.

Merlinius joctus **(Siddiqi, 1971) Sher, 1974**

Female

Body slightly arcuate; annules averaging 1 µm wide near middle; lateral fields 3/11 to 1/4 as wide as body, outer incisures crenate. Lip region rounded, marked off from body by gradual expansion, 7 µm wide and 3 µm high from anterior border of basal plate; framework lightly sclerotized with outer margins extending to seven to nine annules from head end. Anterior and posterior cephalids indistinct, at nought and eight to ten annules behind outer margins of cephalic framework, respectively. Spear slender, more than twice labial width long, with needle-like conus 9 µm long and posteriorly sloping basal knobs 3 µm across; spear shaft shrunk in some specimens giving a lesser value for spear length and greater value for the ratio m. Median and basal bulbs of oesophagus in lectotype measure 19 by 11 µm and 27 by 12 µm, respectively; distinct nucleus of dorsal gland anterior to middle of basal bulb; oesophago-intestinal valve rounded. Excretory pore 82-92 (88) µm from anterior end, just behind 3-4 µtm long hemizonid; deirids at level of posterior margins of hemizonid, distance from centre of median oesophageal bulb to deirids is almost equal to that from the latter to base of oesophagus. Vulva on a raised ventral protuberance of body, epiptygma sunken; spermatheca bilobed, with sperms. Tail elongate-conoid, slightly arcuate, with 56, 59 and 69 ventral annules and a minutely rounded smooth terminus (indented in one paralectotype female) lacking a mucro or process. Phasmids in lectotype at about two anal body widths from anus, right and left ones at 46% and 50% of tail, respectively.

***Hemicycliophora onubensis* sp. n*.** (Van den Berg, 1985).

Female

Body straight or slightly ventrally arcuate. Annuli rounded and with ornamentation appearing as small blocks inside annuli. Cuticular sheath somewhat detached from inner cuticle, distinctly detached in pharyngeal and tail regions. Lip region following contour of body, not offset, rounded, with two annuli and a slightly elevated labial disc, 7.7 ± 0.6 (7.0-8.0) µm wide, body annuli more than 240. Lip patterns, as observed with SEM, consisting of a slightly ovoid labial plate or first lip annulus of larger diam. than second. First lip annulus with a slightly elevated and rectangular labial (= oral) disc showing in some specimens a slit like-stoma. Labial disc dorsoventrally orientated, flanked by large and crescent-shaped amphids. Lateral lip sectors fused with dorsal and ventral lip sectors that are partially fused with labial disc. Pseudolips not observed. Pattern of lateral fields marked by anastomoses or breaks. In SEM view, two longitudinal ridges apparent in lateral field, but there is no demarcation of lines. Stylet slightly curved and flexible, with rounded knobs, slightly directed posteriorly with a small cavity present. Excretory pore posterior to pharyngeal basal gland. Vulva closed with modified lips, anterior and posterior vulval lips elongated, vulval sleeve long, spermatheca rounded

***Xiphinema* sp. (Lamberti et al. 1983)**

Female

The morphometric characters of a single female of Xiphinema sp. found in the rhizosphere of grapevine at Becej are: L = 2.4 mm; a = 57.5; b = 5.1; c = 57; c' = 1.3; V = 43; odontostyle = 131 fJ.m; odontophore = 64 f1.m; oral aperture to guiding ring = 113 f1.m; tail = 42 f1.m; J =6.5 f1.m; body diameter at lip region = 12.5 f1.m; body diameter at guiding ring = 34 fJ.m; body diameter at base of oesophagus = 38 fJ.m; body diameter at vulva = 41.5 f1.m; body diameter at anus = 31.5 fJ.m; body diameter at beginningof J = 14 f1.m.

***Aphelenchoides* sp.**

Female

The identification of Aphelenchoides sp. was done based on the descriptions of Allen (1952). Body of adult female was slender, straight or arcuate when relaxed. Length of adult female was 557 µ, a (ratio of body length and maximum body width) = 27.85, b (ratio of body length and esophageal length) =5.05 and c (ratio of body length and tail length) = 15.91. Lip region is smooth, elevated. Stylet slender about, 10-11 µ long; basal bulb minutebut distinct. Esophagous aphelenchoid type. Esophageal gland elongate, extending over intestine. Vulva transverse slit. Ovary single anteriorly outstretched. Tail elongate conoid. Adult male has well developed spicule (Christie, 1942).

***Panagrolaimus* sp.**

This Panagrolaimus sp. is readily cultured. The generation time from egg to adult at 20 C on NGM plates is about 8 days. Males are necessary for reproduction. Morphometric measurements of this nematode are as follows: Female (n = 10): L = 1.05-1.45 mm (1.22 --- 0.15 mm); a = 15-21; b = 6.5-8; c = 14.5-18; v = 57- 60%; Male (n = 10): L = 0.80-1.05 mm (0.91 + 0.08 mm); a = 18-21; b = 5-6; c = 18-25; spicules = 28 +- 3 µm. A comparison of these measurements with published data was not conclusive and necessitated that a male species was needed for further identification (Bostrom, 1995).

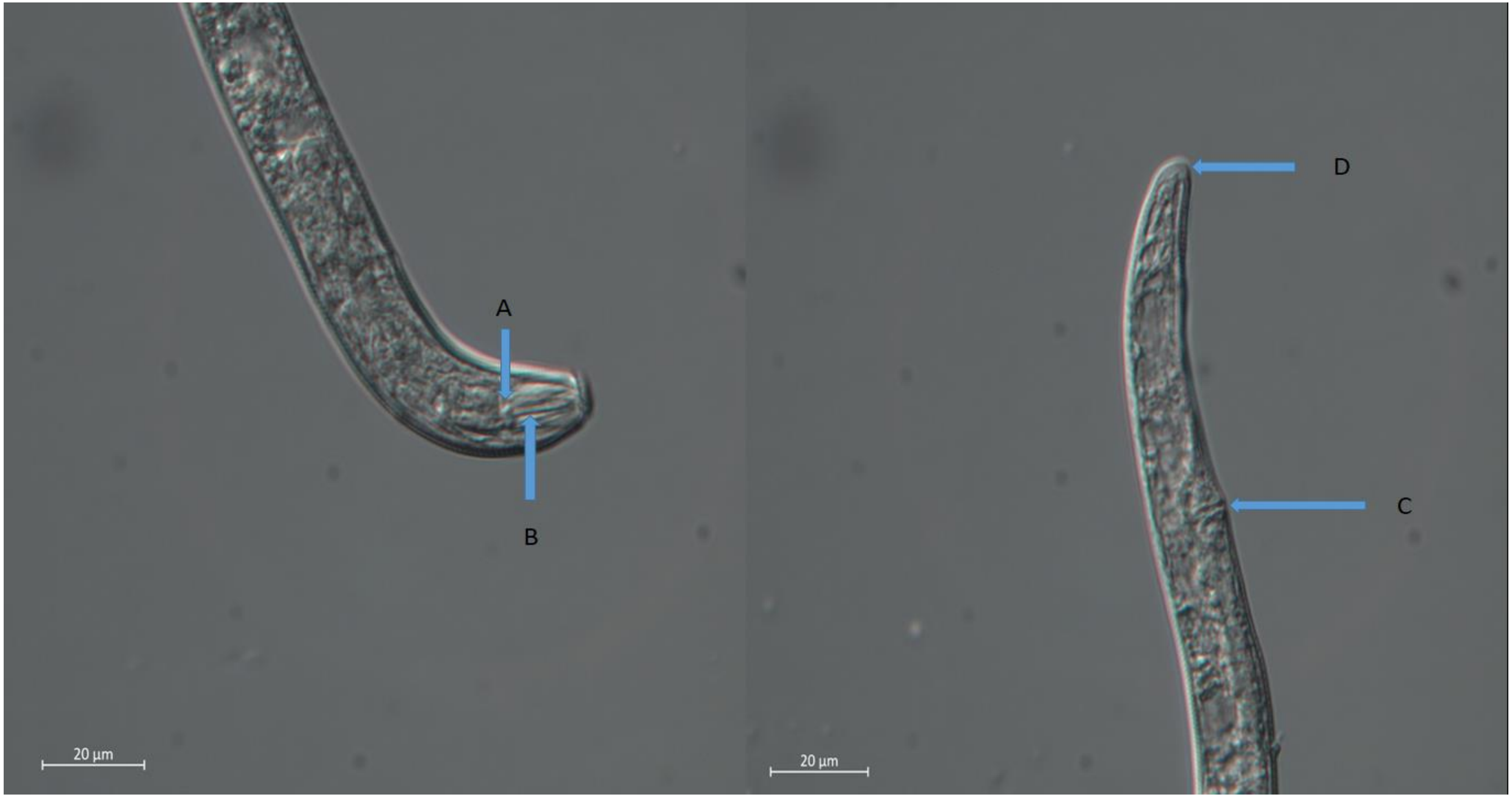

Plate 4.1: Morphological features of the female *Pratylenchus* sp. (*Pratylenchus thornei*). **(A & B)** stylet and stylet knobs well developed; **(C)** Vulva situated about 80% of body length; **(D)** tail blunt; it has the finest annulation, sometimes appears to have smooth cuticle

Plate 4.2: Morphological features of the female *Scutellonema* sp. (*Scutellonema brachyurus*). **(A)** Stylet well developed, 26-30 µm long, in two almost equal parts; **(B)** basal knobs prominent, rounded with slightly flattened anterior surfaces, 4.5 µm across **(C)** lip region broadly rounded, well set off with 4-6 annuli; **(D)** Phasmids enlarged, 3-4 µm in diameter.

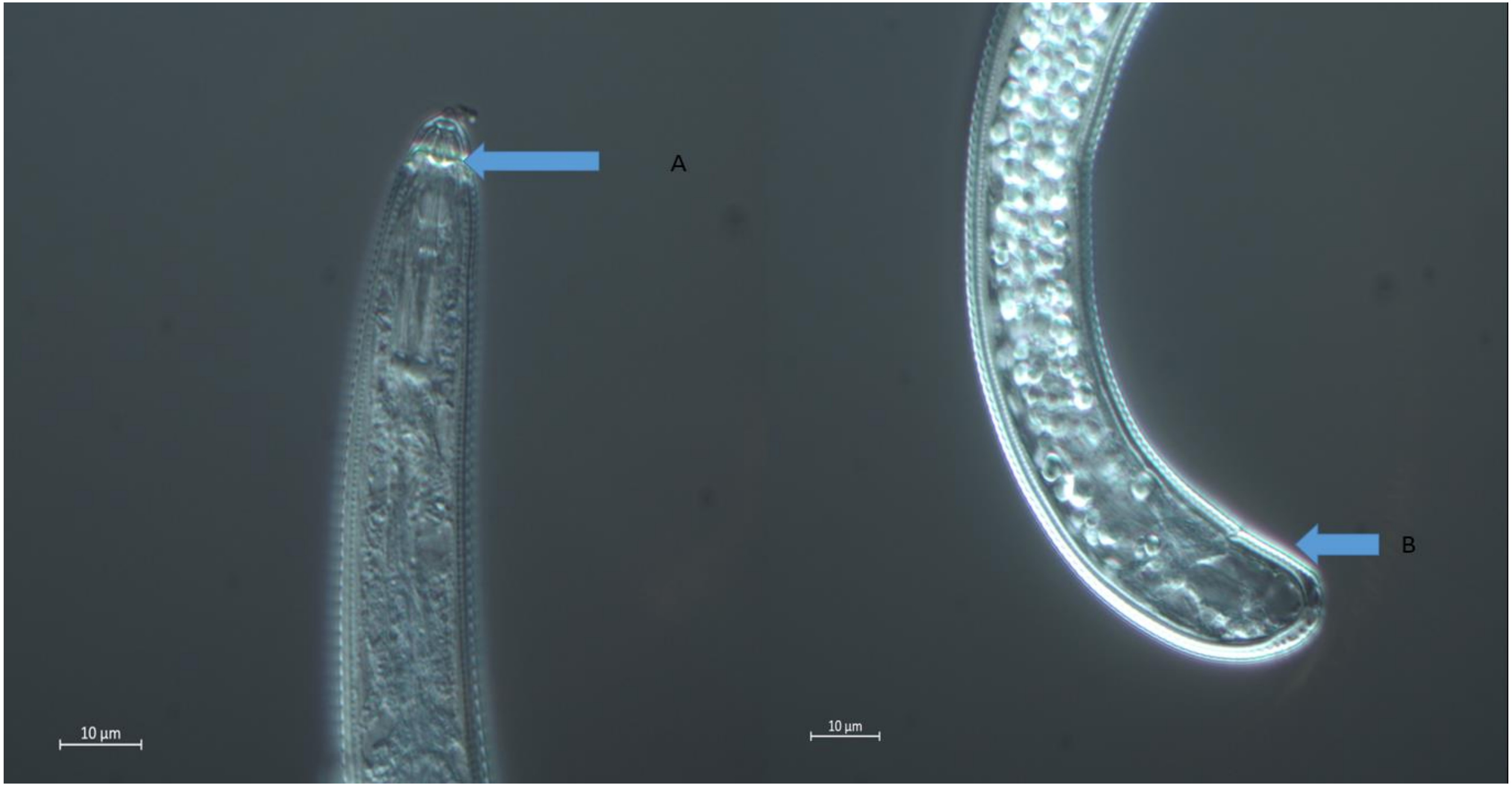

Plate 4.3: Morphological features of the female *Rotylenchus* sp. (*Rotylenchus unisexus*). **(A)** sclerotized head and DGO distance from stylet end; **(B)** lateral lines joined at tail tip and rounded tail. Scale bars: 20 µm

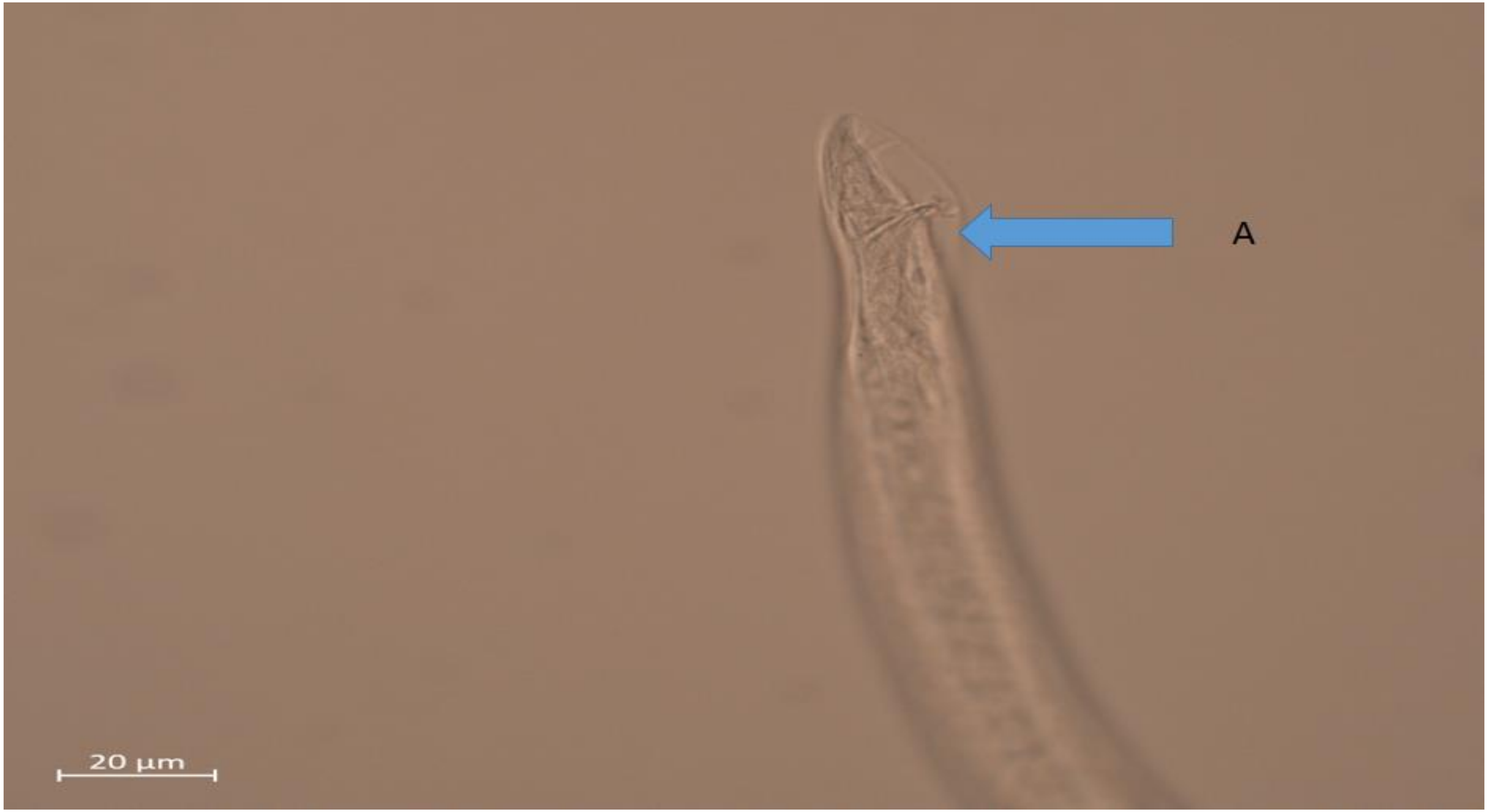

Plate 4.4: Morphological features of male *Dolichodorus* sp.: Caudal alae are wing-like and tri-lobed. **(A)** spicules most generally with prominent flanges

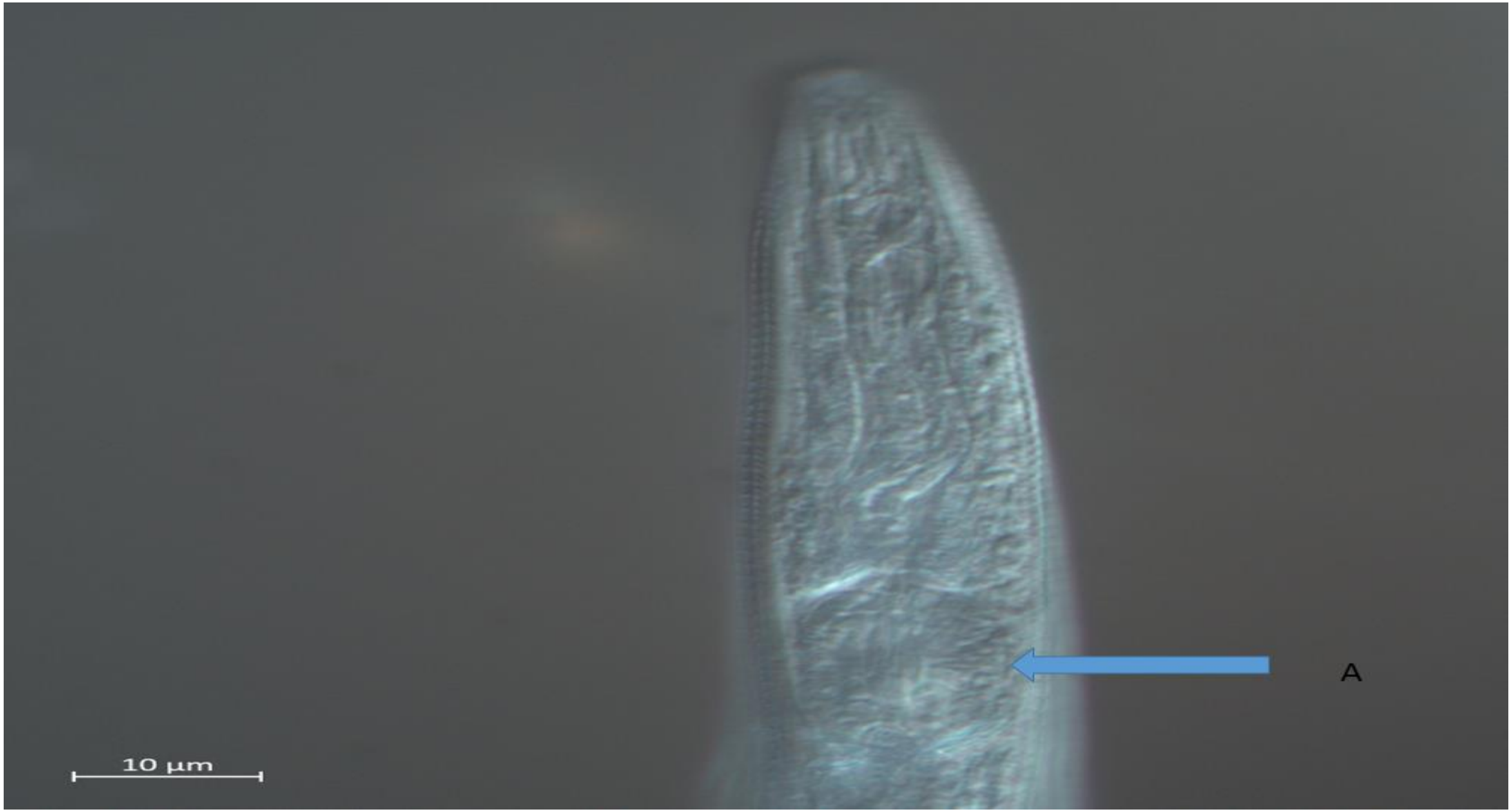

Plate 4.5: Morphological features of *Aphelenchoides* sp. is characterized by; **(A)** large metacorpus (median bulb)

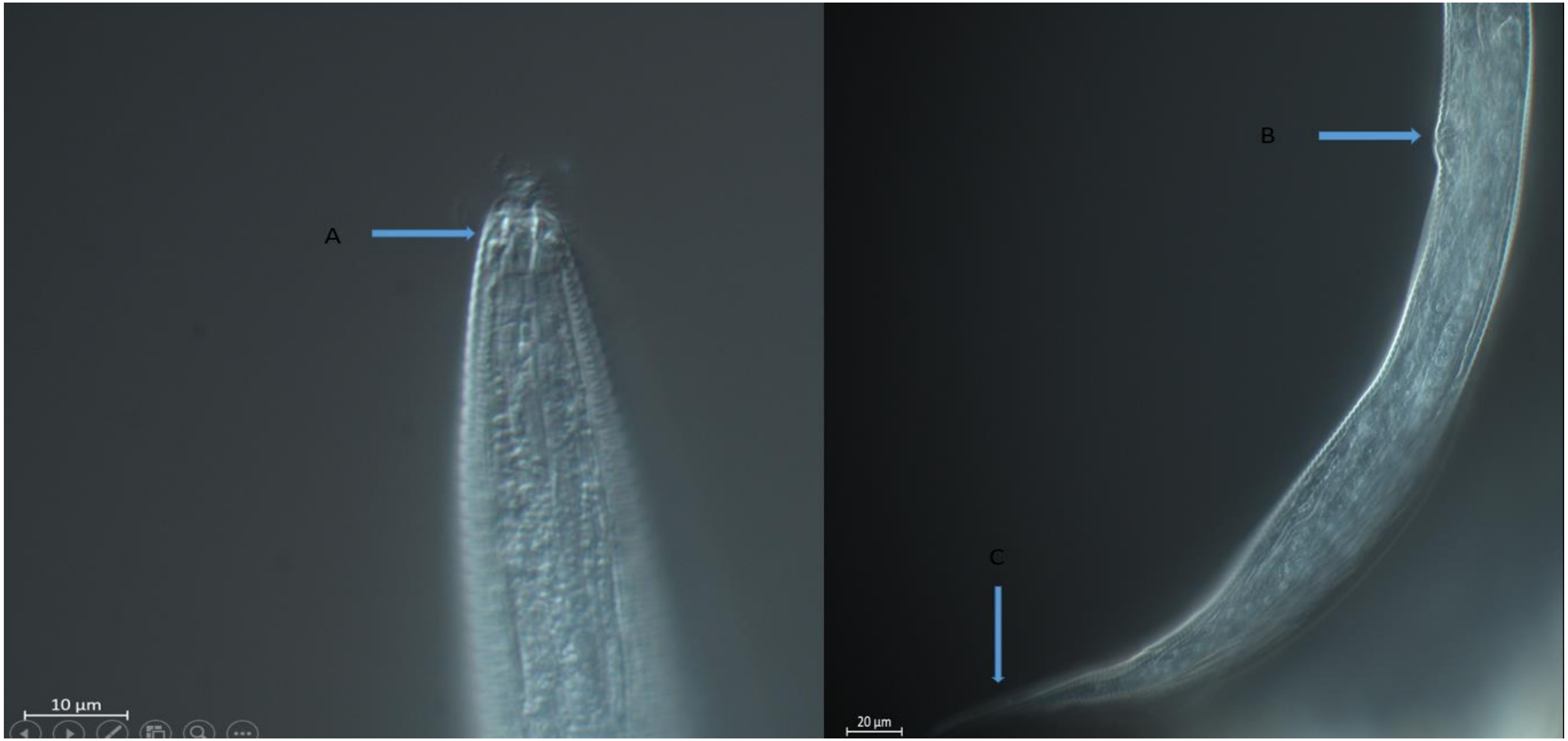

Plate 4.6: Morphological features of *Panagrolaimus* sp (*Panagrolaimus rigidus*); (A) head with lip regions (B) body narrows behind vulva, body diameter about 50 µm anterior to vulva; (C) tail slightly dorsally convex with a pointed or bifurcated tip Bostrom, (1995)

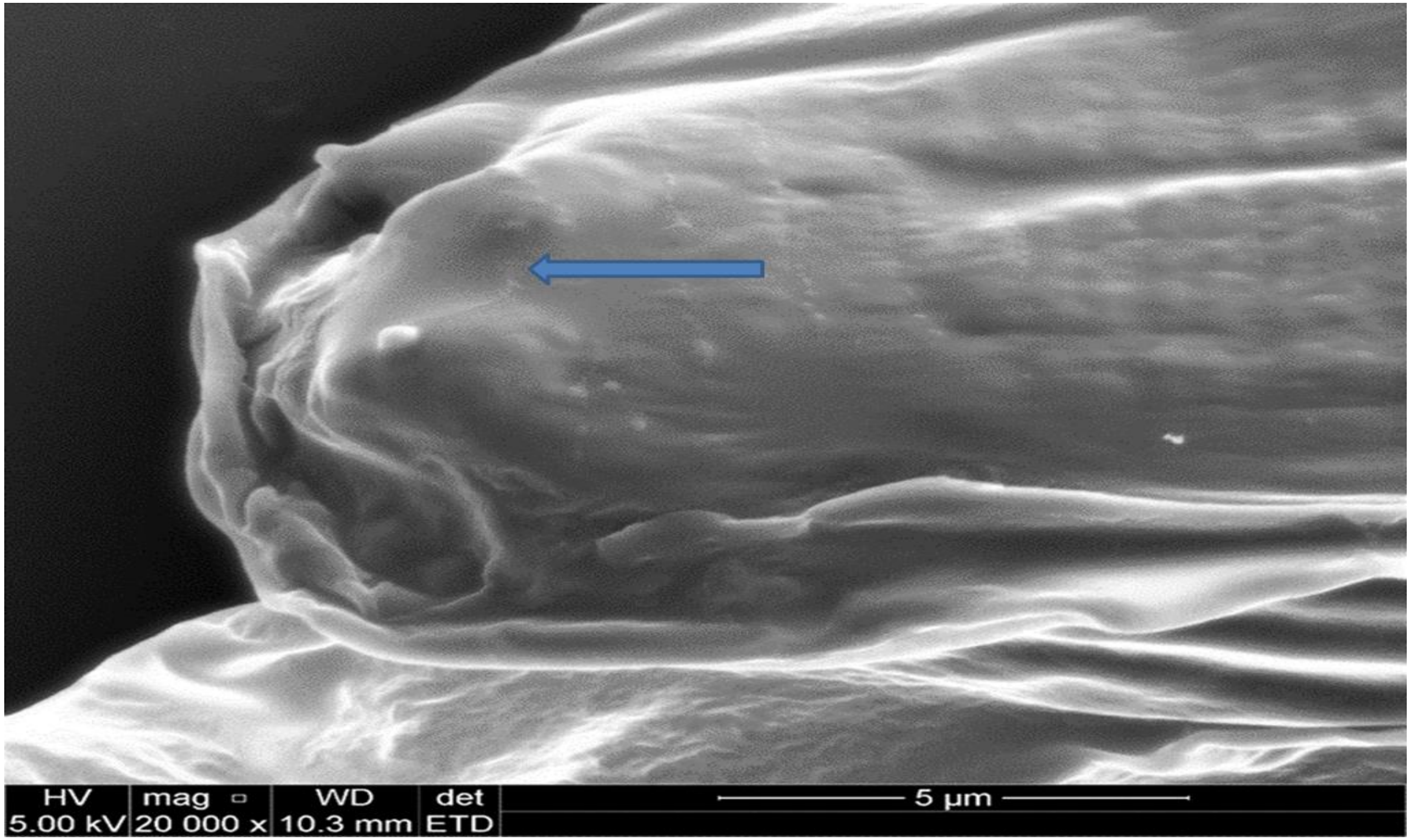

Plate 4.7: Scanning electronic microscope (SEM) image (*P. rigidus*),showing head 10,5-13 11m wide with distintly six prominent lips amalgamated into three pairs. The arrow pointing in between the amalgamed two lip rigion. Lateral lips somewhat lower and smaller than subventral and subdorsal lips (williams,1982)

4.4.1 Western Cape Province

Wheat nematodes detected in soil and roots of plants

Pratylenchus species, data presented in Table 4.3, indicated that the *Pratylenchus* sp. in soil was detected in 85.6% of the field sampled in Western Cape Province (WC). The lowest recorded number of abundance of *Pratylenchus* sp. detected in WC was observed in Koperfontein to be 0.25, while the highest abundance was found in Malmesbury to be 3321 species in composition. The Prominence values (PV) was observed to be 0.03 in Koperfontein and 575.53 in Malmesbury. The frequency of occurrence in percentage (FO%) per field sampled, ranged from 12.5% in Langgewens Experimental Farm (LAE) to 100.0% in Bredasdorp, De Vlei, Hopefield and Wellington, respectively. The highest number of individual *Pratylenchus* species was recorded in Malmesbury to be 8750 (per 200 g) soil sample. Using the 1 way ANOVA test ($P < 0.05$) to check the population densities across the field sampled, significant differences in *Pratylenchus* sp. abundance found in soil was observed across the entire agricultural fields sampled to be $P < 0.0001$ (Figure 4.1; 4.3). The mostly identified *Pratylenchus* genus, were *Pratylenchus thornei* and *Pratylenchus neglectus*

The *Pratylenchus* sp. was detected in 85.6% of the 5 g root samples collected in WC (Figure 4.2) The abundance ranged from 0.63 to 794.23 species composition in Koringplass and De Vlei A, respectively. The FO ranged from 7.1% in Hopefield to 100.0% in Alpha, Bredasdorp, De Vlei, Wellington and Malmesbury. The PV ranged from 2.41 in Hopefield to 892.00 in Bredasdorp. In 50 g roots samples, *Pratylenchus* sp. was detected in 30% of the samples collected. The abundance in species composition ranged from 0.27 in Roodebloem to 62.38 in De Vlei A. The lowest FO was observed in Roodebloem (20.0%), while the highest was recorded in De Vlei A (100.0%). The PV in Roodebloem and Tigerhoek ranged from 3.71 to 475.49, respectively (Table 4.3; Figure 4.3).

The t-test ($P < 0.05$), showed significant differences in *Pratylenchus* sp. abundance between soil and 5 g roots in Malmesbury ($P < 0.0262$), Bredasdorp ($P < 0.0402$) and De Vlei ($P < 0.0105$). Between the soil and 50 g roots samples, the significant differences of *Pratylenchus* sp. were only observed in Roodebloem and De Vlei to be $P < 0.0014$ and $P < 0.0059$, respectively (Figure 4.3). The t- test ($P < 0.05$), between the 5 and 50 g roots sampled from Roodebloem and De Vle A, also showed significant differences in *Pratylenchus* sp. abundance with $P < 0.0200$ and $P < 0.00142$, while Koperfontein and Tigerhoek showed no significant differences with the $P > 0.0896$ and $P > 0.0142$, respectively (Figure 4.3).

Table 4-3: Distribution and prominence of major wheat nematodes in wheat farms of Western Cape, South Africa

		Soil (200g)			Roots (5g)			Roots (50g)		
Locality	**Nematode genera**	**Abundance**	***FO (%)**	****PV**	**Abundance**	***FO (%)**	****PV**	**Abundance**	***FO (%)**	***PV**
Alpha	*Meloidogyne*	-	-	-	-	-	-	-	-	-
	Pratylenchus	20.43	64.29	16.05	13.64	85.71	176.83	-	-	-
	Rotylenchus	316.14	100.00	309.82	19.93	100.00	279.00	-	-	-
	Scutellonema	124.18	87.50	102.00	-	-	-	-	-	-
	Helicotylenchus	5.00	14.29	1.85	-	-	-	-	-	-
	Aphelenchus	1.00	21.43	0.45	-	-	-	-	-	-
	Tylenchus	0.21	14.29	0.08	-	-	-	-	-	-
Bredasdorp	*Pratylenchus*	272.38	100.00	348.64	557.75	100.00	892.00	-	-	-
	Spiral	256.00	100.00	327.68	-	-	-	-	-	-
	Criconema	-	-	-	-	-	-	-	-	-
	Tylenchus	186.06	87.50	222.78	-	-	-	-	-	-
De vlei	*Meloidogyne*	-	-	-	-	-	-	-	-	-

Table 4.3: (*Continued*)

	Pratylenchus	243.40	100.00	486.80	402.65	100.00	853.00	-	-	-
	Tylenchus	14.10	30.00	15.45	-	-	-	-	-	-
De vlei (A)	*Pratylenchus*	16.92	100.00	14.30	794.23	84.62	830.62	62.38	100.00	444.85
	Spiral	8.31	69.23	5.84	19.46	46.15	196.00	1.31	46.15	63.35
	Tylenchus	0.23	15.38	0.08	-	-	-	-	-	-
Hopefield	*Pratylenchus*	5.00	14.29	1.85	0.64	7.14	2.41	-	-	-
	Aphelenchus	-	-	-	-	-	-	-	-	-
	Tylenchus	0.64	7.14	0.17	-	-	-	-	-	-
Koperfontein	*Pratylenchus*	0.25	12.50	0.03	27.75	87.50	561.25	2.00	37.50	146.24
	Spiral	0.75	12.50	0.08	2.63	87.50	53.09	-	-	-
Koringplass	*Spiral*	0.25	12.50	0.03	-	-	-	-	-	-
	Aphelenchus	-	-	-	-	-	-	-	-	-
	Tylenchus	1.38	50.00	0.31	-	-	-	-	-	-
	Pratylenchus	-	-	-	0.63	12.50	6.93	-	-	-

Table 4.3: (*Continued*)

Langgewens Experimental Farm	*Tylenchus*	9.78	33.33	2.29	-	-	-	-	-	-
Langrug	*Meloidogyne*	-	-	-	0.80	20.00	2.98	-	-	-
	Pratylenchus	7.20	40.00	0.57	417.00	60.00	269.72	-	-	-
	Spiral	7.80	80.00	0.87	1.80	40.00	9.49	-	-	-
	Tylenchus	0.20	20.00	0.01	-	-	-	-	-	-
Malmesbury	*Pratylenchus*	3321.50	90.00	575.53	611.80	100.00	611.8	-	-	-
Roodebloem	*Meloidogyne*	10.93	60.00	9.53	2.47	26.67	19.11	-	-	-
	Pratylenchus	14.47	86.67	15.15	8.67	60.00	100.70	0.27	20.00	3.71
	Spiral	103.13	100.00	116.03	13.93	80.00	186.94	0.40	13.33	4.55
	Xiphinema	29.00	100.00	32.63	-	-	-	-	-	-
	Aphelenchus	8.73	93.33	9.49	-	-	-	-	-	-
	Tylenchus	11.33	86.67	11.87	5.33	66.67	65.32	0.53	20.00	7.43
Tiger Hoek	*Pratylenchus*	-	-	-	420.63	87.50	314.67	10.38	62.50	475.49
	Spiral	-	-	-	16.38	87.50	122.54	-	-	-
	Aphelenchus	-	-	-	4.63	75.00	32.04	-	-	-

Table 4.3: (*Continued*).

Wellington	*Pratylenchus*	397.25	100.00	127.12	285.63	100.00	245.40	-	-	-
	Tylenchus	21.00	50.00	4.75	-	-	-	-	-	-

*FO; Frequency of occurrence;

**PV; Prominence value

*** (-); Not detected

Using the 1 way ANOVA test ($P < 0.05$), significant differences in *Pratylenchus* sp. abundance found in roots was observed across the entire agricultural fields sampled to be $P < 0.0001$ (Figure 4.1).

The *Meloidogyne* sp. found in soil was detected in 7.6% of the agricultural fields sampled in WC. The only abundance recorded was in Roodebloem to be 10.93 and the FO to be 60% (Table 4.3). The highest number of individual species observed was 57 (per 200 g) of sample collected and the PV to be 9.53. Using the 1 way ANOVA test ($P < 0.05$), significant differences in *Meloidogyne* sp. abundance in soil was observed across the entire agricultural fields sampled to be $P < 0.0001$ (Figure 4.1).

The *Meloidogyne* sp. was only detected in 15.4% of the 5 g root samples collected in WC. The abundance ranged from 0.80 to 2.47 species composition in Langrug and Roodebloem, respectively (Table 4.3). The t-test ($P < 0.05$), showed significant differences in abundance of *Meloidogyne* sp. between the soil and 5 g roots in Roodebloem with $P < 0.0493$. Using the 1 way ANOVA test ($P < 0.05$), No significant differences in *Melodogyne* sp. abundance found in roots was observed across the entire agricultural fields sampled to be $P > 0.7712$.

In the soil, the spiral nematodes such as, *Rotylenchus* sp.*, Scutellonema* sp. and *Helicotylenchus* sp. were detected in 61.5% of the agricultural fields sampled. The lowest abundance of Spiral sp. detected in WC was observed in Koringplass to be 0.25 and the highest abundance was found in Alpha to be 316.14 species composition. The PV was recorded to be 0.03 in Koringplass and 327.68 in Bredasdorp. The FO ranged from 12.5% in Koperfontein and Koringplass to 100.0% in Alpha, Bredasdorp, De Vlei A, and Roodebloem. The highest number of individual Spiral species found was recorded in Alpha to be 1225 (per 200 g) of sample (Table 4.3). Using the 1 way ANOVA test ($P < 0.05$), significant differences in Spiral nematodes abundance found in soil was observed across agricultural fields sampled to be $P < 0.0118$ (Figure 4.1). *Rotylenchus unisexus, Scutellonema brachyurus* and *Helicotylenchus dihystera* were the most prevalent species identified in Alpha with *Rotylenchus unisexus* recording the highest value (316.14) of abundance than the both *Scutellonema* and *Helicotylenchus* sp. (Table 4.3).

Spiral nematodes were detected in 46.1% of the 5 g root samples in collected in WC. The abundance value recorded in Langrug and Alpha, ranged from 1.80 to 19.93, respectively. The FO ranged from 40.0% to 100.0% in Langrug and Alpha. The PV ranged from 9.49 in Langrug to 279.00 in Alpha. In 50 g roots samples, Spiral nematodes sp. were detected in 15.3% of sampled collected. The abundance ranged from 0.40 to 1.31 species composition in Roodebloem and De Vlei A, respectively. The highest value recorded was observed in Alpha to be 60 individual nematodes species (per 50 g) of roots sample. Spiral nematodes abundance sampled in WC showed significant differences between the soil and 5g root samples in Roodebloem (P < 0.0001) and Alpha (P <.0.0093), while in koperfontein, De Vlei A and Langrug showed no significant differences in population densities with P > 0.0791, P > 0.2580 and P > 0.3398, respectively. In the soil and 50 g root samples, significant differences in abundance were only observed in Roodebloem (P < 0.0216) and De Vlei (P < 0.0048). In Roodebloem, WC, Spiral nematodes showed significant differences in abundance between the 5 and 50 g roots samples with P < 0.007. Using the 1 way ANOVA test (P < 0.05), significant differences in Spiral sp. abundance found in roots was observed across the entire agricultural fields sampled to be P < 0.0118 (Figure 4.3).

Tylenchus sp. in soil was detected in 76.9% of the fields sampled WC. The lowest abundance of *Tylenchus* sp. recorded in WC was observed in Alpha (0.20) and the highest abundance was found in Bredasdorp to be 186.06 species composition. The PV was observed to be 0.01 in Langrug and Bredasdorp to be 222.78. The FO% ranged from 7.1% in Koperfontein and Koringplass to 87.5% in Roodebloem. The highest number of individual *Tylenchus* species found in soil was recorded in Bredasdorp to be 840 (per 200 g) of soil sample. Using the 1 way ANOVA test (P < 0.05), significant differences in *Tylenchus* sp. abundance found in soil was observed across the entire agricultural fields sampled to be P < 0.0001 (Figure 4.3).

In Roodebloem, no significant differences in *Tylenchus* sp. abundance were observed between the soil and 5 g roots samples with P > 0.1283. Significant differences in *Tylenchus* sp. abundance were only observed in Roodebloem between the soil and 50 g roots with P < 0.0016 (Figure 4.1, 4.2; 4.3). In Roodebloem, WC, *Tylenchus* sp., showed significant differences in abundance between the 5 and 50 g roots samples with P < 0.00107. Using the 1 way ANOVA test (P < 0.05), significant differences in *Tylenchus* sp. abundance found in roots was observed across the entire agricultural fields sampled to be P < 0.0107 (Figure 4 3).

The most abundant species found in soil were *Paratylenchus thornei, Pratylenchus neglectus, Scutellonema brachyurus, Helicotylenchus dihystera, Rotylenchus unisexus, Mesocriconema sphaeocephalum, Tylenchorhynchus maximus, Merlinius brevidens* and *Nanidorus minor* (Table 4.3). Other species with lower prevalence and relative abundances, but found occasionally at high densities, were *Coslenchus rhombus* (840 per 200 g of soil), *Ditylenchus dispsaci, Pratylenchus bolivianus, Paratrichodorus minor, Helicotylenchus rotundicauda, Helicotylenchus crenacauda, Amplimerlinius globigerus, Bitylenchus maximus Amplimerlinius paraglobigerus, Melodogyne sp.* (juveniles) and *Hoplolaimus* sp. (Table 4.3).

Table 4-4: Distribution and prominence of major wheat nematodes in wheat farms of Free State, South Africa

		Soil (200g)			**Roots (5g)**			**Roots (50g)**			**Wheat (20g)**		
Locality	**Nematode genera**	**Abundance**	***FO (%)**	****PV**	**Abundance**	***FO (%)**	****PV**	**Abundance**	***FO (%)**	****PV**	**Abundance**	***FO (%)**	***PV**
Arlington	*Spiral*	0.30	20.00	0.07	-	-	-	-	-	-	-	-	-
	Aphelenchus	2.48	52.38	3.95	0.10	4.76	1.83	0.14	9.52	3.23	-	-	-
	Criconema	0.33	9.52	0.23	-	-	-	-	-	-	-	-	-
	Meloidogyne	0.10	4.76	0.05	1.52	38.10	82.95	0.14	9.52	3.23	-	-	-
	Pratylenchus	1.71	42.86	2.47	0.05	4.76	0.92	-	-	-	-	-	-
Clarens	*Scutellonema*	9.19	85.71	18.76	1.43	42.86	82.49	0.62	19.05	19.81	-	-	-
	Rotylenchus	4.50	90.48	38.79	-	-	-	-	-	-	-	-	-
	Helicotylenchus	0.03	14.29	1.13	-	-	-	-	-	-	-	-	-
	Panagrolaimus	-	-	-	-	-	-	-	-	-	2007.83	100.00	531.69
	Tylenchus	7.90	95.24	17.01	-	-	-	-	-	-	-	-	-
Harrismith	*Aphelenchus*	17.05	70.00	28.53	1.20	30.00	52.58	-	-	-	-	-	-
	Meloidogyne	1.80	15.00	1.39	-	-	-	-	-	-	-	-	-

Table 4.4: (*Continued*).

Pratylenchus	86.10	55.00	127.71	0.95	35.00	44.96	-	-	-	-	-	-
Spiral	7.20	55.00	10.68	0.80	25.00	32.00	-	-	-	-	-	-
Xiphinema	1.40	15.00	1.08	-	-	-	-	-	-	-	-	-

*FO; Frequency of occurrence;

**PV; Prominence value

***(-); Not detected

4.4.2 Free State Province

Wheat nematodes in soil and root of plants

Pratylenchus sp.: data presented in Table 4.4, indicated that the *Pratylenchus* sp. in soil was detected in 66.7% of the field sampled in Free State Province (FS). The lowest abundance of *Pratylenchus* sp. detected in FS was observed in Clarens to be 1.71 and the highest abundance was recoded in Harrismith to be 86.10 species composition. The PV was observed to be 2.47 in Clarense and 127.71 in Harrismith (Table 4.4). The FO ranged from 42.9% in Clarens to 55.0% in Harrismith. The highest number of individual *Pratylenchus* species was recorded in Harrismith to be 1056 (per 200 g) of soil sample. Using the 1 way ANOVA test ($P < 0.05$), no significant differences in *Pratylenchus* sp. abundance found in soil was observed across the entire agricultural fields sampled with $P > 0.072$ (Figure 4.4; 4.5; 4.6). The mostly identified *Pratylenchus* sp. in FS were *Pratylenchus thornei* and *Pratylenchus neglectus*

The *Pratylenchus* sp. was detected in 66.7% of the 5 g root samples collected in WC. The abundance ranged from 0.05 to 0.95 species composition in Clarens and Harrismith, respectively. The FO ranged from 4 76% in Clarens to 35.0% in Harrismith. The PV ranged from 0.92 in Clarens to 44.96 in Harrismith. The highest value recorded was observed in Clarens to be 60 individual nematodes species (per 200 g) of soil sample. Using the 1 way ANOVA test ($P < 0.05$), significant differences in *Pratylenchus* sp. abundance between the soil and 5 g roots were observed in Clarens to be $P < 0.0177$. The significant differences in *Pratylenchus* sp. abundance between 5 and 50 g root sample was observed in Clarens to be $P < 0.0140$ (Figure 4.5).

The *Meloidogyne* sp. found in soil was detected in 66.7% of the agricultural fields sampled in FS. The abundance ranged from 0.10 to 1.80 species composition in Clarens and Harrismith, respectively. The FO ranged from 4.76% in Clarens to 15.0% in Harrismith. The PV ranged from 0.05 in Clarens to 1.39 in Harrismith. The highest value recorded was observed in Clarens to be 17 individual (per 200 g) of sample Using the 1 way ANOVA test ($P < 0.05$), significant differences in *Meloidogyne* sp. abundance in soil was observed across the agricultural fields sampled with $P < 0.0001$ (Figure .4.6).

In roots, the *Meloidogyne* sp. was only detected in 33.3% of the 5 g root samples collected in FS. The abundance was 1.52, the FO was 38.1% and the PV was 82.95 in Clarens, respectively. The t-test ($P < 0.05$), between the soil and 5g roots samples showed the significant differences in *Meloidogyne* sp. abundance with P < 0.0418, while between the soil and 50 g roots showed no significant differences with the P > 0.7477 (Figure 4.4).

Spiral nematodes in soil i.e. *Rotylenchus* sp.*, Scutellonema* sp. and *Helicotylenchus* sp. were detected in 100.0% of the agricultural fields sampled in FS (Table 4.4). The lowest abundance of Spiral sp. detected in FS was observed in Arlington with 0.30 and the highest abundance was found in Clarens with 9.91 species composition. The highest PV was recorded to be 38.79 in Clarens. The FO ranged from 14.3 to 90.5% in Clarens. The highest number of individual Spiral species found was recorded in Harrismith to be 59 species per sample. Using the 1 way ANOVA test (P < 0.05), no significant differences in Spiral nematodes abundance found in soil were observed across agricultural fields sampled with P > 0.0544 (Figure 4.4). *Rotylenchus unisexus, Scutellonema brachyurus and Helicotylenchus dihystera* were the most prevalent species identified in Alpha with *Scutellonema brachyurus* recording the highest value (9.19) of abundance than the both Rotylenchus and Helicotylenchus sp. (Table 4.4).

In root samples, spiral nematodes were detected in 66.7% of the 5 g root samples collected in FS. The abundance ranged from 0.80 to 1.43 species composition in Harrismith and Clarens, respectively. The FO ranged from 25.0% in Harrismith to 42.9% in Clarens. The PV ranged from 32.00 in Clarens to 82.49 in Clarens (Table 4.4). The highest value recorded was observed in Clarens to be 9 in 5 g and 43 in 50 g individual per sample. Significant difference in abundance of spiral nematodes in Clarens, FS, between the soil and 5 g was observed with P < 0.0028, while in between the soil and 50 g, no significant difference was observed with P > 0.02370. No significant difference was observed between the soil and 50 g root samples in Harrismith, FS, with P > 0.0678 (Figure 4.4; 4.5; 4.6).

The *Aphelenchus* sp. found in soil was detected in 66.7% of the agricultural fields sampled in FS. The abundance ranged from 2.48 to 17.05 species composition in Clarens and Harrismith, respectively. The FO ranged from 52.4% in Clarens to 70.0% in Harrismith. The PV ranged from 3.95 in Clarens to 1.39 in Harrismith. The highest value recorded was observed in Clarens to be 126 individual per sample Using the t-test (P < 0.05), significant differences in *Aphelenchus* sp. abundance in soil was observed across all agricultural fields sampled to be P < 0.0281 (Figure .4.6).

The *Aphelenchus* sp. was only detected in 33.3% of the 5 g root samples collected in Clarens, FS. The abundance ranged from 0.10 to 1.20 species composition in Clarens and Harrismith, respectively. The FO ranged from 4.8% in Clarens to 30.0% in Harrismith. The PV ranged from 1.83 in Clarens to 52.58 in Harrismith. The highest value recorded was observed in Clarens to be 2 individual per sample Using the t-test ($P < 0.05$), significant differences in *Aphelenchus* sp. abundance in roots was observed across the entire agricultural fields sampled to be $P < 0.0281$. The t-test ($P < 0.05$), the soil and 5 g roots samples showed significant differences in *Aphelenchus* sp. abundance with $P < 0.0317$ (Figure 4.5).

Wheat samples

Table 4.4, indicates that the *Panagrolaimus* sp. in wheat was only detected in Clarens (33.3%) of the wheat kernels collected in FS. The abundance was 2007.83, the FO was 100.0% and the PV was 531.69 in Clarens, respectively. The abundance was 6.70 in species composition. The highest number of individual *Panagrolaimus* species found in wheat was recorded to be 3750 (per 20 g) of wheat sample. No *Panagrolaimus* sp. observed in roots sample (Table 4.4; Figure 4.4).

The most abundant species found in soil were *Pratylenchus thornei, Pratylenchus neglectus, Scutellonema brachyurus, Helicotylenchus dihystera, Rotylenchus unisexus, Mesocriconema sphaeocephalum, Tylenchorhynchus maximus, Merlinius brevidens* and *Nanidorus minor*. Other species with lower prevalence and relative abundances, but found occasionally at high densities, were *Tylenchus* sp. *Helicotylenchus rotundicauda, Helicotylenchus crenacauda*, *Meloidogyne* sp. (juveniles) and *Hoplolaimus* sp.

Table 4-5: Distribution and prominence of major wheat nematodes in wheat farms of Northern Cape, South Africa

		Soil (200g)			Roots (5g)			Roots (50g)			Wheat (20g)		
Locality	**Nematode genera**	**Abundance**	***FO (%)**	****PV**	**Abundance**	***FO (%)**	****PV**	**Abundance**	***FO (%)**	****PV**	**Abundance**	***FO (%)**	****PV**
Barkley West	*Pratylenchus*	4.82	22.73	5.56	2.14	4.55	12.57	-	-	-	-	-	-
	Paratrichodorus	0.27	4.55	0.14	-	-	-	-	-	-	-	-	-
Douglas	*Meloidogyne*	-	-	-	-	-	-	-	.	-	-	-	-
	Pratylenchus	3.33	28.57	3.93	3.05	47.62	59.60	-	-	-	-	-	-
	Criconema	14.29	47.62	21.74	-	-	-	-	-	-	-	-	-
	Spiral	-	-	-	-	-	-	-	-	-	-	-	-
	Paratrichodorus	1.05	14.29	0.87	-	-	-	-	-	-	-	-	-
	Tylenchus	25.76	76.19	49.58	-	-	-	-	-	-	-	-	-
Prieska	*Meloidogyne*	-	-	-	-	-	-	-	-	-	-	-	-
	Pratylenchus	50.05	95.00	97.57	91.60	85.00	798.74	-	-	-	-	-	-
	Criconema	6.65	35.00	7.87	-	-	-	-	-	-	-	-	-
	Spiral	0.90	5.00	0.40	-	-	-	-	-	-	-	-	-

Table 4.5: ***(Continued)***

	Paratrichodorus	1.80	15.00	1.39	-	-	-	-	-	-	-	-	-
Hopetown	*Aphelenchus*	-	-	-	-	-	-	-	-	-	6.70	35.00	105.00
	Criconema	0.43	4.76	0.21	-	-	-	-	-	-	-	-	-
	Pratylenchus	15.81	71.43	29.46	8.19	52.38	145.56	-	-	-	-	-	-
	Spiral	5.33	28.57	6.29	-	-	-	-	-	-	-	-	-
	Aphelenchus	-	-	-	-	-	-	-	-	-	6.67	42.86	131.96
Modderriver	*Longidorus*	0.79	15.79	0.57	-	-	-	-	-	-	-	-	-
	Pratylenchus	175.37	26.32	162.38	9.00	31.58	182.58	-	-	-	-	-	-
	Aphelenchus	-	-	-	-	-	-	-	-	-	1.58	31.58	47.63
Orania	*Pratylenchus*	0.86	9.52	0.58	-	-	-	-	-	-	-	-	-
	Spiral	13.00	47.62	19.78	-	-	-	-	-	-	-	-	-
	Xiphinema	0.14	4.76	0.07	-	-	-	-	-	-	-	-	-
	Tylenchus	4.71	38.10	6.42	-	-	-	-	-	-	-	-	-

*FO; Frequency of occurrence;
PV; Prominence value*
***(-); Not detected

4.4.3 Northern Cape Province

Wheat nematodes in soil and roots of plants

Pratylenchus sp.: Data presented in Table 4.5, indicated that the *Pratylenchus* sp. in soil was detected in 100.0% of the field sampled in Northern Cape (NC). The lowest abundance of *Pratylenchus* sp. detected in NC was observed in Orania to be 0.86 and the highest abundance was found in Modderriver to be 175.37 species in composition. The PV was observed to be 0.58 in Orania and 162.38 in Modderriver. The FO ranged from 9.5% in Orania to 95.0% in Prieska. The highest number of individual *Pratylenchus* species found in soil was recorded in Modderriver to be 3185 per sample. Using the 1 way ANOVA test ($P < 0.05$), statistical significant differences in *Pratylenchus* sp. abundance found in soil was observed across the entire agricultural fields sampled with $P < 0.0001$ (Figure 4.7). The mostly identified *Pratylenchus* sp. in NC was *Pratylenchus thornei* and *Pratylenchus neglectus.*
The *Pratylenchus* sp. was detected in 100.0% of the 5 g root samples collected in NC. The abundance ranged from 2.14 to 91.60 species composition in Barkley west and Prieska, respectively. The FO% ranged from 4.6% in Barkley West to 85.0% in Prieska. The PV ranged from 12.57 in Barkley West to 798.74 in Prieska. The highest value recorded was observed in Prieska to be 613 individuals' species per sample. Using the 1 way ANOVA test ($P < 0.05$) to check the population densities, no significant differences of abundance in *Pratylenchus* sp. between the soil and 5 g roots was observed in all the fields with P values; Orania ($P > 0.0329$), Douglas ($P > 0.8785$), Hopetown ($P > 0.1321$), Prieska ($P > 0.02448$), Barkly West ($P > 0.3295$) and Modderriver ($P > 0.3346$) (Table 4.5; Figure 4.7). No *Meloidogyne sp*. was observed in NC.

In soil samples, spiral nematodes (*Rotylenchus, Scutellonema* and *Helicotylenchus* sp.) were detected in 50.0% of the agricultural fields sampled in NC (Table 4.5). The lowest abundance of Spiral sp. detected in NC was observed in Prieska with 0.90 and the highest abundance was found in Orania with 13.00 species composition. The highest PV was recorded to be 19.78 in Orania. The FO ranged from 5.0 to 47.6% in Prieska and Orania, respectively. The highest number of individual Spiral species found was recorded in Orania to be 123 species (per 200 g) of soil sample. Using the 1 way

ANOVA test ($P < 0.05$), significant differences in Spiral nematodes abundance found in soil were observed across agricultural fields sampled with $P < 0.0136$ (Figure 4.7.). *Rotylenchus unisexus, Scutellonema brachyurus and Helicotylenchus dihystera* were the most prevalent species identified in NC. No spiral sp. observed in roots sample.

Table 4.5, indicates that the *Criconema* sp. in soil was detected in 50.0% of the fields sampled NC. The lowest abundance of *Criconema* sp. recorded in NC was observed in Hopetown (0.43) and the highest abundance was found in Douglas with 14.29 species composition. The PV was observed to be 0.01 in Langrug and Bredasdorp to be 222.78. The FO ranged from 4.7% in Hopetown to 47.6% in Douglas. The highest number of individual *Criconema* species found was recorded in Douglas to be 114 (per 200 g) of soil sample. Using the 1 way ANOVA test ($P < 0.05$) to check the population densities, no significant differences in *Criconema* sp. abundance found in soil was observed across the all agricultural fields sampled with $P > 0.5927$ (Figure 4.7). No *Criconema* sp. observed in roots sample (Figure 4.7).

In soil, *Paratrichodorus* sp. was detected in 50.0% of the fields sampled NC (Table 4.5). The lowest abundance of *Paratrichodorus* sp. recorded in NC was observed in Barkley West (0.27) and the highest abundance was found in Prieska to be 1.80 species composition. The PV was observed to be 0.14 in Barkley West and Prieska to be 1.39. The FO ranged from 4.5% in Barkley West to 15.0% in Prieska. The highest number of individual *Paratrichodorus* sp. found in soil was recorded in Douglas to be 18 individual (per 200 g) of soil sample. Using the 1 way ANOVA test ($P < 0.05$), no statistical significant differences in *Paratrichodorus* sp. abundance found in soil was observed across the entire agricultural fields sampled with $P > 0.4483$ (Figure 4.7). No *Paratrichodorus* sp. observed in roots sample (Table 4.5)

Wheat kernels

In respect of wheat kernels, Table 4.5, shows that the *Aphelenchus* sp. it was detected in 50.0% of the fields sampled NC. The lowest abundance of *Aphelenchus* sp. recorded in NC was observed in Modderriver to be 1.58 and the highest abundance was found in Prieska to be 6.70 species composition. The PV was observed to be 47.63 in

Modderriver and 131.96 in Hopetown. The FO ranged from 31.6% in Modderriver to 42.9% in Hopetown. The highest number of individual *Aphelenchus* species found in wheat samples was recorded in Douglas to be 67 nematodes species (per 200 g). Using the 1 way ANOVA test ($P < 0.05$), no significant differences in *Aphelenchus* sp. abundance found in soil was observed across the entire agricultural fields sampled with $P > 0.6220$ (Figure 4.7). Some of the genera were found relatively in low numbers only in irrigation fields (Table 4.4; 4.5; 4.6), such as *Longidorus, Paralongidorus, Ditylenchus, Hoplolaimus, Tylencholaimus, Hemicyclophora* and *Paratrichodorus.*

4.4.4 KwaZulu-Natal, Mpumalanga, Limpopo and North West Provinces

Wheat nematodes in soil and roots plants

Samples collected from KwaZulu-Natal (KZN), showed relatively high numbers of individual species per samples found in soil (per 200 g); *Meloidogyne* (420), *Pratylenchus* (420), *Criconema* (1470) and Spiral (350), while other species were found in low numbers such as *Tylenchus* (245) and *Longidorus* (21) (Table 4.6). Using the t-test ($P < 0.05$), *Pratylenchus* sp. abundances in KZN, Showed statistical significant differences between the soil and 5 g roots with the $P < 0.0477$ (Table 4.6; Figure 4.8). Samples from Cedara, KZN, showed no significant differences in *Meloidogyne* sp. abundance between the soil and 5 g roots with $P > 0.332$. Pratylenchus (86) was found in low numbers in soil and few numbers of Rotylenchus (5) were also detected in Limpopo. No nematodes were detected in roots samples. Thus no statistical test was conducted to compare the distribution. Mpumalanga had fewer than 5 individual *Pratylenchus* species detected in soil and nothing was found both in roots and kernels. No statistical was also conducted in Mpumalanga. In North West, the *Amplimerlinius sp.* was the dominant species and no other PPNs were detected other than free living nematodes. No nematodes were observed in both the roots samples and kernels.

Table 4-6: Distribution and prominence of major wheatnematodes in wheat farms of KwaZulu-Natal, South Africa

		Soil (200g)			**Roots (5g)**		
Locality	**Nematodes genera**	**Abundance**	***FO (%)**	****PV**	**Abundance**	***FO (%)**	****PV**
Cedara	*Criconema*	623.64	100.00	377.30	-	-	-
	Longidorus	6.36	90.91	3.67	-	-	-
	Meloidogyne	91.55	72.73	47.23	189.64	100.00	294.60
	Pratylenchus	135.27	81.82	74.03	30.09	100.00	364.10
	Spiral	160.82	100.00	97.30	2.73	36.36	19.90
	Tylenchus	33.45	27.27	10.57	-	-	-

*FO; Frequency of occurrence;
**PV; Prominence value
***(-); Non detected

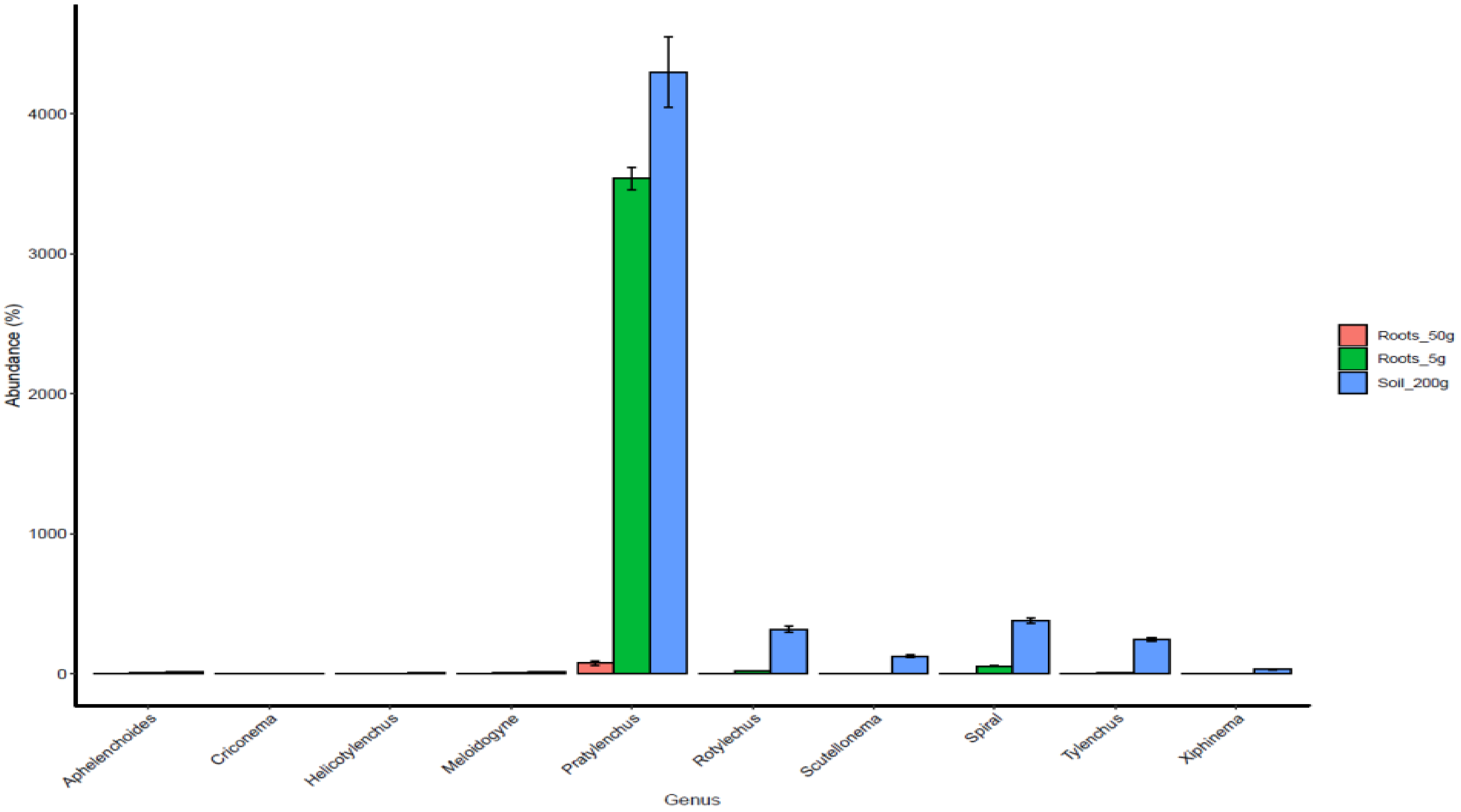

Figure 4-1: Distribution and prominence of major plant-parasitic nematodes between the soil, 5 g roots and 50 g roots samples from wheat farms of Western Cape, South Africa

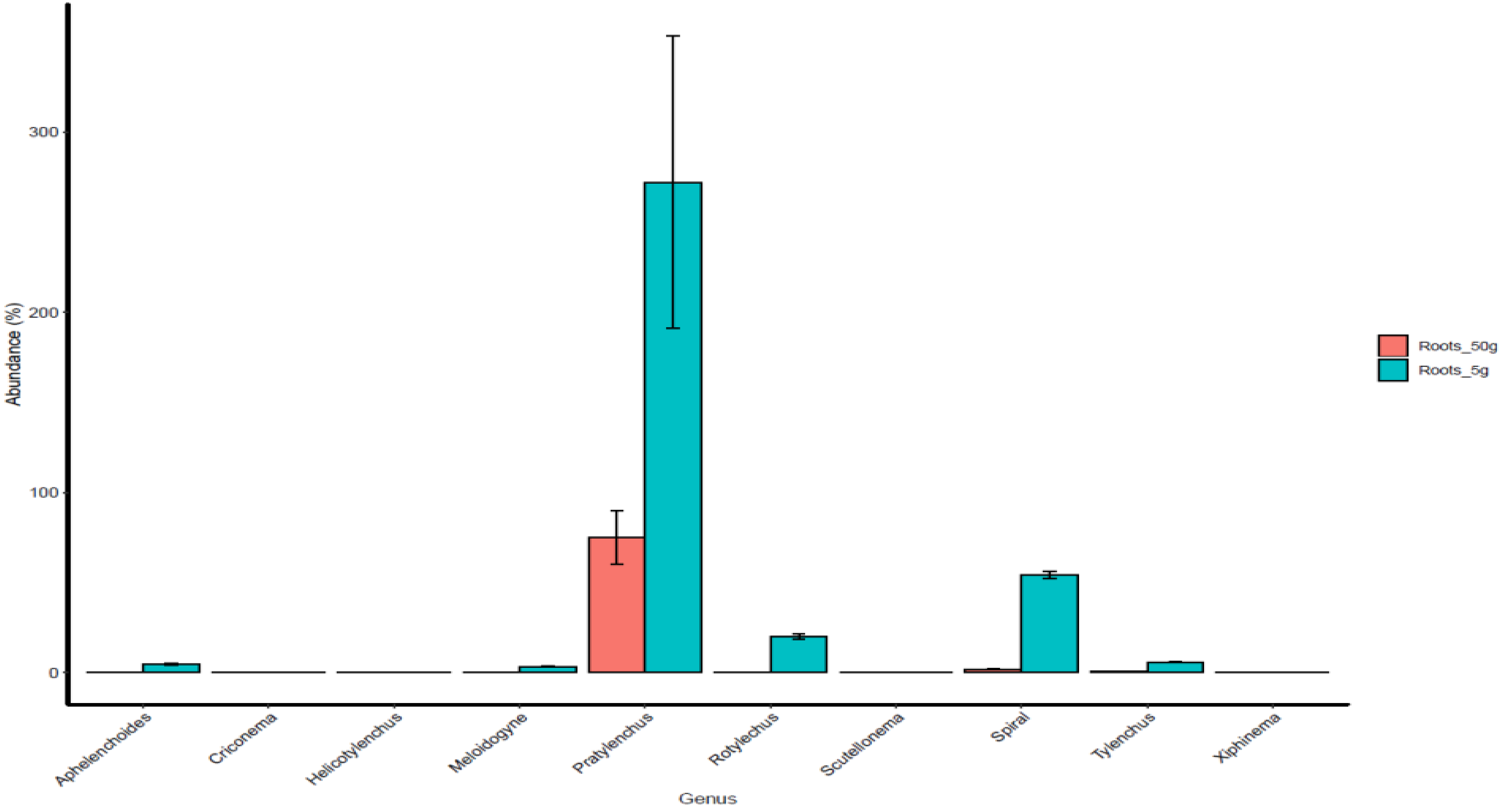

Figure 4-2: Distribution and prominence of major plant-parasitic nematodes between 5 and 50 g roots in wheat farms of Western Cape, South Africa

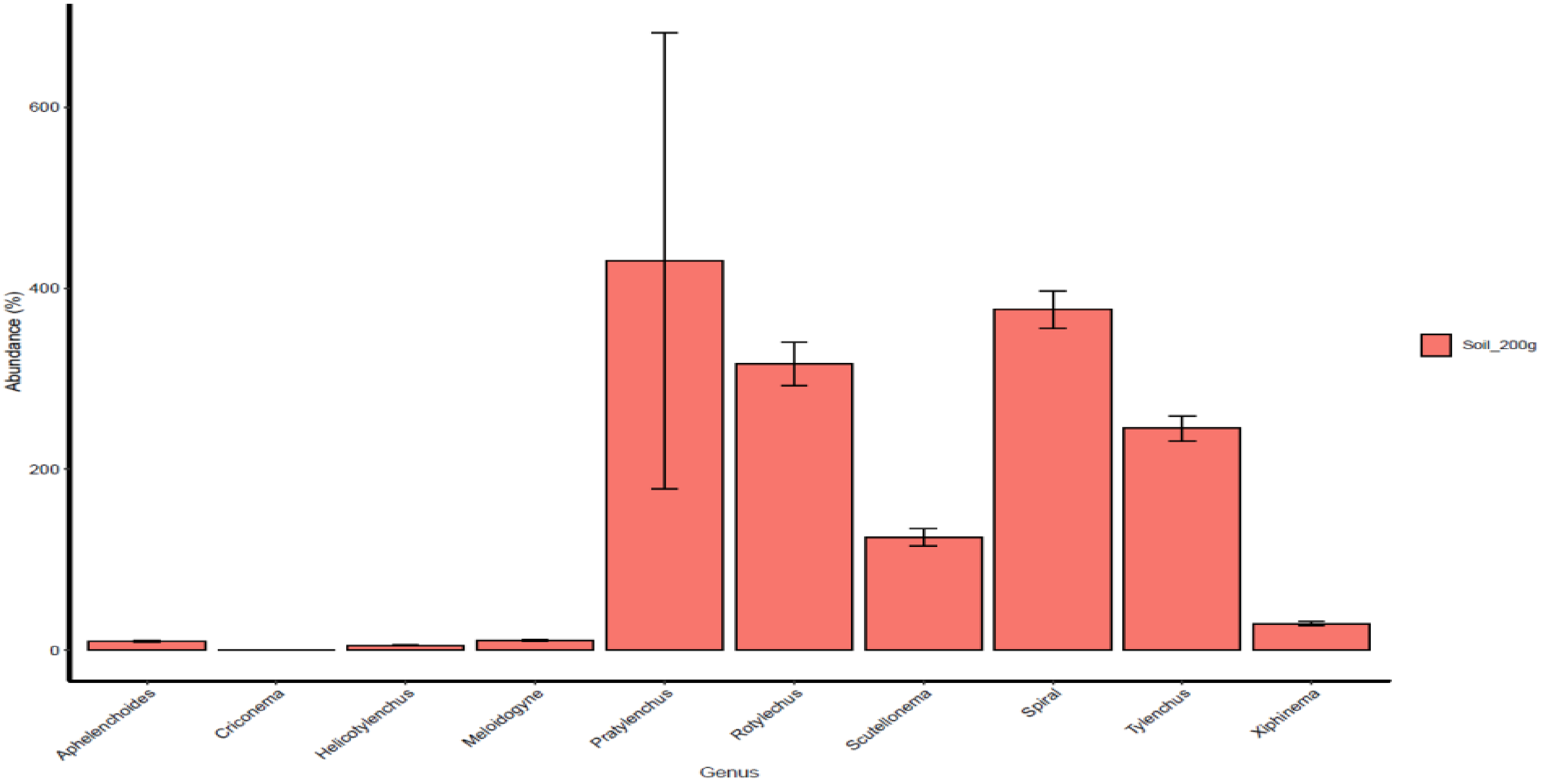

Figure 4-3: Distribution and prominence of major plant-parasitic nematodes found in soil of wheat farms of Western Cape, South Africa

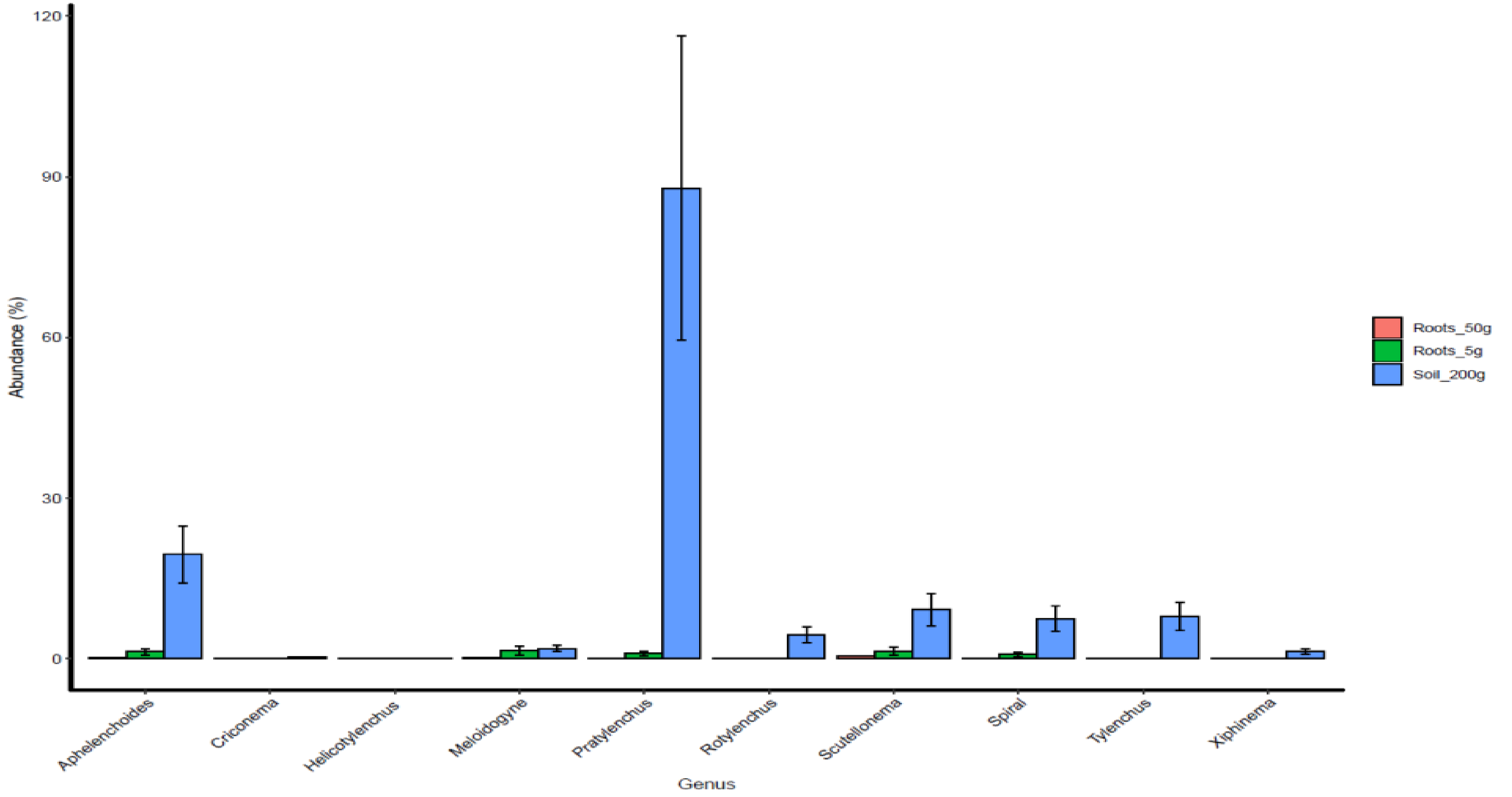

Figure 4-4: Distribution and prominence of major plant-parasitic nematodes between the soil, 5 and 50 g roots in wheat farms of Free State, South Africa

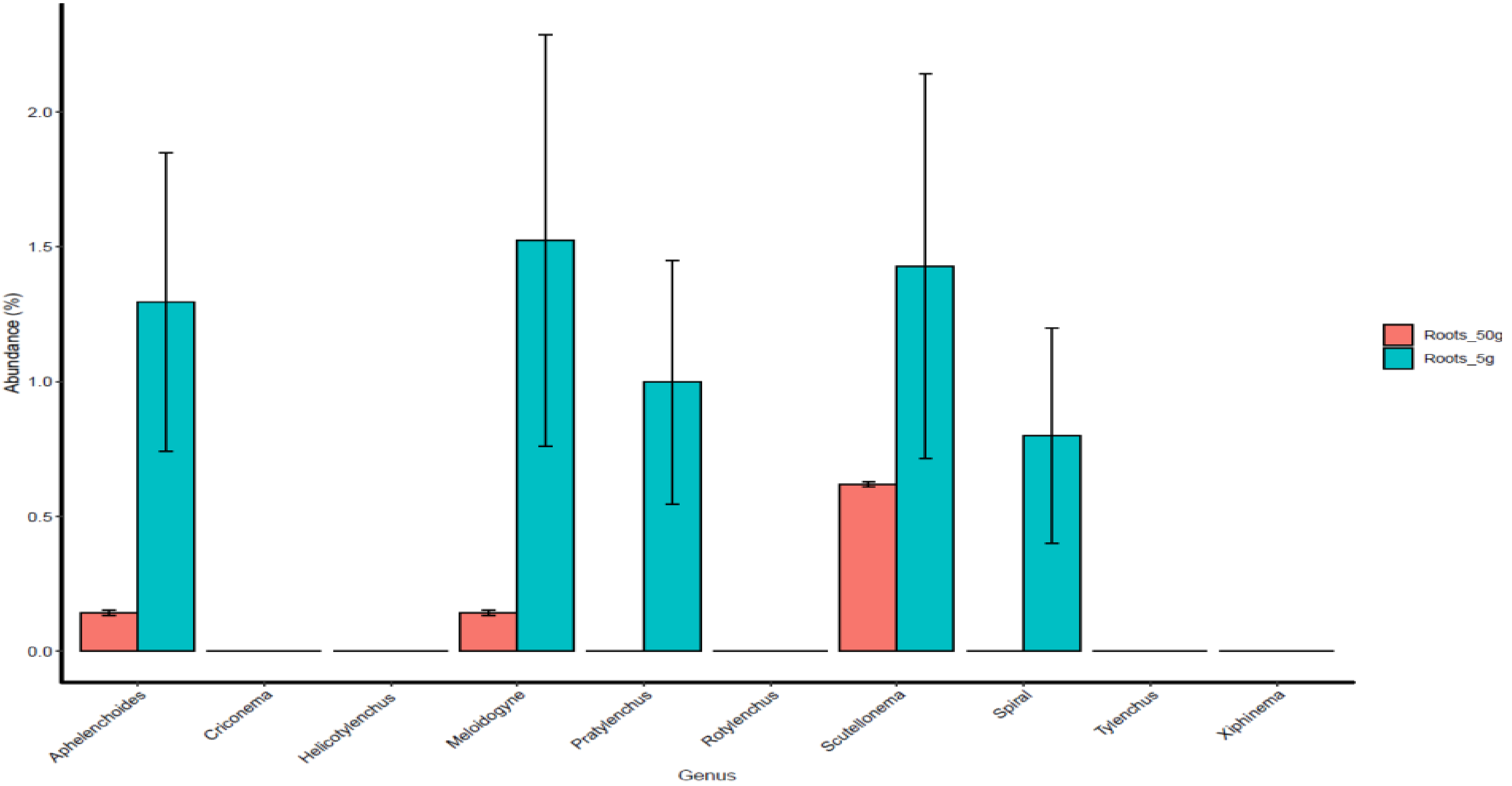

Figure 4-5: Distribution and prominence of major plant-parasitic nematodes between 5 and 50 g roots from wheat farms of Free State, South Africa

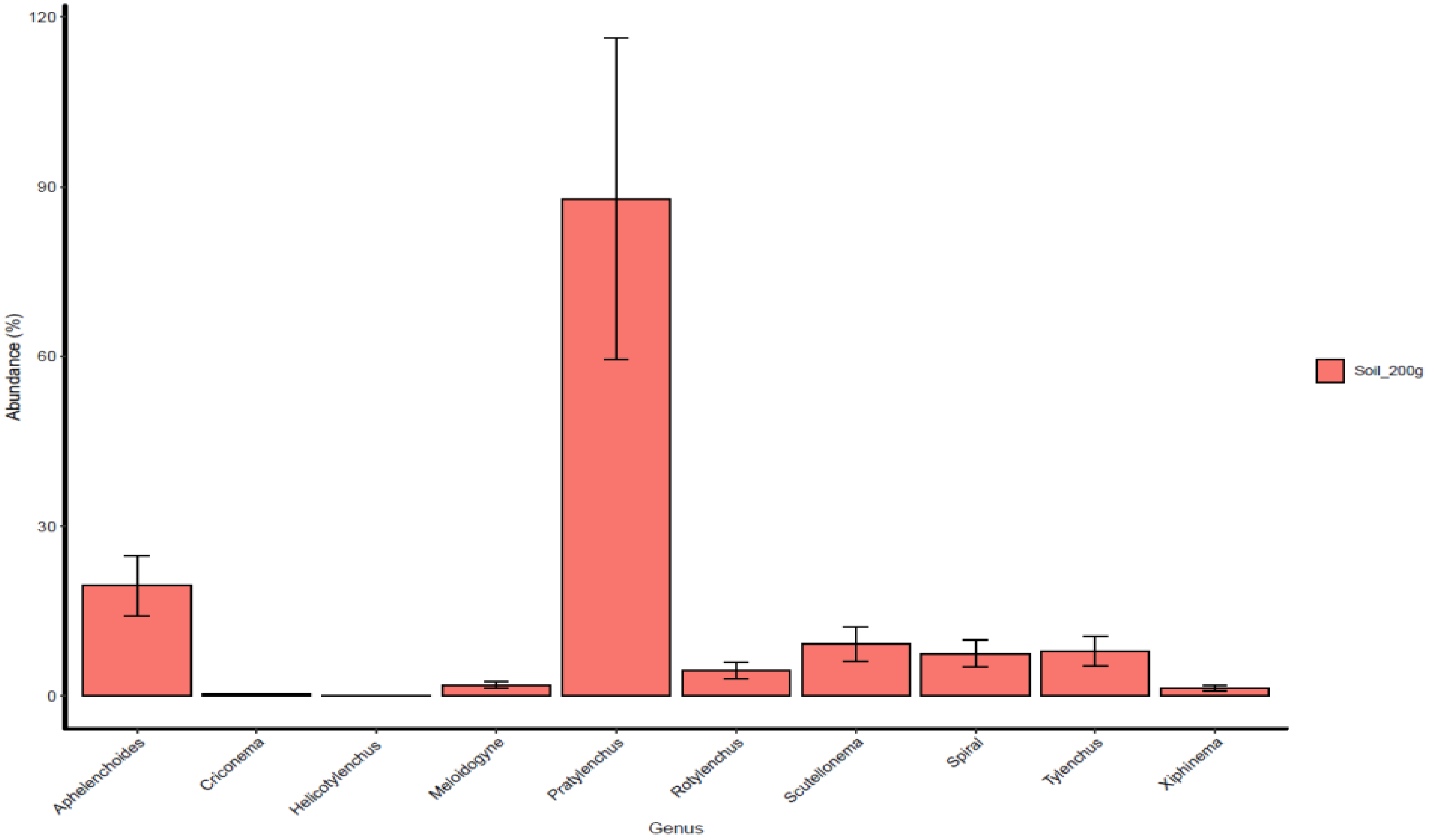

Figure 4-6: Distribution and prominence of major plant-parasitic nematodes in soil from wheat farms of Free State, South Africa

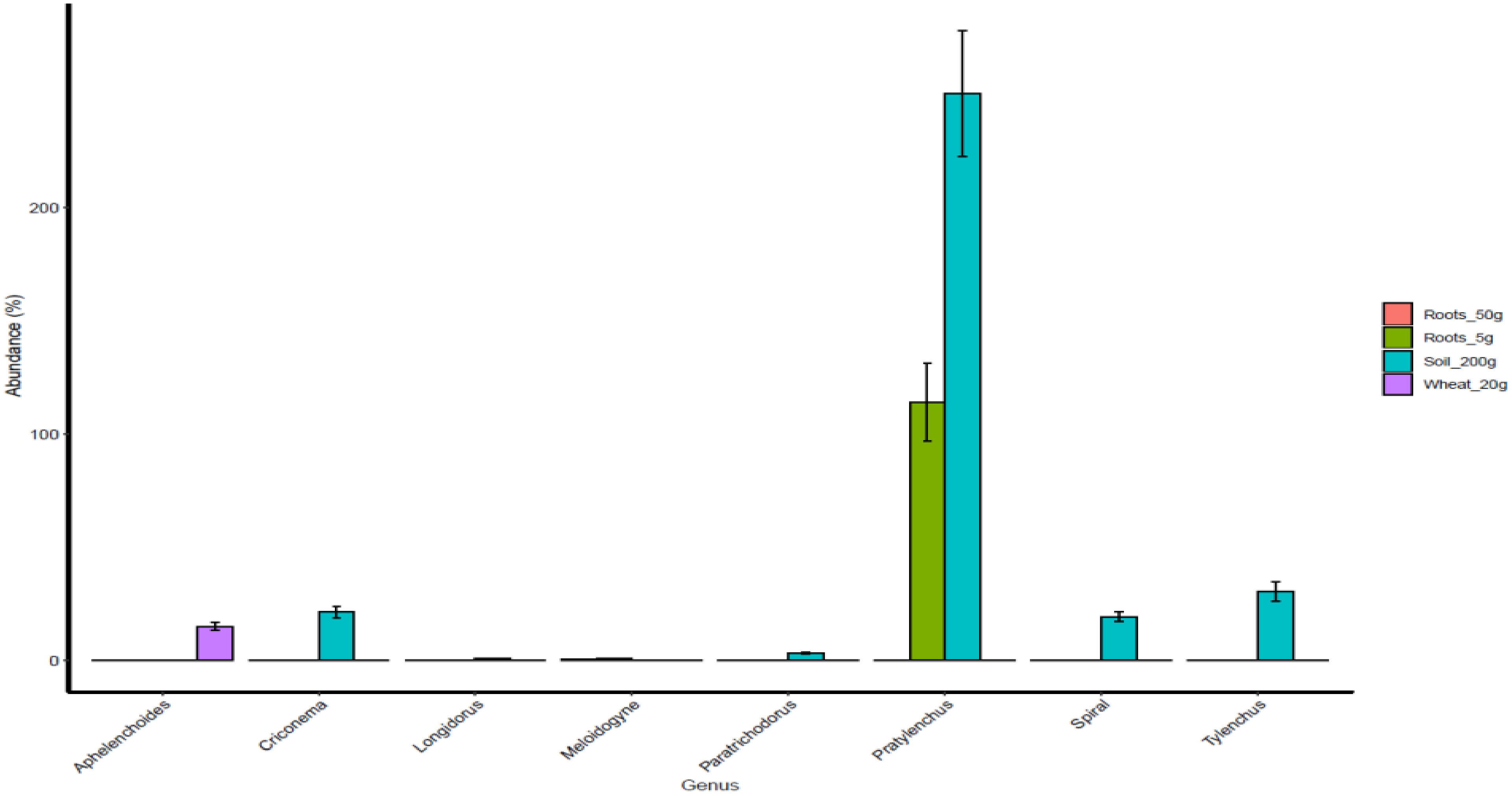

Figure 4-7: Distribution and prominence of major plant-parasitic nematodes between the soil, 5 and 50 g roots and wheat kernels from wheat farms of Northern Cape, South Africa

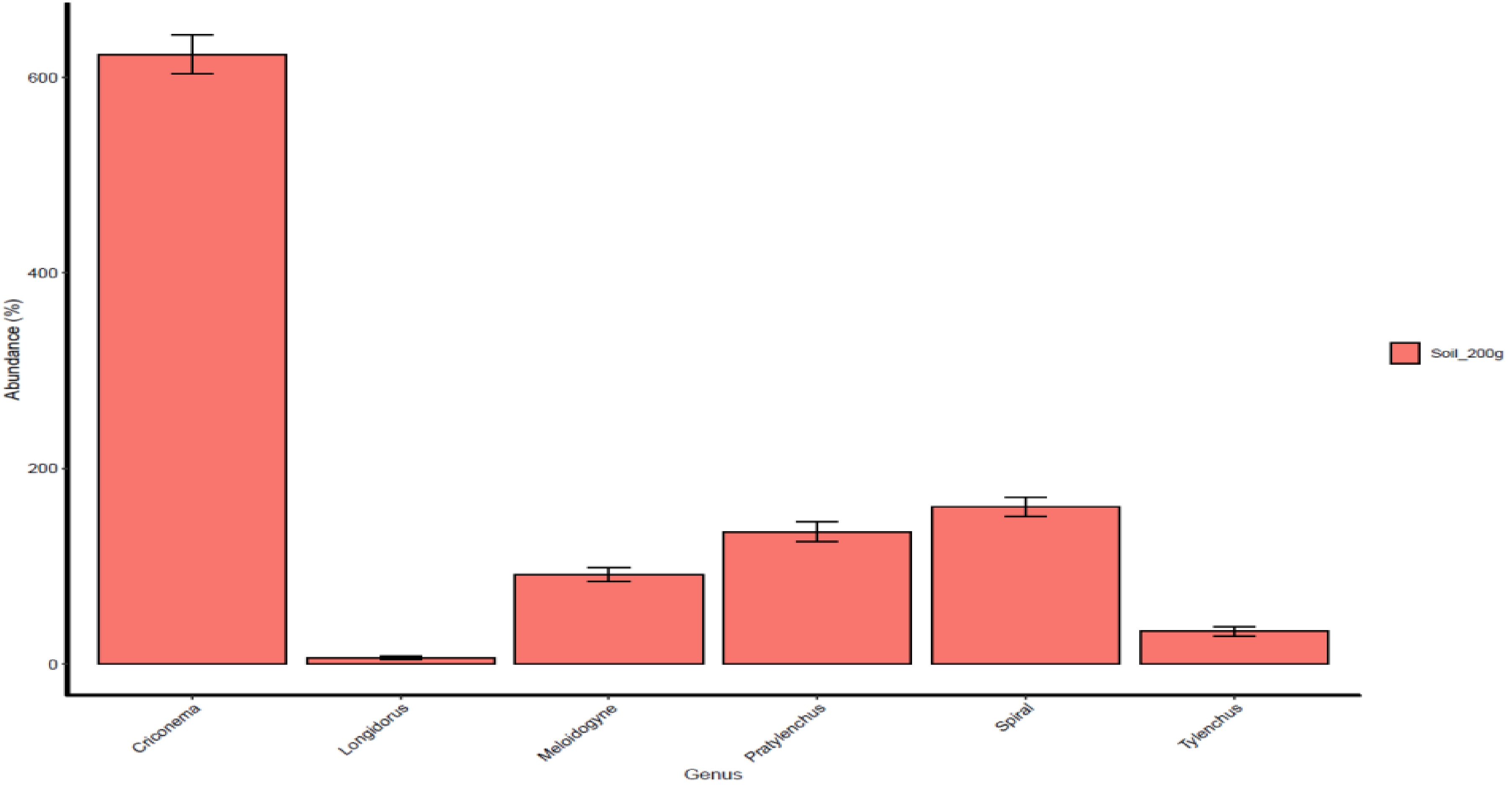

Figure 4-8: Figure 4.8: Distribution and prominence of major plant-parasitic nematodes in soil from wheat farms of KwaZulu-Natal, South Africa

4.5 Molecular identifications

Molecular identification was done to supplement morphological identification of the nematode population. A total of 124 soil, 61 roots and 16 wheat samples, DNA was successfully extracted. DNA samples were amplified by PCR for different plant-parasitic nematodes and some dominant free-living nematodes. 94% of positive DNA samples were successfully amplified using 18S rRNA as a target gene which produced a single fragment of approximately 900 bp (Figure 4.9). *Pratylenchus thornei* and *P. neglectus* were detected from 89.2% (50/56) of localities sampled using the 18S rRNA as a target gene (Table 4.7) (Floyd et al. 2005). PCR from ITS1 primers successfully amplified 33% of DNA positive samples which produced a single fragment of approximately 1080 bp (Figure 4.10) (Ferris et al. 1983). Spiral nematodes (*Rotylenchus sp*. and *Helicotylenchus* sp*.)* were successfully identified in 84% (42/56) of the locality using both the 18S rRNA and ITS1 genes. *P. thornei* and *P. neglectus* were also successfully identified using the ITS1 as a target gene. The amplification of D2-D3 expansion segments of the 28S containing region produced a single fragment of approximately 460 bp (Nunn, 1992; Kumari and Subbotin, 2012). Only 12% of DNA positive samples were successful amplified using the 28S with the majority of sequences being too short. *P. thornei, Mesocriconema sphaerocephalum* and *Merlinius brevidens* were identified using the D2-D3 expansion segments of the 28S containing region as the target gene (Table 4.7). The *P. bolivianus* was only detected in Limpopo using the D2-D3 expansion segments of the 28S containing region gene. The 18S rRNA and ITS1 genes were also used to identify some dominant free-living nematodes (Table 4.8). The sequences generated in this study were submitted to the NCBI database to generate new accession numbers (Table 4.7; 4.8) (Appendix I). Generated sequences matched with the species sequences available in GenBank when subjected to BLASTn (www.ncbi.nlm.nih.gov/blast) (Appendix I and II). The sequences on average were 99% identical (Table 4.7) with most deposited published sequences of nematodes species.

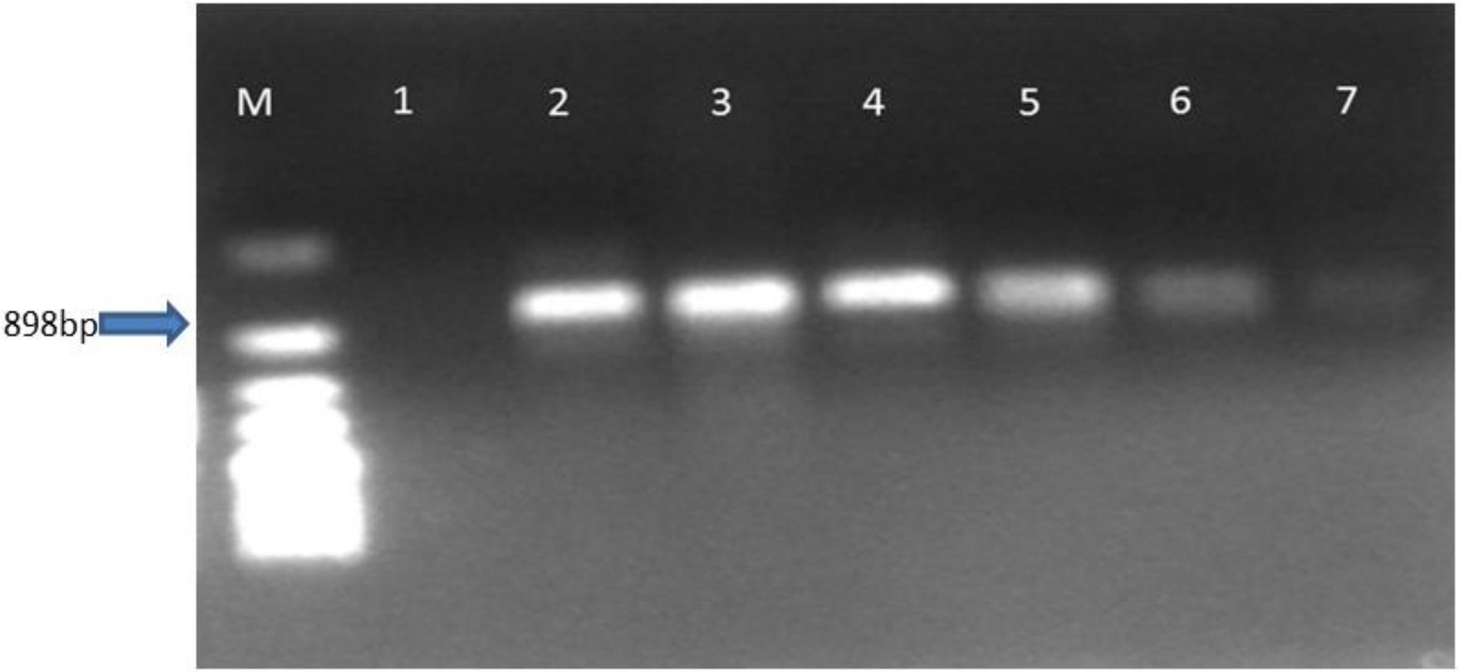

Figure 4-9: Agarose gel electrophoresis of the PCR products amplified with the 18S rRNA primers set of gap gene for DNA extracted from analyzed nematodes isolates. Lane M: GeneRuler 1kb plus DNA ladder; lane 1: negative control (DDW); lane 2: positive control (*Pratylenchus thornei*); lanes 3-7: showing amplified gap gene in isolates

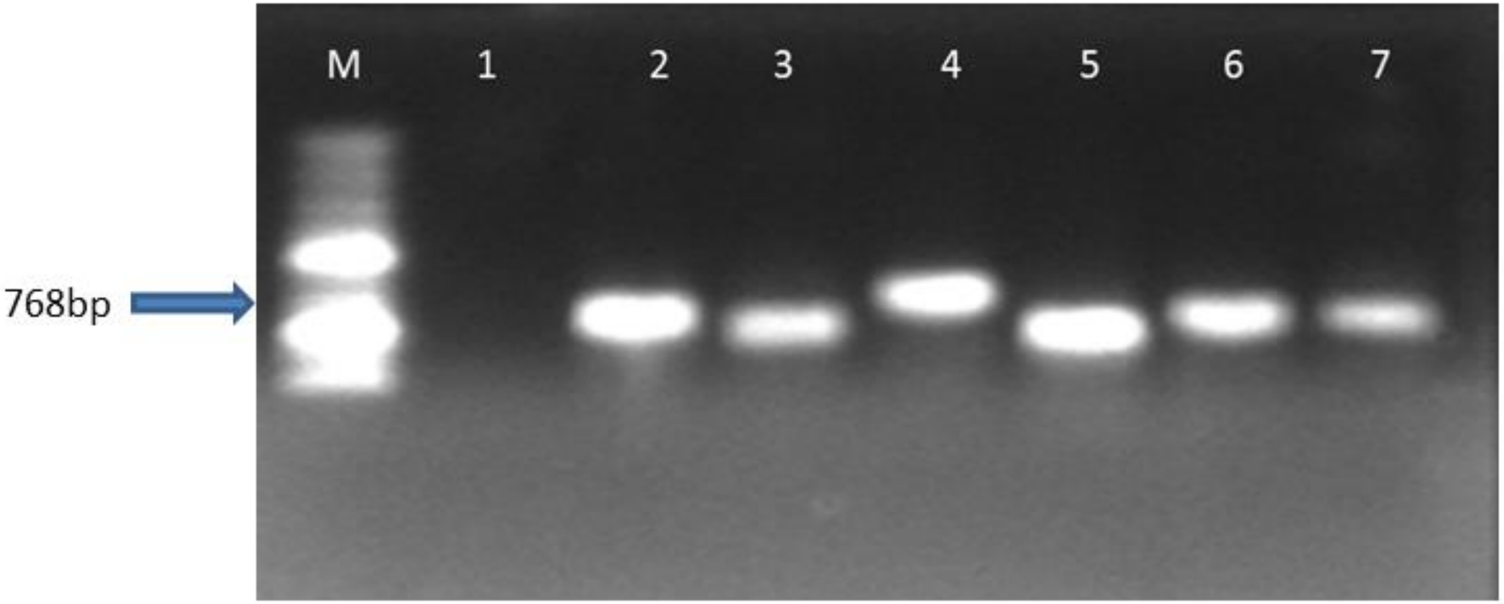

Figure 4-10: Agarose gel electrophoresis of the PCR products amplified with the ITS1 rRNA primers set of gap gene for DNA extracted from analyzed nematodes isolates. Lane M: GeneRuler 1kb plus DNA ladder; lane 1: negative control (DDW); lane 2: positive control (*Pratylenchus thornei*); lanes 3-7: showing amplified gap gene in isolate

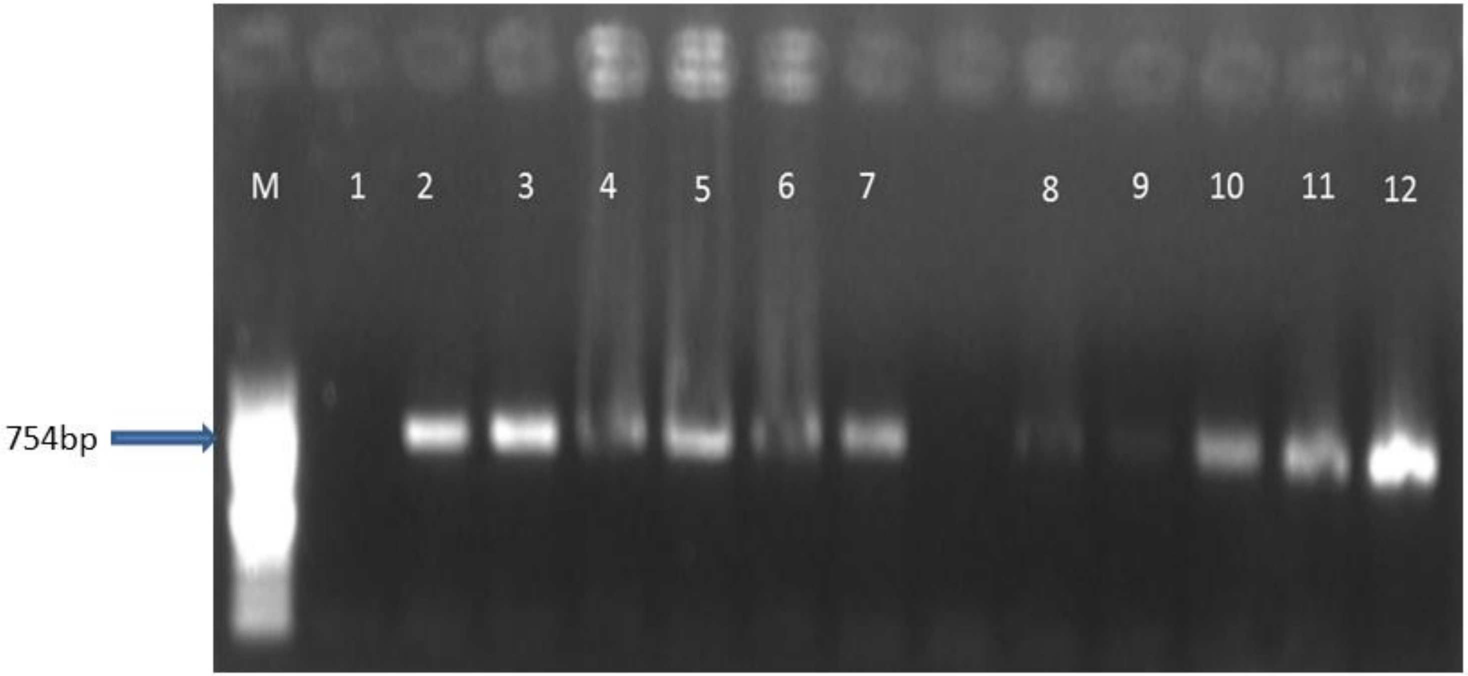

Figure 4-11: Agarose gel electrophoresis of the PCR products amplified with the D2-D3 expansion segments of the 28S rDNA primers set of gap gene for DNA extracted from analyzed nematodes isolates. Lane M: GeneRuler 1kb plus DNA ladder; lane 1: negative control (DDW); lane 2: positive control (*Pratylenchus thornei*); lanes 3-7: showing amplified gap gene in isolates

Table 4-7: Indicates species, target genes, and accession numbers during molecular detection of major plant-parasitic nematodes in wheat farms, South Africa

Nematode species	**18S rRNA**	**ITS1**	**D2-D3**
Pratylenchus neglectus	WC; MK779916 LP; MK779918 WC; MK779922 WC; MK779923	WC; MK786619	-
Pratylenchus thornei	FS; MK779918 WC; MK779921 FS; MK809266	WC; MK786617 WC; MK786618 WC; MK874501 WC; MK874503	WC; MK779924
Pratylenchus bolivianus	LP; MK779917 LP; MK779919 LP; MK779920	-	-
Paralongidorus bikanerensis	KZN; MK796432	-	-
Coslenchus rhombus	WC; MK796434	WC; MK874505 WC; MK874506	
Rotylenchus unisexus	FS; MK809259 FS; MK809263	-	-
Pararotylenchus brevicaudatus	-	FS; MN262443 FS; MN262444 FS; MN262445 FS; MN262446 FS; MN262447	FS; MN262454 FS; MN262456
Bitylenchus maximus	FS; MK796427 FS; MK796428	-	-
Bitylenchus ventrosignatus	-	FS; MN262442	
Helicotylenchus dihystera	WC; MK796429 LP; MK796430 FS; MK796435	LP; MN262448 FS; MN262451	-

Table 4.7*, (Continue)*

Helicotylenchus pseudorobustus	-	LP; MN262452	-
Tylenchorhynchus capitatus	-	-	-
Mesocriconema sphaeocephalum	-	KZN; MK874497	FS; MN262453
Merlinius brevidens		-	WC; MN262457
Merlinius joctus	KZN; MK809265	-	-
Tylencholaimus sp.		-	-
Hoplolaimus galeatus	WC; MK809260 WC; MK809261	-	-
Paramerlinius hexagrammus	-	-	-
Amplimerlinius paraglobigerus	NC; MK809267 NC; MK809269 FS; MK809268 LP; MN262449 WC; MN262450	WC; MK874504 NC; MK874507	-

WC: Western Cape; FS: Free State; NC: Northern Cape; KZN: KwaZulu-Natal; L: Limpopo; MP: Mpumalanga; NW: North West

(-); Not sequenced

Table 4-8: Indicates species, target genes, and accession numbers during molecular detection of some free-living nematodes in wheat farms, South Africa

Nematode species	**18S rRNA**	**ITS1**	**D2-D3**
Panagrolaimus Cf rigidus	FS; MK547102		
Ecumenicus monohystera		NC; MK874500	
Pararhyssocolpus paradoxus	NC; MK796431	NC; MK874502	
Acrobeles complexus		NC; MK874498	
Aporcelaimellus obtusicaudatus	NC; MK796433 NC; MK809262		
Aporcella simplex		NC; MK874498 FS; MK874499	NC; MN262455
Alaimus sp	NC; MK809264		

WC: Western Cape; FS: Free State; NC: Northern Cape; KZN: KwaZulu-Natal; L: Limpopo; MP: Mpumalanga; NW: North West

(-); Not sequenced

4.6 Phylogenetic analysis

The 18S rRNA and ITS1 gene generated from this study and the isolates obtained from the gene bank, were used to investigate phylogenetic relationships between the nematodes species. Each sequences generated from this study matched with the sequences available in GenBank when subjected to BLASTn (www.ncbi.nlm.nih.gov/blast). Each nematode species was aligned against a reference sequence from GenBank and its nucleotide composition analysed. For each nematode species sequence geneerated from this study, four simililar species sequences form the GenBank were used as references for Maximum Likelihood (ML) phylogenetic analysis. Similarity match scores above 50% were concidered as signgificant. For the 18S rRNA gene, 8 major clades were resolved with 10000 bootstrap support values. The outgroup was chosen as a *Haemonchus contortus* (Figure 4.12) For the ITS1 gene, 17 major clades were resolved with 10000 bootstrap support values. The outgroup was also chosen as a *Haemonchus contortus*.(Figure 4.13). The overall avarage *Pratylenchus* species 18S gene pairwise distance nucleotide differences wasfound to be 143.464 (Table 4.9). The collected nuclear ribosomal RNA gene and mitochondrial protein-coding gene sequences from diverse *Panagrolaimus* species and strains obtained from gene bank, which included the newly discovered isolates from Clarens, Free State, used to investigate phylogenetic relationships in this nematode genus were used (Figure 14).

4.7

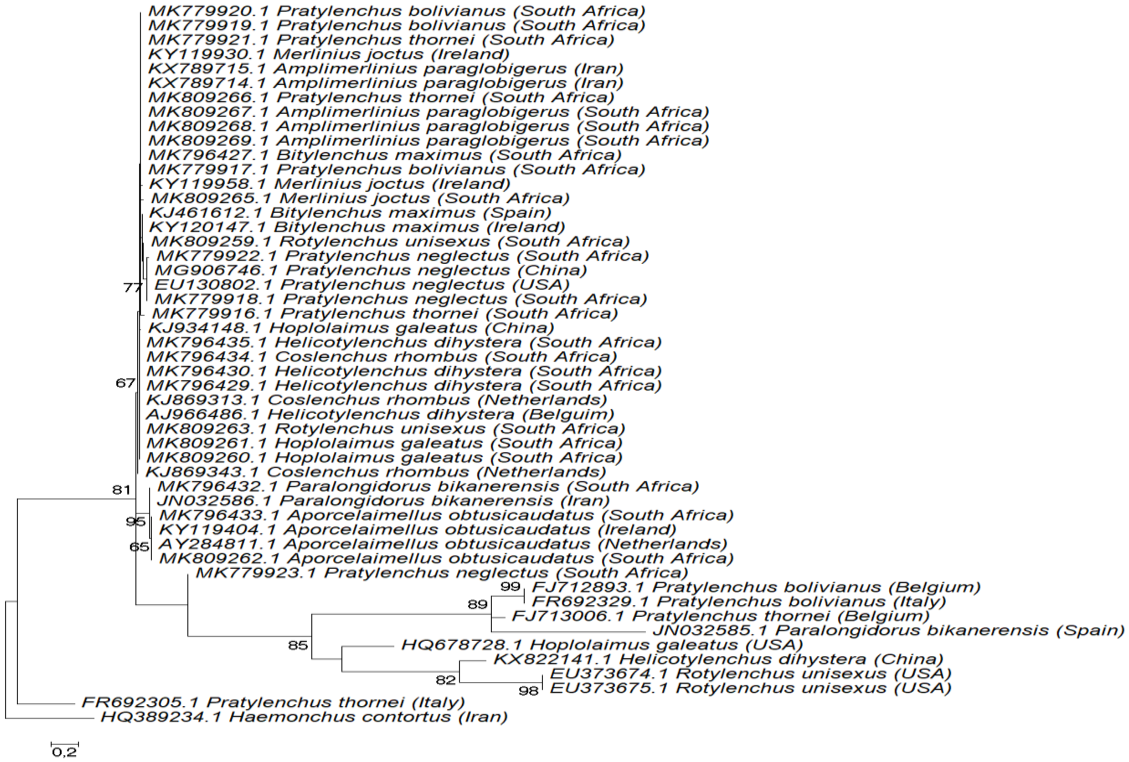

Figure 4-12: A phylogenetic tree of nematodes based on 18S rRNA gene. Plant-parasitic species belonging to the different families based on 18S rRNA sequences from GenBank, including the new sequences of species detected in this study from the Western Cape, Free State, Northern Cape, KwaZulu-Natal, Limpopo, Mpumalanga and North West provinces of South Africa. Tree was constructed by using the Maximum Likelihood method based on the Tamura 3-parameter model (Tamura , 1992)

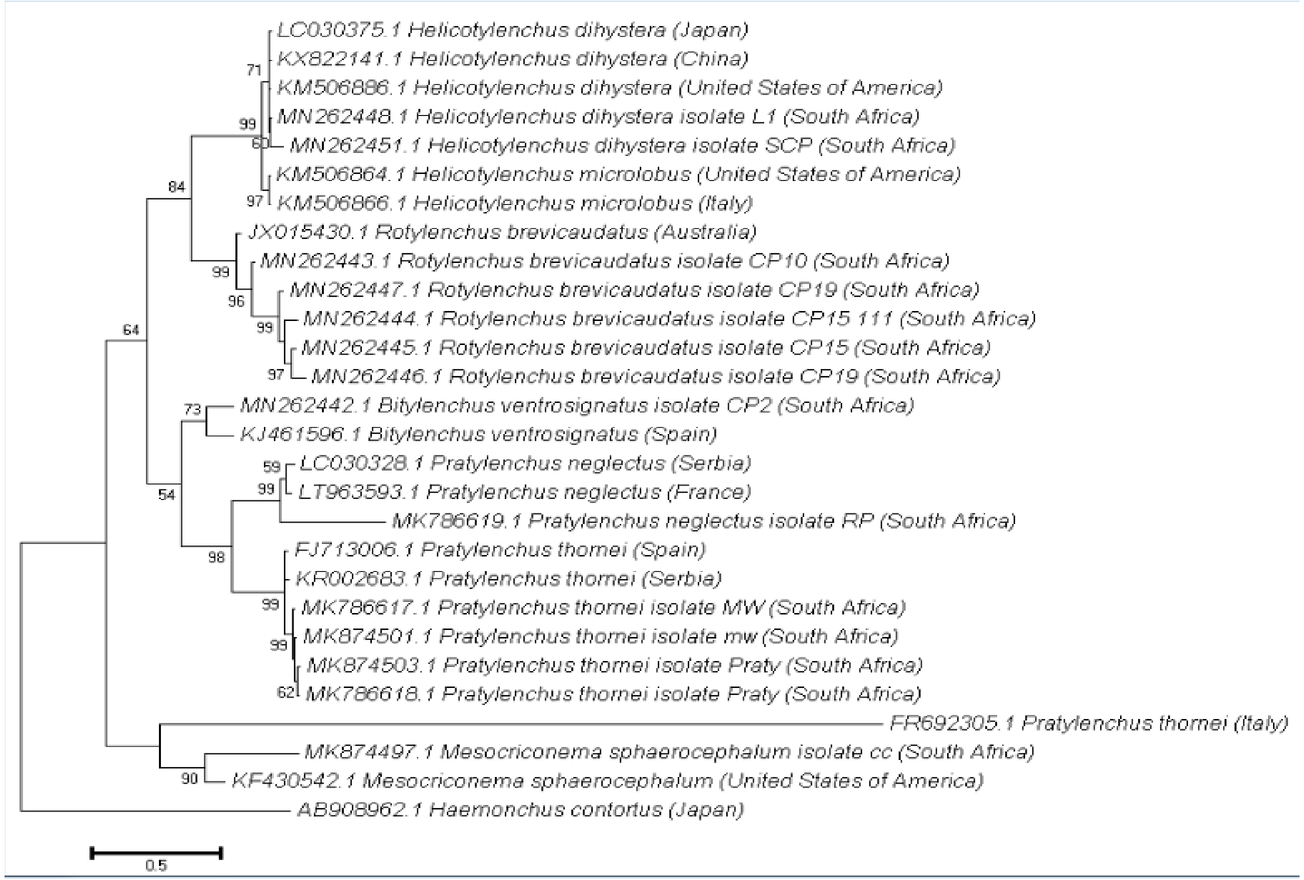

Figure 4-13: A phylogenetic tree of nematodes based on ITS1 gene. Plant-parasitic species belonging to the different families based on ITS1 sequences from GenBank, including the new sequences of species detected in this study from the Western Cape, Free State, Northern Cape, KwaZulu-Natal, Limpopo, Mpumalanga and North West provinces of South Africa. Tree was constructed by using the Maximum Likelihood method based on the Tamura 3-parameter model (Tamura , 1992)

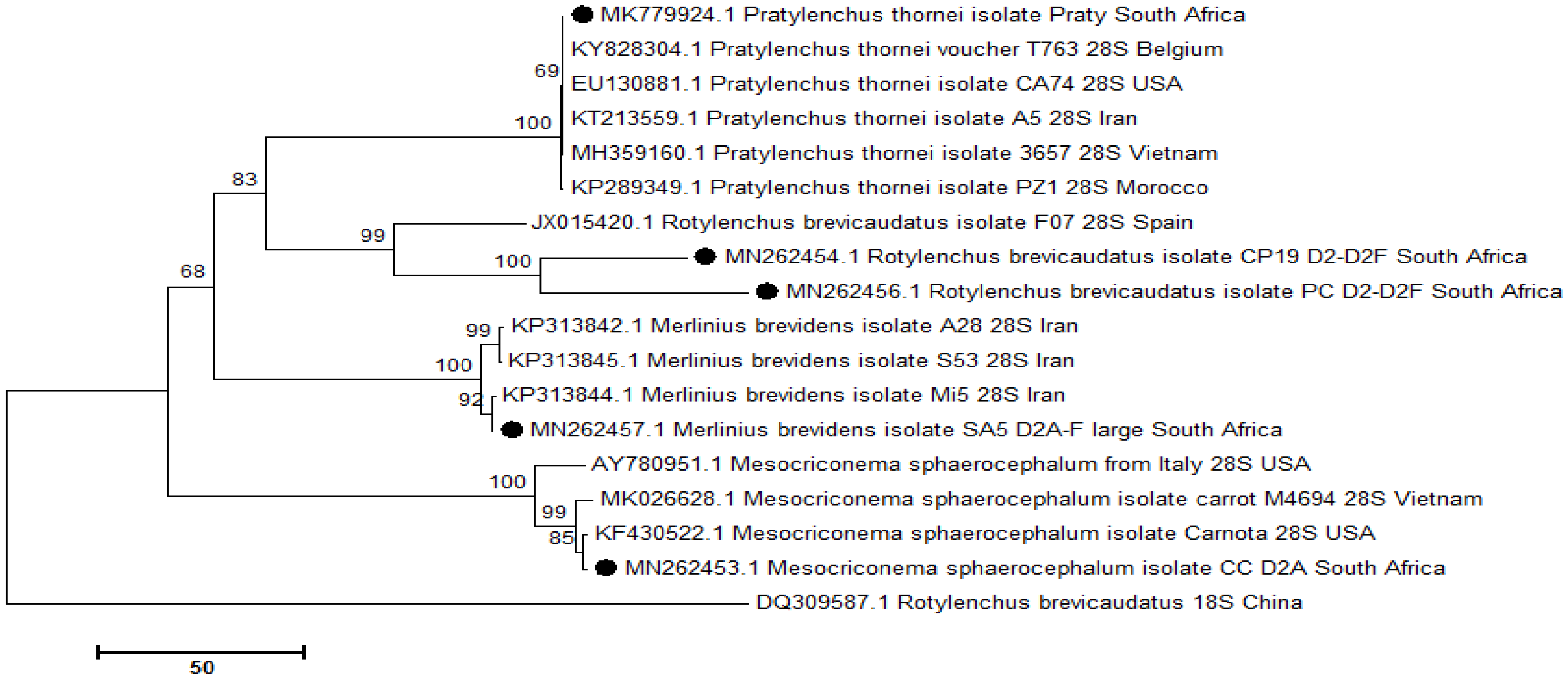

Figure 4-14: The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The optimal tree with the sum of branch length=693.62500000 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (10000 replicate) are shown above the branches (Felsenstein, 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the number of differences method (Nei and Kumar, 2000) and are in the units of the number of base differences per sequence. The analysis involved 18 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 501 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 (Tamura et al. 2013).

Figure 4-15: The evolutionary history of species belonging to the family Panagrolaimidae based on 18S rRNA sequences from GenBank, including the new sequence of *Panagrolaimus rigidus* from the Free State, South Africa, was inferred by using the Maximum Likelihood method based on the Tamura 3-parameter model (Tamura, 1992). The tree with the highest log likelihood (-3528.1154) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches

Table 4-9: *Pratylenchus* species 18S gene pairwise distance nucleotide differences found among the taxa

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
*Pratylenchus_bolivianus_* MK779919.1		3.519	8.486	9.626	12.428	8.160	3.597	9.298
*Pratylenchus_bolivianus_* MK779920.1	15.000		8.111	9.392	12.516	8.262	3.313	9.370
*Pratylenchus_neglectus_* MK779918.1	92.000	86.000		6.483	12.275	9.139	8.211	10.281
*Pratylenchus_neglectus_* MK779922.1	120.000	119.000	51.000		12.332	9.393	9.429	10.437
*Pratylenchus_neglectus_* MK779923.1	302.000	298.000	290.000	284.000		12.500	12.500	12.588
*Pratylenchus_thornei_* MK779916.1	95.000	93.000	110.000	132.000	304.000		8.188	8.145
*Pratylenchus_bolivianus_* MK779917.1	16.000	13.000	91.000	118.000	300.000	96.000		9.060
*Pratylenchus_thornei_* MK779921.1	117.000	114.000	130.000	143.000	296.000	80.000	112.000	

CHAPTER FIVE

DISCUSSION

5.1 Occurrence of nematodes

Soil environment is an important ecosystem on itself consisting of a mixture of physical, chemical and biological mediums. Soils are very dynamic in their nature having the physical, chemical and biological properties determining its nature. Soil organic compounds introduce may either affect the availability of trace metals (chemical), increase or decrease soil metal mobility (physical) and alter the behaviour of soil organisms and plants (biological) (Halbrendt, 1996). Classifications of retention, transformation and transport processes are broadly defined by Cheng (1992). Nematodes can be affected by several environmental factors and soil antagonists. This study focus on the physical and chemical factors of the soil which might have a role on the distribution of nematodes across the seven provinces sampled during this project. Wheat is planted mostly in sandy loam and sandy soil which are the preferred conditions for wheat plantations in South Africa (Grain, SA, 2015). Catroux et al. (1987) and Hassink et al. (1991) found that the net mineralization of soil organic matter was more rapid in sandy soils than in clay soils. Van Veen and Kuikeman, (1990), stated that the lower net mineralization rate found in clay soils was assumed to be due to a greater degree of physical protection of soil organic matter. For example, the organic matter is located in places relatively inaccessible to microbes. Jones, (1982) concluded by stating that nematodes are restricted to pores of 30 µm diameter. This demonstrates that the rate of organic matter is influenced by decomposition and mineralization rate, which is strongly related to the particle size of the soil mineral fraction, greater decomposition rates being associated with the larger sand-size particles (Elliot et al., 1980; Van Veen and Kuikman, 1990). Large free-living nematodes such as *Longidorus* can act as an indicator of the state of the soil. The addition of organic materials usually improves soil structure and thus improving the capacity of the soil to hold water and exchange ions so that together with the nutrients released by the organic matter. The improved soil structure promotes root growth of plants. Predatory nematodes such as *Mononchus* sp. are comparatively large and are therefore favoured by coarse soils of high organic matter content (Akhtar and Malik, 2000). This explains why a lot of free-living nematodes (*Dorylamida* sp*., Rhysocolpus pararoxus, Pararhyssocolpus paradoxus* and *Acrobeles complexus simplex)* were found in irrigation areas in this study where soil organic amendments are practised. The pH of 4.1 recorded in samples from Douglas seems to be acidic for planting, but it is easily encountered by adding lime soil, if the other minerals are sufficient in soil. Similar results were also observed from dry land. Release of nutrients in soil such as nitrogen, phosphorus and potassium depends on the decomposition and the demand made by various populations of soil organisms. According to the literature (Johnson, 1959; Mankau and Minteer, 1962; Rodriguez-Kabana et al. 1987) reported that addition of organic matter to soil stimulates the activity of bacteria, fungi, algae, and other microorganisms. Thus, increase microbial activity in amended soil causes enhanced enzymatic activities (Rodriguez-Kabana et al.1983) and accumulation of decomposition end products and microbial metabolites, which may be detrimental to plant-parasitic nematodes. In addition, the single most important fertilizer input is nitrogen and is required in the largest quantities for crop production and can be applied periodical if necessary.

Burrowing nematodes (*Pratylenchus* sp*. Rotylenchus* sp*.* and *Scutellonema* sp.) were the most common plant-parasitic nematodes recorded in both non-irrigation and irrigation agricultural fields. It is not clear if these observations could be attributed to the soil factors or the host plants. Generally, it is assumed that for PPNs, the presence of their host plant is the main determinant of their population densities (Yeates, 1987). In general, the most prevalent wheat crops in South African agricultural fields were maintenance or good hosts of two *Pratylenchus* sp. (*P. neglectus* and *P. thornei*), followed by *Rotylenchus unisexus*, *Scutellonema brachyurus* and *Helicotylenchus* sp., as most prevalent species, which may explain their wide distribution. The two species of *Pratylenchus (P. neglectus* and *P. thornei*) were included among the most abundant nematodes in the area, as 85.6% of the nematodes recovered belonged to one of these two species. *Pratylenchus* was clearly the dominant genus among plant-parasitic nematodes of South African wheat fields, occurring at 98% of the sites and with relative abundances of 3321 per 200 g of soil, 794 per 5 g of roots. A similar report by Yeates (1987) and Nombela et al. (1999), found that *Pratylenchus* species, particularly *P. neglectus* and *P. thornei* were also widespread in Southern Spain region with 54.9 and 41.2% of the sites, respectively. Furthermore, *P. neglectus* and *P. thornei,* showed similar host range, have been reported as pests of grasses and herbage legumes

(Townshend et al. 1973; Tobar & Verdejo, 1978; Kimpinski et al. 1988) and thus could be considered as potential pests of southern Spain grasslands (Talavera and Navas, 2002). The *P. bolivianus* detected only in Limpopo might be attributed to the agricultural practice. The similar finding is reported by Swart & Marais, (2002).. These reports suggest that, the presence or population of the species depends more on the plant species planted. Pants are often planted in soils which are regarded as suitable for that crop, but soil amendments or manipulations are sometimes necessary to improve the soil fertility for plant growth (Akhtar and Malik, 2000). As mention above, this might suppress nematodes population and increase plant growth. It is assumed that the application of organic matter to the soil and the beneficial effect of organic amendments are generally due to the provision to the crops of extra nutrients of some sort (Akhtar and Malik, 2000). According to Page (1966), plants grown in plots receiving organic manures are always larger than those receiving inorganic fertilizers. Furthermore, very often, when there is a decrease in the population of soil pathogens, there is a consequent increase in crop yield. However, nematode control is by no means always followed by increased yield. Talavera and Navas, (2002), regarded 188 and 122 nematodes per 100 cm^3 as maximum densities which could significantly cause yield reductions in agricultural wheat production. The 3321 nematodes per 200 g reported in this current survey is 20 times higher than the maximum threshold that is described by the latter. Pin nematodes (*Paratylenchus*) have been found to be damaging in semi-arid regions, where rainfed agriculture is usually practiced (Ortiz-Monasterio and Nicol, 2004)). Similar observation on the dominance by pin nematodes in dry land pastures has also been reported by Yeates (1987) and Nombela et al. (1999).

Spiral (*Rotylenchus, Scutellonema* and *Helicotylenchus)* and stunt nematodes (*Tylenchorhynchus* sp.) are often associated with pastures and grasslands, but there is little evidence of damage in wheat field situations. *Xiphinema* sp. (a*mericanum* species complex) and *Ditylenchus* sp. have been cited as major pests of pasture legumes and grasses (Cook and Yeates, 1993), but their relative abundances in the sampled area were fewer in soil and it seems unlikely that they cause any damage to the crops. Nevertheless, *Ditylenchus* sp. populations in soil are generally low and populations increase rapidly in plants when environmental conditions are favourable, but its pathogenicity has not been determined on wheat. Leaf or foliar nematodes, *Aphelenchoides* species were found on wheat in relatively low numbers and can also increase rapidly in plants when environmental conditions are favourable, but its pathogenicity on wheat has also not been determined. Agricultural fields that planted wheat, *Meloidogyne* and *Heterodera* spp. were little to absent, except in the localities where there is the performance of agricultural experiment using different crops, such as Cedara where *Meloidogyne* juveniles were found (Table 4.5). Talavera & Navas, (2002), reported the absence of *Meloidogyne* spp., which is also considered as an important pest of grasslands. The reports by Yeates (1987) and Nombela et al. (1999) strongly suggest that nematodes population densities are associated with host plant species rather than the soil factors. The results from this study found no correlation between the soil minerals and nematodes, which suggests that nematodes population densities depends on the presence of the host plant.

5.2 Molecular characters

The use of DNA sequences to identify nematodes was used to supplement traditional morphological identification. Almost all species included in this study have a unique sequence combination or signature; most exceptions are in cases where the validity of the species identification is questionable. In the present study, the approach was successfully used for the detection of several nematodes species in agricultural infested field samples, including wheat roots and soil, indicating the high potential of this method. Sequenced PCR amplicons of the 18S rRNA, D2-D3 expansion segments of the 28S rDNA and the ITS1 containing region produced a single fragment of approximately 900 bp and 1080 bp genes which matched with nematodes sequences available on GenBank database with 99% -100% identity score which confirmed observations made with the morphological identification (Table 4.14). Several rRNA fragments have been proposed as appropriate markers for barcoding nematodes by Floyd et al. (2002) and De Ley et al. (2005). This includes the comparison of 18S and ITS1 rRNA gene regions included in this study show that the D2–D3 expansion segment of 28S rRNA seems to be a better target than partial 18S rRNA for barcoding with respect to recognizing a higher degree of interspecific genetic variability (Table 4.14). The detection and sensitivity of gene target regions has also been reported by Castillo and Vovlas (2007), and Peng et al. (2013). For example, using morphological identification, *P. thornei* and *P. neglectus* were described across the field sampled, while the molecular assay revealed an additional *P. bolivianus* and other *Pratylenchus* species with uncertainty sequences which were not listed in this study. Similar findings were reported by Castillo and Vovlas (2007). According to Castillo and Vovlas (2007), the reliability of *Pratylenchus* morphological character species, often diagnosed on few characters, some of them polymorphic, which may sometimes result in doubtful considering the potential of similar character sets converging across disparate geographies. Furthermore, alternative hypotheses or assumptions are that a species is consistent but may be widely distributed because it is ancient, or that it has been dispersed. This is seen on the modern global anthropogenic practices, often in conjunction with agriculture. Therefore, the monophyl of several morphological species can be tested by examining an independent character set, using sequences shown to evolve at about the level of extant species in relation to worldwide populations. Regarding the current study, even where several geographically divergent isolates are included, most species are reassuringly monophyletic. Exceptions can generally be accounted for by misidentifications, in spite of some cases of species validity might be questioned and subsequently tested by sequencing topotypes, where available (Subbotin et al. 2008).

The study showed the number of main *Pratylenchus* clades varied depending on genes used. The BI analysis of the combined 18S rRNA and ITS1 dataset revealed at least six clades with *Pratylenchus* species. While the *Pratylenchus* sp. 18S gene pairwise distance nucleotide differences test value was too high and unusual. This might be due to the different is species (Table 9). The three major highly supported root-lesion nematode groups were evident from all analyses (Figure 4.12; 4.13; 4.13). *Helicotylenchus dihystera, Amplimerlinius paraglobigerus, Bitylenchus maximus, Merlinius joctus, Paralongidorus bikanerensis, Hoplolaimus galeatus* and *Rotylenchus unisexus* each formed clades with strains of the same species obtained from the Genbank using the 18S rRNA gene. *Helicotylenchus dihystera, Helicotylenchus microbolus, Rotylenchus brevicaudatus, bitylenchus ventrosignatus* and *Mesocriconema sphaerocephalum* also formed clades with the strains of the same species obtained in the Genbank when using the ITS1 gene. The two live specimens collected from Free State, South Africa formed their own clade, based on the phylogenetic tree, suggesting that, the two shared a common ancestor in the very recent evolutionary past (Figure 4.14). The latter also applies to the species in Figure 4.12 and Figure 4.13. *Panagrolaimus* cf. *papillosus*, was found to be more closely than the others. The Antarctic species *P. davidi* was found to be very closely related to two *Panagrolaimus* species from Iran. Phylogenetic and molecular clock analyses suggested that *P. davidi* and *P. facetus* from Iran shared a common ancestor in the very recent evolutionary past. Phylogenetic analysis, and cluster analysis showed similarities between several nematode species in the area (Be specific). High similarities between rare species can be caused by single random occurrences within the same sample and thus were not taken into account. Significant differences ($P < 0.05$), of PPNs abundance found in soil were observed across the entire agricultural fields sampled with $P < 0.0001$, especially *Pratylenchus, Rotylenchus, Scutellonema* and *Helicotylenchus* species. The significant differences were also observed between soil and root samples. Similar observations were reported by Peng et al. (2013). Furthermore, significant differences were observed between soil and roots and also variations of PPNs distributions between cultivars were observed. Ferris (1984), reported variation of PPNs population densities between different cultivars and seasons. It is worthy to note that, the presence of a plant-parasitic species does not automatically mean that it will affect the plant. According to Yeates, (1987), plants occur in associations depending on various physical and ecological factors, and it is generally assumed that for PPNs the presence of their host plant is the main determinant of nematode population densities. Therefore, impacts or effects of PPNs are expected to be associated with the host plant and the species population densities and reproduction rates. In this present study, it was challenge to connect these associations to any ecological factor due to the lack of proper environmental data, but further studies including host plant associations and environmental data should provide stronger evidence. Yeates (2003), reported the use of diversity indices to assess variations in nematode fauna in pastures, but other authors (Freckman and Ettema, 1993) considered that diversity is not very reliable in showing differences among treatments, systems or crops. Furthermore, Nombela et al. (1999) argued that diversity based on genera was not influenced by the time passed after the last cropping in a pasture system. In addition, it suggested that a more detailed analysis at a species level could be more sensitive to differences in nematode diversity (Yeates, 2003). In this present study, diversity indices based on species identification were not useful in distinguishing differences among ecological areas either. However, different physical ecological, climatic conditions and types of agricultural practices were taken into consideration, owing to the relative homogeneity of the habitat sampled.

Hoplolaimus galeatus was found in Clarens, FS, often in sufficient numbers and can cause considerable damage to the roots of wheat and other plants and it is considered quarantine according to the literature (Thorne and Malek, 1968), perhaps new diagnostic assays are necessary to confirm the introduction of this species to FS. Furthermore, a new possible new species of *Rotylenchus* spp. was found in Roodebloem, WC, but new diagnostic assays are necessary to confirm the species since morphological identification suggests it might be different from the previously listed *Rotylenchus* spp*.* In addition, a variety of genera and species, which could be potential major pest if their respective population were to build up or increase were found in FS and this, raises concerns. The effect of *Aphelenchus* and *Panagrolaimus* ssp on the leaf of wheat necessitates more research to be done. Although *Panagrolaimus* is not a plant-parasite, it is crucial to note this, since it can possible form associations with fungi and bacteria that causes rot. (Siddiqui and Mahmood, 1998; Bjorsell et al. 2017) (such, as *Anguina* and *Subanguina*) that kills animals when they feed on such plant parts.

5.3 Conclusion

The study was able to meet its objectives, by identifying species which are found in wheat agricultural fields of South Africa. The study successfully identified nematodes both morphologically and molecularly. The study also provided the knowledge on the nematodes population densities that are found in both soil and roots. This current study gives an idea of which nematodes species affects plant roots, which eventually affect the plant growth. These are the species which are considered as economical important species or genus. Furthermore, successfully provided or gave update on the patterns of distributions and describe common and rare species. However, such patterns must be studied periodically in order to track the population densities. There is serious lack of knowledge or minimal awareness on nematode pests among farmers, extension workers and politicians on the extent of t damage nematodes could cause. This lack of information poses numerous challenges in securing improved crop security through PPN control, including the procurement of essential funding. During the collections of samples and interactions with farmers, it became clear that main targets of these funding control initiatives have been for the more conspicuous insects and fungal pathogens that attack the plant above ground. Furthermore, the extent to which nematodes can significantly cause damage on the crops was not well understood by the famers. This necessitates that, the damage caused by nematodes and the benefits attained from their control must be adequately demonstrated to the farmer so that meaningful success can be achieved. This project has tried to bridge the gap in knowledge regarding the current status of plant-parasitic nematodes and their prevalence in South Africa agricultural fields. The present study is only the first step on the way to gain better a understanding of the common or rare species distribution and their effect on South African wheat. This project forms a basis for which more research can be done regarding their control strategies.

5.4 Recommendations

- To explore the impact of different environmental conditions, the patterns of distributions and describe common and rare species and such patterns must be released periodically to track changes in population densities and the introduction of new species.

- Conduct greenhouse and field experiments to demonstrate their extent of the damage.

- Develop multiplex molecular diagnostic assays including LAMP and qPCR for the detection of economically important parasitic nematodes of wheat cultivars in South Africa.

- Since PPNs can be major facilitators of secondary infection by fungi and bacteria, more research is still necessary to understand the extent of damage on wheat cultivars as a result of their (PPNs, fungi and bacteria) associations.

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ANNEXURE 1: PLANT PARASITIC NEMATODE GENE SEQUENCES

#MK779916.1_*Pratylenchus_thornei*_isolate_142_small_subunit_ribosomal_RNA_gene_partial_sequence

TGCCATTGATATCAACCTGGTAAACTGGGGGAATTGTAGAAYTAATACATGCACCA
AAGCTCCGACCCGCGAGGGGAGAGCGCATTTATTAGAACAAAACCAAGCGGCTTC
GGCCGTCCATTGTTGACTCAGAATAACTAAGCTGATCGTACGGTCTTGTACCGACG
ACGTGTCTTTCAAGTTTCTGCCTTATCAACTTTCGATGGGAGTGTATCTGACTACCC
TGGTGGGGACGGGTAACGGAGGATAAGGGGGCGACTCCAGAGAAAGGGGCTGA
GAAATGGCCACTACGGCTAAGGATGGGAGCAGGCGCGCAAATTACCCACTCTCAA
TGCGAGGAGGTAGTGACGAGAAATAACGAGGGCGCTCTCTTTGAGGCCGGTCATC
GGAATGGGTACAATTTAAACCCTTTAACGAGTATCTATGAGAGGGGAAGTCTGGGG
CCAGCAGCCGCGGTAATTCCAGCTCTCTAAATGCATAGAAATATTGCTGCGGTTAA
AAAGCTCGTAGTTGGATCTGTGCCTGTGCTCTGGTCCACCTCGCGGGGCGCACTG
GGTGTCACAGGGTTTCCTGCAATGTTCAGCCCCGGCGCCTTCACCGGCACCGGTG
TGTTGATGCTGCAAGTTTACTTTGAACAAATCGGAGTGCTCCAAACAGGCGTTTGC
TTGAACGTTCGTGCATGGAATAATAGAAGAGGATTTCGGTTCAATTTTATTGGTTTT
ATTGGCCGAGATAATGGTTAACAGAGACAAACGGGGGCATTTGTATTGCTGCGTG
AGAGGTGAAATTCTTGGACCGTAGCAAGACAAACTACCGCGAAAGCATTTTGCCAA
GAATGTCTTCATTAATCAAGAACGAAAGTCAGAGGTTCGAAGGCGATCAGAATACC
CGCCCCACA

#MK779917.1_*Pratylenchus_bolivianus*_isolate_CW_small_subunit_ribosomal_RNA_gene_partial_sequence

GGGTGTCGCTCAAATATACCTGCCAAACTGTGGTAATTCTAGAGCTAATACATGCA
CCAAAGCTCCAACCCGCAAGGGAAGAGCGCATTTATTAGAACAAAACCAAGCGGC
TTCGGCCGTCCAAAGTTGACTCAGAATAACTAAGCTGATCGCATGGTCTTGTACCG
GCGACGTGTCTTTCAAGCATCTGCCTTATCAACTTTCGATGGTAGTGTATCTGACTA
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#MK796427.1_*Bitylenchus_maximus*_isolate_CP2_small_subunit_ribosomal_RNA_gene_partial_sequence

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#MK796428.1_*Bitylenchus_maximus*_isolate_CP3_small_subunit_ribosomal_RNA_gene_partial_sequence

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#MK796429.1_*Helicotylenchus_dihystera*_isolate_CRT_small_subunit_ribosomal_RNA_gene_partial_sequence

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#MK796430.1_*Helicotylenchus_dihystera*_isolate_L1_small_subunit_ribosomal_RNA_gene_partial_sequence

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#MK796435.1_*Helicotylenchus_dihystera*_isolate_SPL_small_subunit_ribosomal_RNA_gene_partial_sequence

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#MK796432.1_*Paralongidorus_bikanerensis*_isolate_PL1_small_subunit_ribosomal_RNA_gene_partial_sequence

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#MK796434.1_*Coslenchus_rhombus*_isolate_RR_small_subunit_ribosomal_RNA_gene_partial_sequence

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#MK786617.1_*Pratylenchus_thornei*_isolate_MW_internal_transcribed_spacer_1_partial_sequence_5.8S_ribosomal_RNA_gene_and_internal_transcribed_spacer_2_complete_sequence_and_large_subunit_ribosomal_RNA_ge

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#MK786618.1_*Pratylenchus_thornei*_isolate_Praty_internal_transcribed_spacer_1_partial_sequence_5.8S_ribosomal_RNA_gene_and_internal_transcribed_spacer_2_complete_sequence_and_large_subunit_ribosomal_RNe

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#MK786619.1_*Pratylenchus_neglectus*_isolate_RP_internal_transcribed_spacer_1_partial_sequence

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#MK874501.1_*Pratylenchus_thornei*_isolate_mw_internal_transcribed_spacer_1_partial_sequence_5.8S_ribosomal_RNA_gene_and_internal_transcribed_spacer_2_complete_sequence_and_large_subunit_ribosomal_RNA_ge

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#MK874497.1_*Mesocriconema_sphaerocephalum*_isolate_cc_internal_transcribed_spacer_1_partial_sequence_5.8S_ribosomal_RNA_gene_complete_sequence_and_internal_transcribed_spacer_2_partial_sequence

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#MK874504.1_*Amplimerlinius_paraglobigerus*_isolate_Rb_internal_transcribed_spacer_1_partial_sequence_5.8S_ribosomal_RNA_gene_and_internal_transcribed_spacer_2_complete_sequence_and_large_subunit_ribosoe

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#MK874507.1_*Amplimerlinius_paraglobigerus*_isolate_Rty_internal_transcribed_spacer_1_partial_sequence_5.8S_ribosomal_RNA_gene_and_internal_transcribed_spacer_2_complete_sequence_and_large_subunit_ribose

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#MK874505.1_*Coslenchus_rhombus*_isolate_Rf_internal_transcribed_spacer_1_partial_sequence_5.8S_ribosomal_RNA_gene_complete_sequence_and_internal_transcribed_spacer_2_partial_sequence

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#MK874506.1_*Coslenchus_rhombus*_isolate_RR_internal_transcribed_spacer_1_partial_sequence_5.8S_ribosomal_RNA_gene_and_internal_transcribed_spacer_2_complete_sequence_and_large_subunit_ribosomal_RNA_g...

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#MN262442.1_*Bitylenchus_ventrosignatus*_isolate_CP2_internal_transcribed_spacer_1_partial_sequence_5.8S_ribosomal_RNA_gene_and_internal_transcribed_spacer_2_complete_sequence_and_large_subunit_ribosomae

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#MN262443.1_*Rotylenchus_brevicaudatus*_isolate_CP10_internal_transcribed_spacer_1_partial_sequence_5.8S_ribosomal_RNA_gene_and_internal_transcribed_spacer_2_complete_sequence_and_large_subunit_ribosomae

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#MN262444.1_*Rotylenchus_brevicaudatus*_isolate_CP15_1111_internal_transcribed_spacer_1_partial_sequence_5.8S_ribosomal_RNA_gene_and_internal_transcribed_spacer_2_complete_sequence_and_large_subunit_ribe

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#MN262445.1_*Rotylenchus_brevicaudatus*_isolate_CP15_internal_transcribed_spacer_1_partial_sequence_5.8S_ribosomal_RNA_gene_and_internal_transcribed_spacer_2_complete_sequence_and_large_subunit_ribosomae

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#MN262446.1_*Rotylenchus_brevicaudatus*_isolate_CP19_internal_transcribed_spacer_1_partial_sequence_5.8S_ribosomal_RNA_gene_complete_sequence_and_internal_transcribed_spacer_2_partial_sequence

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#MN262447.1_*Rotylenchus_brevicaudatus*_isolate_CP19_internal_transcribed_spacer_1_partial_sequence_5.8S_ribosomal_RNA_gene_and_internal_transcribed_spacer_2_complete_sequence_and_large_subunit_ribosomae

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#MN262448.1_*Helicotylenchus_dihystera*_isolate_L1_internal_transcribed_spacer_1_partial_sequence_5.8S_ribosomal_RNA_gene_and_internal_transcribed_spacer_2_complete_sequence_and_large_subunit_ribosomal_e

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#MN262451.1_*Helicotylenchus_dihystera*_isolate_SCP_internal_transcribed_spacer_1_partial_sequence_5.8S_ribosomal_RNA_gene_and_internal_transcribed_spacer_2_complete_sequence_and_large_subunit_ribosomale

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#MN262452.1_*Helicotylenchus_microlobus*_isolate_SPL_internal_transcribed_spacer_1_partial_sequence

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#MN262450.1_*Amplimerlinius_paraglobigerus*_isolate_SA5_internal_transcribed_spacer_1_partial_sequence_5.8S_ribosomal_RNA_gene_and_internal_transcribed_spacer_2_complete_sequence_and_large_subunit_ribose

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#MK779924.1_*Pratylenchus_thornei*_isolate_Praty_large_subunit_ribosomal_RNA_gene_partial_sequence

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#MN262453.1_*Mesocriconema_sphaerocephalum*_isolate_CC_D2A_large_subunit_ribosomal_RNA_gene_partial_sequence

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#MN262454.1_*Rotylenchus_brevicaudatus*_isolate_CP19_D2D2F_large_subunit_ribosomal_RNA_gene_partial_sequence

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#MN262456.1_*Rotylenchus_brevicaudatus*_isolate_PC_D2D2F_large_subunit_ribosomal_RNA_gene_partial_sequence

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#MN262457.1_*Merlinius_brevidens*_isolate_SA5_D2AF_large_subunit_ribosomal_RNA_gene_partial_sequence

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ANNEXURE 2: FREE-LIVING NEMATODE SEQUENCES

#MK547102.1_*Panagrolaimus_rigidus*_isolate_*rigidus*1_smal_subunit_ribosomal_RNA_gene_partial_sequence

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#MK809262.1_*Aporcelaimellus_obtusicaudatus*_isolate_CP23_small_subunit_ribosomal_RNA_gene_partial_sequence

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#MK796433.1_*Aporcelaimellus_obtusicaudatus*_isolate_PotD_small_subunit_ribosomal_RNA_gene_partial_sequence

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#MK796431.1_*Pararhyssocolpus_paradoxus*_isolate_PD1_small_subunit_ribosomal_RNA_gene_partial_sequence

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#MK874499.1_*Acrobeles_complexus*_isolate_CF2_internal_transcribed_spacer_1_partial_sequence_5.8S_ribosomal_RNA_gene_complete_sequence_and_internal_transcribed_spacer_2_partial_sequence

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#MK874500.1_*Ecumenicus_monohystera*_isolate_Htf_internal_transcribed_spacer_1_partial_sequence_5.8S_ribosomal_RNA_gene_complete_sequence_and_internal_transcribed_spacer_2_partial_sequence

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#MK874502.1_*Pararhyssocolpus_paradoxus*_isolate_OD_internal_transcribed_spacer_1_partial_sequence_5.8S_ribosomal_RNA_gene_and_internal_transcribed_spacer_2_complete_sequence_and_large_subunit_ribosomale

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#MN262455.1_*Aporcella_simplex*_isolate_HTD_D2A_large_subunit_ribosomal_RNA_gene_partial_sequence

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