Nematode fauna in the Telperion Nature Reserve

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“It seems to me that the natural world is the greatest source of excitement; the greatest source of visual beauty; the greatest source of intellectual interest. It is the greatest source of so much in life that makes life worth living.”

Sir David F. Attenborough

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ABSTRACT

The Nematology Unit, Biosystematics, Agricultural Research Council–Plant Health and Protection (ARC–PHP) founded the South African Plant-Parasitic Nematode Survey (SAPPNS) in 1987. The aim of the SAPPNS was to make a comprehensive assessment of the nematode biodiversity resources of South Africa with the following main objectives, namely to: i) make an inventory, ii) study the biogeography, iii) establish an electronic database of and iv) compile distribution maps of the different species of all the plant–parasitic nematodes of South Africa. To contribute to the assessment of nematode biodiversity in South Africa and the knowledge of nematode diversity in grassland and in protected areas, the present survey of the Telperion Nature Reserve was conducted, the results of which will be incorporated in the SAPPNS database. This reserve is situated in Mpumalanga, stretches over 7 350 ha and forms part of the Rand Highveld Grassland of the important Grassland Biome. The Grassland Biome is one of the largest and most important vegetation types in South Africa and the Rand Highveld Grassland is considered endangered with only 1 % protected. The aim of the study was to conduct an extensive nematode survey of the Telperion Nature Reserve, including all trophic levels, to determine the nematode diversity in a pristine, natural habitat in the Grassland Biome.The nematode survey of the Telperion Nature Reserve was conducted over four consecutive seasons during 2015 to 2016 (autumn, winter, spring and summer, in this order). Samples were collected from 30 different sites which were divided into the following: 1) 26 terrestrial sites which were subdivided into open grassland, covered rocky grassland and water related habitats; 2) four freshwater sites where samples were collected from the Wilge River, a wetland and two other freshwater sources in the reserve. Soil and root samples were collected from the terrestrial sites, water sediment samples from the freshwater sites and finally grass seed samples from the terrestrial sites during the summer season. Nematodes were extracted from these various substrates and fixed and mounted using standard methods. Nematode genera and species were identified using traditional morphological methods and the present thesis therefore includes descriptions of species based on morphological and morphometric methods only.As expected from a pristine habitat within the Grassland Biome, the diversity of nematodes found at the Telperion Nature Reserve was remarkable. A total of 109 nematode genera were recorded from all the habitats sampled, with 59 species identified. Of the plant–parasitic nematodes 47 species belonging to 30 genera were identified. *Pratylenchus pseudopratensis* is reported for the first time in South Africa. Undescribed species of the genera *Helicotylenchus, Hemicycliophora, Lindseyus, Subanguina, Trichodorus, Tylenchorhynchus, Xiphinema* and *Xiphinemella* were also found and will be

described in future publications. Seventy nematode genera were found in soil samples from the terrestrial habitats of which four genera are reported for the first time in South Africa. These included *Discomyctus, Drilocephalobus, Heterocephalobellus,* and *Seinura*. From the grass seed samples six nematode species from three genera were identified which included four new species reports for South Africa i.e. *Aphelenchoides lichenicola, A. rutgersi, A. spicomucronatus* and *Panagrolaimus leperisini*. The samples from the freshwater habitats yielded 48 genera of which 17 species were identified. Here three new species were found *Aphanolaimus strilliae* n. sp., *Ironus telperionensis* n. sp. and *Makatinus africanus* n. sp. as well as two new species reports for South Africa *viz. Chronogaster aspinata* and *Paraphanolaimus behningi*. Several specimens of *Chronogaster africana, Eutobrilus annetteae* and *Neotobrilus ampiei* (previously found in South Africa) were collected from the freshwater sites and are described herein for the first time using Scanning Electron Microscopy. As three species of the genus *Pratylenchus* were found during this study the need for a compendium as well as a key to the South African species of the family Pratylenchidae was identified and is therefore included in this thesis. Also, several members of Pratylenchidae are well–known in South Africa due to their economic importance in agriculture and the correct identification of the members of this family is crucial. The nematode survey in the Telperion Nature Reserve resulted in new and novel information and broadens our knowledge of nematode diversity not only in South Africa, but also on a global scale. The potential for future work, to use as reference data in other fields of Nematology such as Ecology, was also identified especially in protected areas of South Africa. The importance of traditional morphological identification was also highlighted and supports the use of these methods in future work.

Keywords: Biodiversity, free-living, freshwater, Grassland Biome, morphology, morphometrics, nematodes, plant-parasitic, systematics, taxonomy.

PREFACE

This thesis follows the article format style as prescribed by the North-West University. Therefore, articles appear in published format, while manuscripts and other chapters are adjusted according to the instructions to authors of internationally accredited, scientific journals. As an additional requirement by the North-West University, Table A details the contributions of authors for each article/manuscript and provides consent for use as part of this thesis.

The following Chapters were included in this work:

Chapter 1 – Introduction, literature review, and thesis structure: **Zootaxa (Magnolia press, referencing style only)**

Chapter 2 – Article 1 (prepared): **Journal of Nematology (Exeley, Inc.)**

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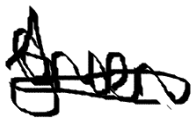
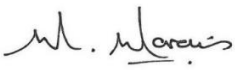
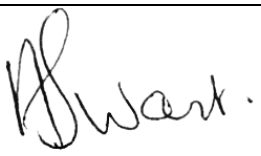
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Chapter 1, Chapter 5 (published, Article 4), Chapter 6 (unpublished manuscript, Article 5) and Chapter 7 were adjusted according to the instructions to authors by Zootaxa (Magnolia press) of which an excerpt is provided in Appendix A. Unpublished manuscript (Chapter 3: Article 2) was adjusted according to requirements of the Agricultural Research Council–Plant Health and Protection (ARC–PHP) Handbook and Manual Series provided in Appendix B. Unpublished manuscript (Chapter 2: Article 1) was adjusted according to the instructions to authors by Journal of Nematology (Exeley, Inc.) provided in Appendix C. Unpublished manuscript Chapter 4 (Article 3) was adjusted according to the format of Koedoe, provided in Appendix D. Permission was obtained from Magnolia Press (Zootaxa) to present Article 4 as part of this thesis (Chapter

5)and is available in Appendix E. Appendix F provides a link to the raw datasets used in this thesis. Permission to collect samples from Telperion Nature Reserve in provided in Appendix G. Style and formatting was kept uniform throughout the thesis, reference style of each chapter is in accordance to that of the selected journal

Table A: Contributions of authors and consent for use.

Author	Article	Contributions	Consent *
C Girgan	Articles 1 – 5	Pinciple investigator: Responsible for field sampling, identification of nematodes, drawing of specimens, and data analysis and interpretation.	
H Fourie	Articles 1 – 5	As promotor, supervised the execution of the study. Also provided intellectual input and writing of manuscripts and the thesis.	
M Marais	Articles 1 – 5	As co-promotor, responsible for original concept of the project, co–ordinator of South African Plant–Parasitic Nematode Survey (SAPPNS) and National Collection of Nematodes (NCN) database, participated in field work, provided guidance in the idenfification of nematode species, and responsible for editing thesis and manuscripts.	
A Swart	Articles 1 – 5	As co-promotor, participated in field work, provided guidance in the idenfification of nematode species, and responsible for editing thesis and manuscripts.	
E Van den Berg	Articles 2 and 3	As co-author, retired specialist researcher with expertise in the families Pratylenchidae, Criconematidae and Hoplolaimidae, responcible for editing manuscripts.	
L Tiedt	Articles 6	Provided Scanning Electron Mircoscopy photographs	

*I declare that the stated contributions are accurate and have approved the use of this article/manuscript as part of the thesis of Mrs C Girgan.

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CHAPTER 1

INTRODUCTION, LITERATURE REVIEW, AND THESIS STRUCTURE

1.1 Introduction

Nematodes, a globally abundant and diverse group of organisms, play an important role in essential soil processes and ecosystem function. Some nematodes are considered important in agriculture due to the damage they cause as pests of agricultural crops. Conventionally, all nematodes that are not animal parasites are called non–parasitic, including the herbivores or plant–parasitic nematodes (Poinar 1983). However, in this study the term non–parasitic, beneficial or free–living nematodes is used in a more restricted sense to refer only to non–plant–parasitic, terrestrial and freshwater nematodes and will be referred to as free–living nematodes.

The Nematology Unit, Biosystematics, Agricultural Research Council – Plant Health and Protection (ARC–PHP) founded the South African Plant–Parasitic Nematode Survey (SAPPNS) in 1987. The aim was to make a comprehensive assessment of the nematode biodiversity resources of South Africa with the following main objectives: i) to make an inventory, ii) to study the biogeography, iii) to establish an electronic database of and iv) to compile distribution maps of the different species of all the plant–parasitic nematodes of South Africa (Marais *et al.* 2017). Nematode biodiversity studies have increased in recent years in South Africa, however these studies frequently only included the diversity of the plant–parasitic nematodes. According to the SAPPNS there are 446 plant–parasitic nematode species currently reported from South Africa with 198 of these species being endemic. Nematode diversity (especially terrestrial, free–living nematodes) in some areas within South Africa is still unknown.

The protected areas of South Africa have been surveyed since the 1960s (National Collection of Nematodes–NCN) in papers on nematode biodiversity by Heyns (1961) and Heyns & Lagerway (1965) with extensive nematode surveys conducted by Prof Juan Heyns, Rand Afrikaans University

(now University of Johannesburg) and his students. Surveys were initiated in the Kruger National Park, with the nematode orders Araeolaimida De Coninck & Schuurmans Stekhoven, 1933, Pearse, 1942, Enoplida Filipjev, 1929, Chromadorida Chitwood, 1933, Monhysterida De Coninck & Schuurmans Stekhoven, 1933, Mononchida Jairajpuri, 1969 and Tylenchida Thorne, 1949 being reported (Botha & Heyns 1990a, b, c; 1991; 1992a, b, c; 1993a, b). From these surveys 59 genera and 93 species were recorded (Botha & Heyns 1990b; 1993b). The Nematology Unit (Biosystematics, ARC–PHP) also conducted surveys in different protected areas, including the Goegap and Witsand Nature Reserves in the Northern Cape Province (Van den Berg *et al.* 2007a), the Wilderness and Tsitsikamma sections of the Garden Route National Park (Van den Berg 1993; 1996) and the Swartberg Nature Reserve in the Western Cape Province (Van den Berg *et al.* 2003). Results of surveys undertaken in the protected areas of the Eastern Cape, KwaZulu–Natal and the Western Cape provinces were collated in three papers written by systematisists of the NCN (Marais *et al.* 2004; Marais & Swart 2013; 2014). Samples were also collected by Dr Lenie Meyer (Arachnology Unit, Biosystematics, ARC–PHP) in various national parks. Taxa descriptions of the nematodes found during these surveys were published in several papers (Van den Berg 1983; 1984; 1989). Various taxa were also described from other protected areas in the Gauteng, Mpumalanga and Western Cape provinces (Van den Berg 1978; 1980; Heyns 1995).

To contribute to the assessment of nematode biodiversity in South Africa and the knowledge of nematode diversity in protected areas, the Telperion Nature Reserve was selected for a systematic survey, the results of which will be incorporated in the SAPPNS database. This reserve is situated in Mpumalanga, stretches over 7 350 ha and forms part of the Rand Highveld Grassland of the important Grassland Biome. This vegetation type is considered endangered with only 1 % protected. To date no nematode surveys have been done in this reserve and the aim of this study was to conduct a full survey of the area. All material collected will be deposited in the NCN housed at Biosystematics, ARC–PHP, Pretoria and the dataset read into the SAPPNS – and NCN databases.

The first part of the study introduces the reader to various aspects of nematology being investigated in South Africa and also globally. Here, nematode taxonomy and methods used in nematode identification are discussed. Nematode diversity on a global scale as well as nematode diversity in South Africa will also be discussed with emphasis on the Grassland Biome. The present study is divided into different sections discussing the nematode fauna found in various substrates (grass seeds, freshwater sediment, plant roots and soil) taken from the Telperion Nature Reserve. The first section is based on the diversity of plant–parasitic nematodes within the reserve and the comparison of these findings with those reported for the rest of South Africa. For this, a checklist of the plant–parasitic nematodes and their morphometrics are given and compared to populations from various parts of the world. This section is followed by a compendium and keys to the members of the family Pratylenchidae in South Africa. This was included in the thesis due to some members of the family being found in the Telperion Nature Reserve and the need for such work on members of this family reported in South Africa. The diversity of the terrestrial free–living nematodes are discussed in the next section, which includes a checklist of these nematodes found within the reserve. Next follows the diversity of nematodes found in grass seeds. These findings are compared to existing knowledge while some new records for South Africa are also added. In the final section of the present study the diversity of free–living freshwater nematodes found in the Telperion Nature Reserve are discussed and new and known species are described.

1.2 Research aims and objectives

1.2.1 General aims

The aim of this thesis was to conduct a nematode survey of the Telperion Nature Reserve, Mpumalanga as part of the SAPPNS to enhance the knowledge of nematode diversity in a protected area in South Africa.

1.2.2 Objectives

The specific objectives of the study included:

I. To use classical morphological and morphometric techniques to identify the plant–parasitic nematodes of the Telperion Nature Reserve and to compile a checklist as part of the SAPPNS.
II. To compile a compendium of, and keys to one of the most agriculturally important nematode families, Pratylenchidae, in South Africa.
III. To compile a checklist of the terrestrial free–living nematodes in the Telperion Nature Reserve.
IV. To determine the diversity of nematodes associated with grass seeds in the grassland Biome of the Telperion Nature Reserve.
V. To determine the diversity of the freshwater nematodes in the Telperion Nature Reserve.

1.2.3 Hypothesis

The following hypotheses were postulated:

I. Telperion Nature Reserve is situated in a protected part of the Grassland Biome and will reflect high nematode diversity in the soil rhizosphere.
II. The expected high diversity will include nematode species new to science and also, species new to South Africa.

1.3 Literature review

It has been estimated that between 80 and 90 % of all multicellular organisms on earth are nematodes (Bloemers *et al.* 1997). Nematodes are the most abundant and diverse Metazoa occurring in various habitats. Historically, species diversity estimates were at about one million nematodes species of which only 25 043 species have been known by 2013 (Zhang 2013). This lack of information on the biodiversity of nematodes is mainly due to the lack of taxonomical expertise and limited funding available in the field of nematode taxonomy. Many scientific fields such as pest management, soil ecology and bioprospecting are dependent on the correct identification of nematode species. Therefore, nematode taxonomy plays a key role in nematological studies and the decline in taxonomical expertise is of great concern (Coomans 2002).

1.3.1. History of nematode classification and taxonomy

In the tenth edition of *Systema Naturae* (1758), Carl Linnaeus, classified nematodes under the Class Vermes (Chitwood 1958; Linnaeus 1958; Andrássy 2005). However, due to the presence of unrelated taxa including segmented and unsegmented worms, this class is no longer in use. The higher classification of nematodes was uncertain for many years and nematodes as a group of organisms were moved from taxa to taxa (Figure 1.1). In the early 1800s, Rudolphi (1808; 1809) proposed the Class Helmintha Rudolphi, 1808 containing five orders: Trematoda Rudolphi, 1808, Cestoidea Rudolphi, 1808, Cystica Rudolphi, 1808, Nematoidea Rudolphi, 1808 and Acanthocephala Rudolphi, 1808. Rudolphi omitted all free–living nematodes, plant–parasitic nematodes and free–living stages of animal parasites in the Class Nematoidea. However, Ehrenberg (1831) included free–living nematodes in the Nematoidea. More than a decade later, Von Siebold (1848) altered the spelling of the Order Nematoidea to the Order Nematodes, which was then changed to Nematoda by Diesing (1851). Here free–living and plant–parasitic nematodes were included as well as the horse–hair worms. The horse–hair worms were then separated from Nematoda and placed in Nematomorpha, in the order Annelida (Vejdovský 1886). Cobb (1917) separated nematodes from all other organisms and placed them in a separate phylum, Nemates. The Phylum Nemates was promoted by Pearse (1936) and the Phylum Nemata also known as

the Phylum Nematoda (as proposed by Chitwood & Chitwood [1950]) was established (Chitwood 1958). The use of the name Nemata Cobb, 1919 and Nematoda (Rudolphi, 1808) Lankester, 1877 remained a debate among nematode taxonomists until Decraemer (2000) proposed the use of one name, Nematoda, for the phylum because, although not the oldest, it is more widely used. This name is therefore adhered to in the present study.

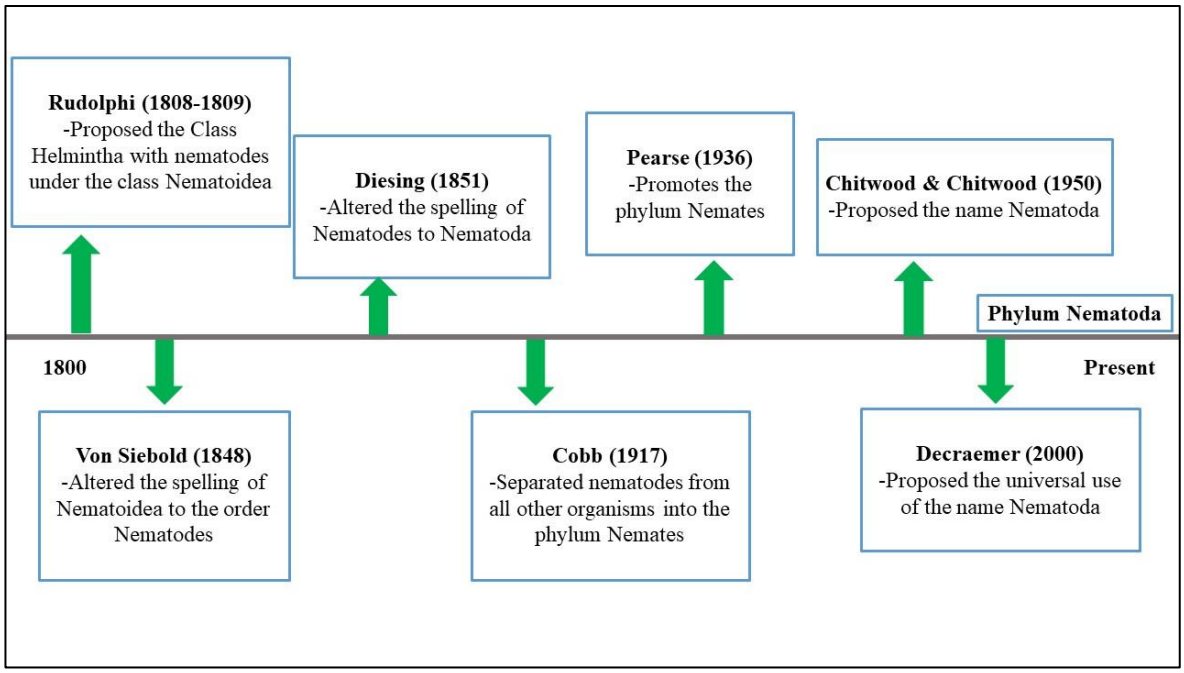


Figure 1.1: Concise history of the classification of nematodes and the establishment of the phylum Nematoda.

In this thesis the following classifications will be used: Maggenti *et al.* (1988) and Geraert (2011) for Tylenchina, Heteroderidae as reproduced in Kleynhans *et al.* (1996), Hunt (1993) for Aphelenchida, Decraemer (1995) and Duarte *et al.* (2010) for Trichodoridae and Andrássy (2005; 2007; 2009) for all free–living nematodes. For detailed classification of the nematodes reported in the present study, see the checklists in Chapter 2–Article 1 (plant–parasitic nematodes), Chapter 4–Article3 (terrestrial, free–living nematodes) and Chapter 6–Article 5 (freshwater, free–living nematodes).

1.3.2 Nematode identification methods:

Conventionally nematode identification is based on morphological characters however, molecular identification and the application of biochemical methods are also used to identify nematodes. In recent research, a combination of these methods is the basis for sound classification and diagnostic procedures (Oliveira *et al.* 2011). Ferris (1994) stated that "History shows that no single approach is without problems and challenges". Both of these methods have advantages and disadvantages, leading to the current norm of combining the two approaches.

1.3.2.1 Morphological identification of nematodes:

Even though molecular characterisation of nematodes has become more established in recent years, morphological identification by using the light microscope (LM), scanning electron microscope (SEM), and morphometrics are still an essential part of species identification and species description (Zullini *et al.* 2001). Identification of feeding group, family, genus and species is based on morphological characteristics and is generally simple to a trained eye (Griffiths *et al.* 2006). However, the identification of nematodes to species level can be a challenge due to their microscopic size, morphological similarities and overlapping morphometrics (Nega 2014). Other limitations of morphological identification are the following: substantial expertise and training is needed; eggs cannot be used for species identification; and morphological characteristics of one specimen is not always sufficient to identify a species due to extensive variations in some genera. However, morphological identification to at least genus level can be rapid and cost effective and can therefore be used in other fields of Nematology, most often in Nematode Ecology (Seesao *et al*. 2017).

Other tools are also available in the morphological identification of nematodes, one of which is the use of SEM. With the introduction of SEM, scientists have been able to study the external features of nematode morphology such as lip region, cuticular morphology and tail region in more detail (Hirschmann 1983; Ferris 1994). SEM can also reveal details on buccal cavities, outlets of pharyngeal

glands and copulatory structures (Coomans 2002). These characteristics have been used in many species descriptions and other morphological studies to support morphological characteristics observed by LM. SEM also adds to species descriptions resulting in more complete morphological characterization (Hirschmann 1983). The transmission electron microscope (TEM) has also been used to clarify minute internal structures observed with LM (Heyns & Jacobs 1989).

1.3.2.2 Biochemical and molecular methods for the identification of nematodes:

Although not used in the present study, the biochemical approach to identify nematode species using protein–based techniques is worth mentioning especially as it is a powerful tool in examining phylogenetic relationships (Seesao *et al*. 2017). In the late 1990s it was suggested that morphological identification alone was not sufficient to interpret nematode diversity (Seesao *et al*. 2017). With the development of Polymerase Chain Reaction (PCR) came a powerful stimulus to the use of molecular methods in nematode systematics (Coomans 2000). In 1985 fragments of genomic DNA was analysed with restriction enzymes to distinguish between *Meloidogyne arenaria* (Neal, 1889) Cobb, 1890 and *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949. This was the first report of deoxyribonucleic acid (DNA) techniques used for taxonomic purposes (Abrantes *et al.* 2004). *Ditylenchus africanus* Wendt, Swart, Vrain & Webster, 1995, was the first South African nematode described using both morphological and molecular characterization (Wendt *et al.* 1995). Since the mid–1980s more DNA–based techniques have been developed to detect genetic variations between genera and species (Subbotin *et al.* 2013). Molecular methods have become established among nematode taxonomists in the last 10 years and can provide a powerful tool in the identification of nematode species of which morphological diagnostic characteristics are scarce and in species with high interspecific variation. The following should be considered:

- Using several genes for one species limits the misidentification of the species. The most frequently used genes for nematode taxonomy are ITS, COXI, COX2, 16S and 28S (Seesao *et al.* 2017).

- The link between molecular identification and morphological identification is crucial as well as the morphological identification of specimens used for molecular work to avoid contamination of samples (Janssen *et al.* 2017).
- Morphological voucher specimens deposited in reference collections should always be linked to molecular sequences deposited, for further study or confirmation of species identity (Janssen *et al*. 2017).
- The clean–up of all the known incorrect sequences in GenBank is imperative. For example, in a case study on *Pratylenchus* spp. done by Janssen *et al.* (2017) it became evident that 12 published ITS sequences of *Pratylenchus goodeyi* Sher & Allen, 1953 were actually sequences from free–living nematodes of the family Cephalobidae (Filipjev, 1934) Chitwood & Chitwood, 1934.

In the present study, traditional morphology and morphometrics are used to identify the nematode taxa. This is done to highlight the importance of these traditional methods as basis for nematode identification. However, the credibility and benefits of molecular methods are not questioned and the use thereof are one of the recommendations for future studies.

1.3.3 Nematode diversity in South Africa

In 2013, Zhang estimated 25 043 nematode species globally with new species being described yearly (Zhang 2013). In South Africa 704 nematode species have been recorded (SAPPNS and NCN databases). Of these 255 species are free–living nematodes from various terrestrial and aquatic habitats (Swart *et al.* 2017), while 449 represent plant–parasitic species (SAPPNS and NCN databases). Although various nematode diversity surveys have been conducted in South Africa, gaps still exist in the knowledge generated for various biomes including the Grassland Biome (Marais *et al*. 2017). This is especially true for the protected areas in the Grassland Biome since only data on protected areas in

KwaZulu–Natal and Free State are available for this biome (Van den Berg 1983, 1989; Marais & Swart 2013).

1.3.3.1 Nematode diversity in protected areas of South Africa

As previously stated (1.1 Introduction), protected areas of South Africa have been surveyed since the mid1960s with various checklists and species descriptions published to report the diversity of nematodes in such areas. These surveys have broaden the knowledge about nematode diversity and included many new species being listed. Some of the most extensive biodiversity studies were conducted in the Kruger National Park, with 59 genera and 93 species reported (Botha & Heyns 1990b; 1993b). Although many other surveys have been conducted in protected areas, these surveys mainly focus on the diversity of only plant–parasitic nematodes. Van den Berg (1993; 1996) reported 19 and 23 plant–parasitic nematode species from the Wilderness and Tsitsikamma sections of the Garden Route National Park, respectively. Forty one and 34 plant–parasitic nematode species were reported from the Swartberg Nature Reserve (Western Cape Province) and Goegap and Witsand Nature Reserves (Northern Cape Province), respectively (Van den Berg *et al*. 2003; 2007a). Marais & Swart (2013; 2014) reported 94 and 80 plant–parasitic species from 18 protected areas in the KwaZulu–Natal Province and from seven protected areas in the Eastern Cape Province, respectively.

Apart from the work done by Prof Juan Heyns and collaborators in the Kruger National Park, very little extensive surveys have been done of the terrestrial and aquatic free–living nematodes in local protected areas. Durand *et al.* (2012) and Mobara (2014) conducted surveys of these nematodes in the Bakwena caves and Seekoeivlei Nature Reserve, respectively. Du Preez *et al.* (2015) conducted a survey of the Wonderfontein caves. Most of the work done on free–living nematodes have been conducted in cultivated fields (Swart *et al.,* 2017; Habig *et al.* 2018). The present study will therefore add a substantial amount of information to our knowledge of the diversity of free–living (terrestrial and freshwater) and plant–parasitic nematodes in the protected areas of the Grassland Biome. In the present study nematodes

from various substrates in the Grassland Biome of the Telperion Nature Reserve were surveyed which included plant–parasitic nematodes from soil and roots, terrestrial free–living nematodes from soil, free–living nematodes from grass seeds and freshwater nematodes from freshwater sediment.

1.3.3.2 Plant–parasitic nematodes in South Africa:

As mentioned in Paragraph 1.1 Introduction, the SAPPNS was founded in 1987 (Marais *et al.* 2017). From the 9 000 localities recorded in the SAPPNS database (Figure 1.2), 449 plant–parasitic nematode species have been recorded belonging to 50 genera. Nonetheless, huge gaps still exist in our knowledge of the Thicket, Grassland, Savanna, Nama– and Succulent Karoo biomes (Marais *et al*. 2017). Of the 9 000 records, 2 205 (25 %) are situated in the Grassland Biome. These records include reports from various host types with 48 % being from crops, 23 % from natural vegetation and the remaining 29 % from other vegetation types including shrubs, succulents and trees (SAPPNS). The present study also forms part of the SAPPNS and is only the second nematode survey from a protected area in the Mpumalanga Province. The first being work by Kruger & Heyns (1987) in the Blyde River Canyon Nature Reserve. Reports from the current survey not only broadens the knowledge we have on the distribution of the plant–parasitic nematodes in South Africa but also adds to our knowledge of the diversity of these nematodes in the country.

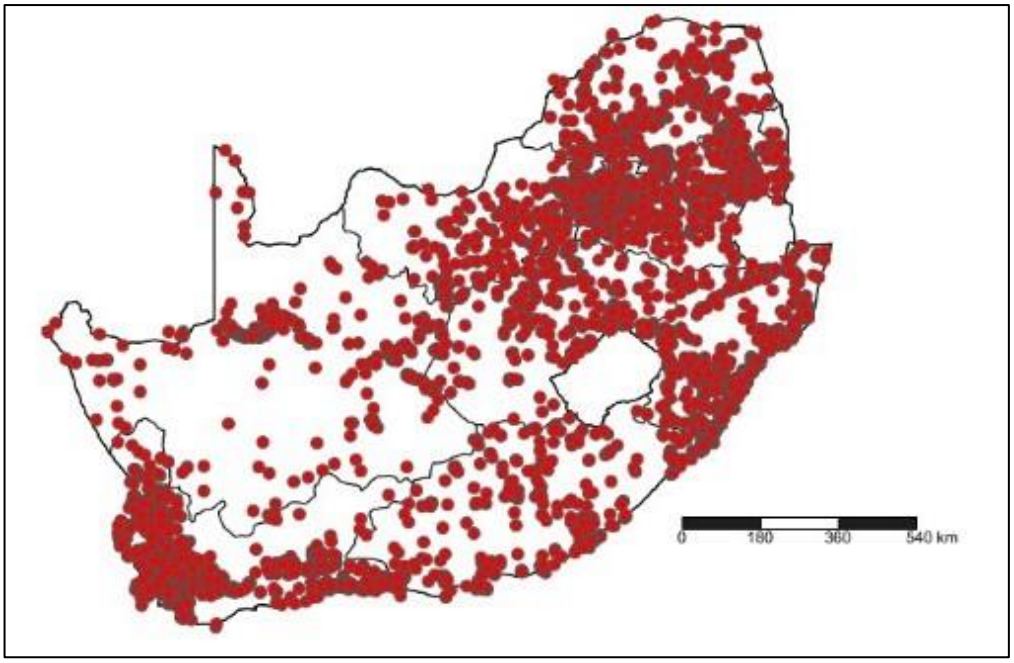


Figure 1.2: Total locality records listed in the South African Plant–Parasitic Nematode Survey where nematodes were recorded from South Africa. Each red dot represent a locality captured in the database (Shorthouse 2010).

1.3.3.3 The Pratylenchidae of South Africa:

Members of the family Pratylenchidae Thorne, 1949 are a major pest species in South Africa and the occurrence of species of this family has increased in the country (Dr Mariette Marais, personal communication, April 1, 2015). The current study also includes a compendium of the family Pratylenchidae with the focus on the species occurring in South Africa. The family Pratylenchidae consists of 11 genera of which *Pratylenchus* Filipjev, 1936 is the most species rich with 101 species (Geraert 2013). Members of the family Pratylenchidae are polyphagous migratory, sedentary in the case of *Nacobbus* Thorne & Allen, 1944, endoparasitic nematodes (Yeates *et al.* 1993; Castillo *et al.* 2012). This family contains some of the most economically important species of nematodes, *Pratylenchus*, *Radopholus* Thorne, 1949, *Hirschmanniella* Luc & Goodey, 1964 and *Nacobbus*. In fact, members of the family Pratylenchidae are considered the second most economically important nematodes after *Meloidogyne* Göldi, 1892 globally and in South Africa (Keetch 1989; Castillo *et al*. 2012). Of the 11 known genera of Pratylenchidae, six have been recorded in South Africa i.e. *Apratylenchoides* Sher,

1973, *Hirschmanniella*, *Pratylenchus*, *Radopholus*, *Radopholoides* De Guiran, 1967 and *Zygotylenchus* Siddiqi, 1963 (SAPPNS).

Members of the family Pratylenchidae are known to parasitize various crops in South Africa and some species are listed as quarantine organisms, e.g. *Radopholus similis* (Cobb, 1893) Thorne, 1949 a quarantine pest of banana (Knoetze *et al.* 2017). *Pratylenchus* is the most common Pratylenchidae genus in South Africa and in many cases the dominant nematode pest in various crops. Cereal crops such as *Zea mays* L., *Sorghum bicolor* (L.) Moench and *Triticum* L. are some of the most susceptible hosts to *Pratylenchus* with *P. zeae* Graham, 1951 and *P. brachyurus* (Godfrey, 1929) Filipjev & Schuurmans Stekhoven, 1941 being most abundant on *Z. mays* (Mc Donald *et al.* 2017). *Pratylenchus brachyurus* is also reported as the most common species of the genus in South Africa associated with *Arachis hypogaea* L. (Fourie *et al.* 2017) and *Solanum tuberosum* L. (Jones *et al.* 2017). *Pratylenchus zeae* is reported as the most abundant species of this genus associated with *Saccharum officinarum* L. (Berry *et al.* 2017) and has an extremely wide host range (according to SAPPNS, 104 recorded host plants in South Africa). Cereals are the major food crops in South Africa, belonging to the grass family (Poaceae or Gramineae). As grasses in natural grassland systems may serve as reservoir hosts of important agricultural pests, such as members of Pratylenchidae, it is therefore important to know their nematode parasites. Also, because the Pratylenchidae includes major agricultural pests and quarantine organisms, correct identification of species is crucial. The compendium compiled in this study includes the host range and geographical distribution of the species reported from South Africa, as well as the morphology and morphometric range of each species. Diagnostic keys to the taxa of Pratylenchidae in South Africa are also provided.

1.3.3.4 Terrestrial free–living nematodes in South Africa

Nematodes form a large percentage of the mesofauna in the soil ecosystem and are considered the most abundant multicellular, eukaryotic organisms. They are diverse, widely distributed, linked to

several key trophic levels in the soil food web, integrators of soil properties and respond rapidly to biotic and abiotic changes in the soil environment (Azmat & Akhter 2009). Because nematodes are one of the largest invertebrate groups in the soil ecosystem, they play an important role in several ecosystem processes and ecosystem services. They transform organic matter and nutrients, contribute to soil food web stability and are involved in nitrogen and phosphor mineralisation (Kalkhoran & Ahangar 2014).These characteristics and ecosystem services make nematodes well suited biological indicators of soil health and have been used in several ecological and pollution based studies (Wilson & Kakouli–Duarte 2009). Free–living nematodes make up a large portion of the nematode communities in various habitats. It is therefore important to include these nematodes in surveys conducted especially in natural habitats. This may establish baseline diversity data for future ecological studies as well as a reference dataset when comparing to cultivated or other soils impacted by anthropogenic activities.

Plant–parasitic nematodes were normally the focus of nematode surveys and apart from the previously mentioned surveys conducted in protected areas (Section 1.1 Introduction) very few nematode surveys conducted in South Africa have included the terrestrial free–living nematodes. Most of the surveys conducted in South Africa that included terrestrial free–living nematodes have been done in agricultural soils, caves and freshwater habitats (Durand *et al.* 2012; Mobara 2014; Du Preez *et al.* 2015; Swart *et al.* 2017). Only a few nematodes surveys that includes both plant–parasitic and free–living nematodes have been conducted in terrestrial habitats in South Africa in the last decade. Such research includes work done by personnel of the National Collection of Nematodes (NCN, Biosystematics, ARC–PHP). These surveys were conducted at the Groenkloof and Rietvlei Dam Nature Reserves in 2013, the Serene Valley as part of the green belt around the Moreleta Spruit, Garsfontein, the Willem Pretorius, Erfenis Dam and Kalkfontein nature reserves (as part of the South African Grassland Survey in 2014 (Dr Mariette Marais, personal communication, February 12, 2019), and the Karoo Biogaps project (SANBI 2018). Based on the scarcity of information on the diversity of terrestrial free–living nematodes, the survey at the Telperion Nature Reserve will not only add to our knowledge

on the diversity of these nematodes in natural habitats but can also be used as baseline ecological data in future studies or as a reference site when compared to less natural soils.

1.3.3.5 Nematodes associated with seeds in South Africa:

Plant–parasitic nematodes can be found in all the different parts of a plant and feed either endo– or ectoparasitically. For example, *Pratylenchus* spp. are endoparasites and *Trichodorus* spp. are ectoparasites of below–ground plants parts; *Ditylenchus dipsaci* (Kühn, 1857) Filipjev, 1936 feeds within above–ground plant tissue; *Aphelenchoides ritzemabosi* (Schwartz, 1911) Steiner & Buhrer, 1932 is a foliar nematode feeding endoparasitically in the leaves and buds of plants (Barker 1998). Globally, various nematode species are associated with seeds of various agriculturally important crops and cause extensive damage and yield loss, e.g., *Anguina tritici* (Steinbuch, 1799) Chitwood, 1935, that are frequently found in seeds of small grain cereal (Dababat & Fourie 2018).

According to the SAPPNS and NCN database a number of nematodes were reported from seeds, from either ornamentals or crops, in South Africa. For example, some free-living nematodes have been recorded from the surface of onion seeds rendered from the diagnostics services done at the Nematology Unit (ARC-PHP, Biosystematics). These included specimens of *Acrobeloides* sp., *Aphanolaimus* sp., *Aphelenchoides dactylocercus* Hooper, 1958, *Aphelenchoides eltayebi* Zeidan & Geraert, 1991, *Aphelenchoides helicus* Heyns, 1964, *Aphelenchus* sp., *Cephalobus* sp., *Cruznema* sp., *Eucephalobus* sp., *Mesorhabditis*, sp., *Monhystera* sp., *Panagrolaimus* sp., *Plectus* sp., *Rhabditis* sp., and *Tobrilus* sp. They are generally considered to be transmitted to the seed by contact with soils.

Anguina spp., *Aphelenchoides* spp., *Ditylenchus* spp. and *Subanguina* spp. were also reported from crops seeds in South Africa. *Anguina agrostis* (Steinbuch, 1799) Filipjev, 1936 was the only *Anguina* sp. reported locally and only from *Lolium perenne* L. in the Western Cape Province (Kleynhans *et al.* 1996). The species was described from *Agrostis capillaris* L. in Germany and vectors the

bacterium *Rathayibacter rathayi* (Smith, 1913) Zgurskaya, Evtushenko, Akimov, Kalakoutskii, 1993, which is the cause of 'sheep staggers' (Steinbuch 1799; Stynes & Bird 1980). *Subanguina wevelli* (Van den Berg, 1985) Fortuner & Maggenti, 1987, was described from *Eragrostis curvula* (Schrad.) Nees seed from two localities in the Free State and Mpumalanga provinces (Van den Berg 1985). During a survey of *Eragrosti*s Wolf, *S. wevelli* was also reported from Bethal, Amersfoort and Standerton (Mpumalanga Province), and Potchefstroom areas (North West Province) (Bekker 2008). First reported as *Ditylenchus destructor* Thorne, 1945, *D. africanus* was described from groundnut seeds in the Schweizer–Reneke district, North West Province (Wendt *et al*. 1995). The species has since been reported from various other localities in South Africa. More recently *Aphelenchoides arachidis* Bos, 1977 was found in the testa and shells of groundnuts in the Vaalharts Irrigation Scheme, Northern Cape (Lesufi *et al.* 2015). As part of the nematode survey of Telperion Nature Reserve, grass seed samples were collected and contained nematodes from various genera discussed in Chapter 5: Article 4.

1.3.3.6 Nematode diversity in freshwater systems in South Africa

Only approximately 7 % (about 1 808) of nematodes species known to science have been reported from freshwater globally with 351 species residing in the Afrotropical region (Eyualem–Abebe *et al.* 2008). In South Africa, research on freshwater nematodes dates back to the mid 1960s by South African nematologist Victoria Coetzee (Coetzee 1965; 1966; 1967a, b; 1968a, b). From the 1970s freshwater nematodes were studied by various nematologists and resulted in the description of several new species (Table 1.1) of which most are endemic to South Africa (Heyns 1976; 2002a, b). Two papers combined the knowledge of the freshwater nematodes from southern Africa (Jacobs 1984; Heyns 2002). However the most recent paper was more almost two decades ago. More recent research on freshwater nematodes in South Africa includes the studies by Du Preez *et al.* (2015); Durand *et al.* (2012); Mobara (2014); Van den Berg *et al.* (2007b); Van den Berg *et al.* (2009). These were conducted in caves and protected areas in South Africa and not only resulted in new taxa but also new distribution records of the freshwater nematodes previously reported from South Africa. In the present study freshwater

sources were sampled to add to and compare to this knowledge of freshwater nematodes in South Africa and globally.

Table 1.1: Nematode species described from freshwater habitats in South Africa

Species	Provinces reported from	Endemic?	Reference
Afractinolaimus capensis (Heyns & Argo, 1969) Vinciguerra & Heyns, 1984	Western Cape	Yes	Heyns & Argo (1969)
Afractinolaimus magaliesmontanus (Heyns & Argo, 1969) Jairajpuri & Ahmad, 1992	North West	Yes	Heyns & Argo (1969)
Allodiplogaster aquatica (Dassonville & Heyns, 1984) Kanzaki, Ragsdale & Giblin–Davis, 2014	Gauteng	Yes	Dassonville & Heyns (1984)
Aphelenchoides africanus Dassonville & Heyns, 1984	Gauteng	Yes	Dassonville & Heyns (1984)
Aporcelaimellus glandus Botha & Heyns, 1991	Mpumalanga; Limpopo	Yes	Botha & Heyns (1991)
Carcharolaimus crassicostatus Heyns & Argo, 1969	Limpopo	Yes	Heyns & Argo (1969)
Chronogaster africana Heyns & Coomans, 1980	Gauteng;North West; Eastern Cape	Yes	Heyns & Coomans (1980)
Chronogaster glandifera Heyns & Coomans, 1980	Gauteng	Yes	Heyns & Coomans (1980)
Chronogaster longicauda Heyns & Coomans, 1980	Gauteng; Mpumalanga	No	Heyns & Coomans (1980)
Chronogaster multispinata Heyns & Coomans, 1980	Limpopo; North West; Free State	Yes	Heyns & Coomans (1980)
Cobbonchus dianae Coetzee, 1965	Free State	Yes	Coetzee (1965)
Cobbonchus megalus Coetzee, 1966	Eastern Cape	Yes	Coetzee (1966)
Coomansus pretoriensis (Coetzee, 1968) Jairaipuri & Khan, 1977	Gauteng	Yes	Coetzee (1968a)
Drepanodorylaimus williamsi (Heyns & Kruger, 1983) Andrássy, 1986	North West	Yes	Heyns & Kruger (1983)
Epitobrilus heptapapillatus (Joubert & Heyns, 1979) Tsalolikhin, 1981	Gauteng; KwaZulu–Natal; Eastern Cape	No	Joubert & Heyns (1979)
Eutobrilus annetteae (Joubert & Heyns, 1979) Tsalolikhin, 1981	Gauteng; Western Cape	No	Joubert & Heyns (1979)
Hemicycliophora lutosa Loof & Heyns, 1969	Mpumalanga; Limpopo	No	Loof & Heyns (1969)
Hirschmanniella kwazuna Van den Berg, Subbotin, Handoo & Tiedt, 2009	KwaZulu–Natal	Yes	Van den Berg *et al.* (2009)
Ironus crassatus Argo & Heyns, 1972	Mpumalanga; Limpopo	Yes	Argo & Heyns (1972)
Ironus ernsti Argo & Heyns, 1972	Eastern Cape; Free State; Western Cape	Yes	Argo & Heyns (1972)
Ischiodorylaimus tessares Kleynhans, 1970	Western Cape	Yes	Kleynhans (1970)
Laimydorus africanus Botha & Heyns, 1993	Limpopo	Yes	Botha & Heyns (1993a)
Laimydorus olifanti Botha & Heyns 1991	Limpopo	Yes	Botha & Heyns (1991)
Lenonchium frimbricaudatum Swart & Heyns, 1991	Mpumalanga	Yes	Swart & Heyns (1991a)
Lenonchium longidens (Furstenberg & Heyns, 1966) Jairajpuri, 1967	Limpopo	Yes	Furstenberg & Heyns (1966)
Limonchulus heynsi Swart & Furstenberg, 1993	KwaZulu–Natal	Yes	Swart & Furstenberg (1993)
Mactinolaimus transkeiensis (Heyns & Argo, 1969) Vinciguerra, 1988	Eastern Cape	Yes	Heyns & Argo (1969)
Mesodorylaimus bainsi Basson & Heyns, 1974	Western Cape	No	Basson & Heyns (1974)
Mesodorylaimus importunes Basson & Heyns, 1974	Limpopo	Yes	Basson & Heyns (1974)
Mesodorylaimus intermedius Dassonville & Heyns, 1984	Gauteng	Yes	Dassonville & Heyns (1984)
Mesodorylaimus kowyni Basson & Heyns, 1974	Mpumalanga	Yes	Basson & Heyns (1974)
Mesodorylaimus paralitoralis Basson & Heyns, 1974	Limpopo	Yes	Basson & Heyns (1974)
Mesodorylaimus pseudosubtilis Basson & Heyns, 1974	Limpopo; Mpumalanga	Yes	Basson & Heyns (1974)

Table 1.1 (continued): Nematode species described from freshwater habitats in South Africa

Species	Provinces reported from	Endemic?	Reference
Monhystera gabaza Joubert & Heyns, 1980	Gauteng	No	Joubert & Heyns (1980)
Monhystera magnacephala Joubert & Heyns, 1980	Gauteng	No	Joubert & Heyns (1980)
Mononchoides gracilis Dassonville & Heyns, 1984	Gauteng	Yes	Dassonville & Heyns (1984)
Mononchus aquaticus Coetzee, 1968	Gauteng	No	Coetzee (1968b)
Namaquanema hanki Heyns & Swart, 1993	Northern Cape	Yes	Heyns & Swart (1993)
Neoactinolaimus barbieri Vinciguerra & Heyns, 1984	Mpumalanga; Limpopo; KwaZulu–Natal; Eastern Cape	No	Vinciguerra & Heyns (1984)
Neoactinolaimus brachydorus Vinciguerra & Heyns, 1984	Mpumalanga	Yes	Vinciguerra & Heyns (1984)
Neoactinolaimus crassidens Heyns & Argo, 1969	Limpopo	Yes	Heyns & Argo (1969)
Neotobrilus ampiei (Joubert & Heyns, 1979) Tsalolikhin, 1981	North West; Eastern Cape	Yes	Joubert & Heyns (1979)
Neotobrilus floridensis (Joubert & Heyns, 1979) Tsalolikhin, 1981	Gauteng; Limpopo	Yes	Joubert & Heyns (1979)
Oncholaimus deconincki Heyns & Coomans, 1977	Eastern Cape	Yes	Heyns & Coomans (1977)
Oncholaimus jessicae Coomans & Heyns, 1986	Mpumalanga	Yes	Coomans & Heyns (1986)
Paractinolaimus xosorum Heyns & Argo, 1969	Eastern Cape	Yes	Heyns & Argo (1969)
Prodorylaimus ensis Kleynhans, 1970	Limpopo	Yes	Kleynhans (1970)
Proleptonchus krugeri Botha & Heyns, 1992	Mpumalanga	Yes	Botha & Heyns (1992b)
Sibayinema natalense Swart & Heyns, 1991	KwaZulu–Natal	Yes	Swart & Heyns (1991b)

1.3.4 Final considerations:

Nematodes occurring in South Africa are as diverse and abundant as in the rest of the world. Major gaps, however, exist in our knowledge of these organisms for various habitats including grassland– and freshwater ecosystems. Many of the nematological studies done in South Africa have focussed on plant–parasitic nematodes, and specifically in agricultural soils. Since nematode communities in protected areas have not received as much attention as those in agricultural areas, a diversity study of nematodes in a protected area is the main focus of this study. Due to the importance of nematodes in food security and the critical scarce skill of nematode taxonomy, the identification of nematodes based on classical methods are extremely important and is a skill that needs to be carried over to new generations of scientists.

1.4 Structure of thesis

This thesis is subdivided into the following chapters

1. **Introduction, literature review and thesis structure** conveys the rational of the study and presents the aims, objectives and hypotheses. Background is given on the history of nematode classification, methods to identify nematodes as well as the diversity of both aquatic and terrestrial nematodes in South Africa.
2. **Article 1** investigates the diversity of plant–parasitic nematodes in a natural grassland and lists the species found during this study with morphometric data given for identified taxa.
3. **Article 2** consists of a compendium of the members of Pratylenchidae reported from South Africa, including morphology, morphometrics and distribution of each species.
4. **Article 3** investigates the diversity of the terrestrial free–living nematodes in a protected area and lists the genera found during this study.
5. **Article 4** reports nematodes species found associated with grass seeds found in the Telperion Nature Reserve with four new reports for South Africa.
6. **Article 5** investigates the diversity of nematodes from freshwater sources in the Telperion Nature Reserve. This paper contains the descriptions of three new species, two new reports for South Africa and three known species.
7. **Conclusions and recommendations** considers the key findings of the PhD study. This final chapter emphasises the importance of nematode surveys in protected grasslands and the diversity of nematodes in this ecosystem. This chapter also discusses and provides recommendations for future studies.

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CHAPTER 2: ARTICLE 1

Plant–Parasitic Nematodes of the Telperion Nature Reserve

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2.1 Abstract

The South African Plant–Parasitic Nematode Survey (SAPPNS) was founded in 1987 by the Nematology Unit of Biosystematics, Agricultural Research Council–Plant Health and Protection. The aim was to make a comprehensive assessment of the plant–parasitic nematode diversity and distribution in South Africa. Since the start of the SAPPNS numerous surveys in protected areas of South Africa have been conducted. The Telperion Nature Reserve is such a protected area in the Grassland Biome of Mpumalanga, South Africa and was selected for a nematode survey as part of the SAPPNS. Soil and root samples were collected from 26 sites in the reserve over four seasons during 2015–2016. Thirty plant–parasitic nematode genera were found, and 47 species were identified. The present paper presents a checklist of the plant–parasitic nematodes found during the survey as well as morphological and morphometric data on the identified species.

Keywords: Criconematidae, Grassland Biome, Hoplolaimidae, Longidoridae, morphology, morphometrics, plant–parasitic nematodes, Pratylenchidae, systematics, taxonomy, Trichodoridae.

2.2 Introduction

The Nematology Unit of Biosystematics, Agricultural Research Council – Plant Health and Protection (ARC–PHP) founded the South African Plant–Parasitic Nematode Survey (SAPPNS) in 1987. The aim was to make a comprehensive assessment of the nematode biodiversity resources of South Africa with the following main objectives: i) to make an inventory, ii) to study the biogeography, iii) to establish an electronic database of and iv) to compile distribution maps of the different species of all the plant–parasitic nematodes of South Africa (Marais et al., 2017a). To date, the SAPPNS database contains 9000 records of 449 plant–parasitic nematode species (Marais et al., 2017a; SAPPNS). Since the start of the SAPPNS various surveys have been conducted to generate data about the diversity of plant-parasitic nematodes in South Africa. The results of these surveys were published in a series of papers between 1993 and 2014 authored by the nematologists at the Nematology Unit of Biosystematics, ARC – PHP (Van den Berg, 1993; Marais and Swart, 1996; Marais and Van den Berg, 1996; Van den Berg, 1996; Marais and Swart, 1998; Marais and Swart, 1999; Swart, 2000; Swart et al., 2000; Marais and Swart, 2001; Marais and Swart, 2002; Marais and Swart, 2003; Van den Berg et al., 2003; Marais et al., 2004; Marais and Swart, 2007; Van den Berg et al., 2007a, 2007b; Marais and Swart, 2013; Marais and Swart, 2014).

However, there are still gaps in our knowledge of the plant–parasitic nematodes from the Thicket, Grassland, Savanna, Nama– and Succulent Karoo biomes (Marais et al., 2017a). The Grassland Biome is one of the largest biomes in South Africa and according to Mucina et al. (2006), 30 % of this biome has been permanently transformed by cultivation, urbanization, plantation forestry and mining. Of the 9 000 records in the SAPPNS, 2 205 are from the Grassland Biome. These records include nematode species associated with various plant species of which 48 % are agri-or horticultural crops, 23 % natural vegetation and the remaining 29 % other vegetation types including shrubs, succulents and trees. The present study, conducted in the Telperion Nature Reserve, also forms part of the SAPPNS and is the second extensive survey in the Mpumalanga Province. The first includes work by Kruger and Heyns (1987) in the Blyde River Canyon Nature Reserve.

The protected areas of South Africa have been surveyed since the 1960s (Heyns, 1961; Heyns and Lagerway, 1965) with extensive nematode surveys conducted in the Kruger National Park by Prof. Juan Heyns, Rand Afrikaans University (now University of Johannesburg) and his students (Botha and Heyns, 1990a, 1990b, 1990c; 1991; 1992a, 1992b, 1992c; 1993a, 1993b). Apart from the surveys in the Kruger National Park, the Nematology Unit of the National Collection of Nematodes (NCN, ARC–PHP) also conducted surveys in other protected areas, including the Goegap and Witsand Nature Reserves in the Northern Cape Province (Van den Berg et al., 2007a), the Wilderness and Tsitsikamma sections of the Garden Route National Park (Van den Berg, 1993; 1996) and the Swartberg Nature Reserve in the Western Cape Province (Van den Berg et al., 2003). Surveys undertaken in the protected areas of the Eastern Cape, KwaZulu–Natal and the Western Cape provinces were collated in three papers written by the personnel of the NCN (Marais et al., 2004; Marais and Swart, 2013; 2014). Samples were also collected by Dr Lenie Meyer (Arachnology Unit, Biosystematics, ARC–PHP) in various national parks and taxa descriptions of the nematodes found during these surveys were published in several papers (Van den Berg, 1983; 1984; 1989). Apart from nematode surveys various taxa were also described from other protected areas in the Gauteng, Mpumalanga and Western Cape provinces (Van den Berg, 1978a, 1978b; 1980; 1983; Heyns, 1995).

In the present paper, the plant–parasitic nematodes found during the survey of the Telperion Nature Reserve are listed and the morphometrics of identified species are given. This information not only broadens the geographical distribution information in the SAPPNS database, but also broadens knowledge of scientists on the morphology and morphometrics of various species encountered during the survey.

2.3 Materials and Methods

2.3.1 Telperion Nature Reserve:

The study was conducted in the Telperion Nature Reserve, Mpumalanga, South Africa (Figure 2.1) during 2015 and 2016. Telperion Nature Reserve is situated in the extreme north of the Grassland Biome in South Africa recognized by Mucina et al. (2006), as the Rand Highveld Grassland in the Mesic Highveld Grassland Bioregion. Telperion is situated on the farm Elandsfontein 493 JR, (between –25.65986, 29.00921 and –25.71322, 29.03384, North to South; and –25.6724, 29.04703 and –25.69527, 28.97136, East to West). The reserve stretches over 7 350 ha and is dominated by grassy plains, interspersed with rocky outcrops dominated by woody plant species (Grobler, 1999).

2.3.2 Collection of samples:

One hundred and four soil and root samples were collected from 26 sites within the reserve (Figure 2.1) during 2015 and 2016 (26 samples per season for four consecutive seasons). Approximately 1 kg soil was collected from each site using a garden trowel. These samples were then stored in a 10 ° C cold room until nematode extraction commenced.

2.3.3 Nematode extraction and preservation:

Nematodes were extracted from the soil and root samples using various extraction methods. Endoparasitic nematodes were extracted from root tissues using the aeration method as described in Marais et al. (2017b). Ecto–parasitic nematodes were extracted from 250 cm^3 soil and root samples using an adapted decanting and sieving method, followed by a sugar flotation method (Marais et al., 2017b). To obtain female specimens of the larger dorylaimid nematodes (Longidoridae and Belondiroidea) an adapted decanting and sieving method followed clarification method (using a flocculant) as described by Loubser (1985) as well as the Flegg modified Cobb technique (EPPO, 2013).

The nematodes were then fixed in a heated 4 % formaldehyde + 1 % propionic acid (FPG) solution, dehydrated in a glycerin solution and mounted in glycerin on permanent glass slides using a wax ring method (Marais et al., 2017b).

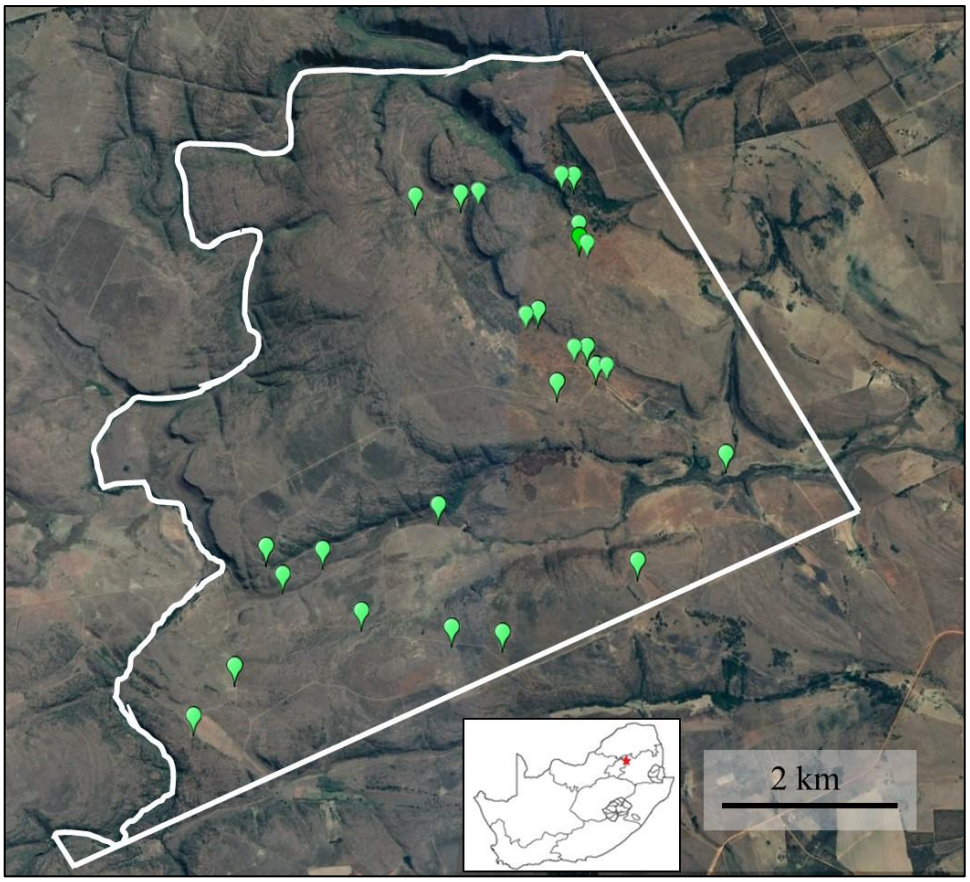


Figure 2.1: Map of Telperion Nature Reserve indicating the nematode sampling sites. Map insert represents South Africa where the red star indicates the location of the Telperion Nature Reserve, Mpumalanga. (Shorthouse, 2010).

2.3.4 Nematode identification and classification:

Nematodes were counted using a De Grisse counting dish with a WILD Heerbrugg dissection microscope and identified to genus using an Olympus BX53F microscope. In this paper, plant–parasitic

nematodes refer to those that feed on plants and is based on the classification by Yeates et al. (1993). Nematode classification was based on a synthesis of Maggenti et al. (1988) and Geraert (2011) for the Tylenchina; Kleynhans et al. (1996) for the Heteroderidae; Decraemer (1995) and Duarte et al. (2010) for the Trichodoridae; and Andrássy (2009) for the dorylaimids. Authorities for genera regarded as valid here are Hunt et al. (2005) for *Criconemoides*; Marais (2001) for *Helicotylenchus*; and Handoo (2000) for *Tylenchorhynchus*.

Measurements and drawings of the mounted specimens were done with a Zeiss Axio Imager A2 equipped with an Axiocam ERc5s camera and iPad with the Labscope imaging application as a drawing tube. All measurements and identifications were done at 1000× magnification. Curved structures were measured along the median line. Morphometrics according to De Man (1884) were used in descriptions; standard morphometric calculations and terms were used throughout this paper. All measurements in the descriptions are given in micrometers (µm) except when stated otherwise. All measurements are given in the form: mean ± standard deviation (range). All specimens were deposited in the NCN, Biosystematics, ARC – PHP, Pretoria.

2.4 Results

Plant–parasitic nematodes of 30 genera were found during the survey (Table 2.1). Forty seven species were identified. Due to the lack of mature females or good quality specimens, not all species could be identified. The genus *Xiphinema* Cobb, 1913 was the most diverse with 13 known species identified. *Pratylenchus pseudopratensis* Seinhorst, 1968 is reported here for the first time in South Africa. The morphometrics of species belonging to the families Pratylenchidae Thorne, 1949; Hoplolaimidae (Filipjev, 1934) Wieser, 1953; Criconematidae (Taylor, 1936) Thorne, 1949; Trichodoridae (Thorne, 1935) Clark, 1961; and Longidoridae Thorne, 1935 are also given in this paper. Species that are listed in the checklist but not morphometrically discussed was due to the poor condition of the specimens.

Table 2.1: Checklist of the plant–parasitic nematodes found at the Telperion Nature Reserve.

Phylum Nematoda (Rudolphi,1808) Lankester, 1877

Class Secernentia Von Linstow, 1905

Subclass Diplogasteria Maggenti, 1982

Order Tylenchida Thorne, 1949

Suborder Tylenchida Chitwood, 1950

Superfamily Tylenchoidea Örley, 1880

Family Tylenchidae Örley, 1880

Subfamily Tylenchinae Örley, 1880

Genus *Tylenchus* Bastian, 1865

Tylenchus spp.[a]

Subfamily Atylenchinae Skarbilovich, 1959

Genus *Coslenchus* Siddiqi, 1978 [a]

Coslenchus spp.[a]

Family Anguinidae Nicoll, 1935 (1926)

Genus *Subanguina* Paramonov, 1967

Subanguina sp.[b]

Family Dolichodoridae Chitwood, 1950

Subfamily Telotylenchinae Siddiqi, 1960

Genus *Tylenchorhynchus* Cobb, 1913

Tylenchorhynchus sp.[b]

Tylenchorhynchus brevilineatus Williams, 1960

Family Pratylenchidae Thorne, 1949

Subfamily Pratylenchinae Thorne, 1949

Genus *Pratylenchus* Filipjev, 1936

Pratylenchus brachyurus (Godfrey, 1929) Filipjev and Schuurmans Stekhoven, 1941

Pratylenchus dellatrei Luc, 1958

Pratylenchus pseudopratensis Seinhorst, 1968

[a] Species not identified because not enough high quality specimens for morphometric characterization.

[b] Indicates possible new species and needs further investigation.

[c] Species identified but not enough high quality specimens for morphometric characterization.

Table 2.1 (continued): Checklist of the plant–parasitic nematodes found at the Telperion Nature Reserve.

Family Hoplolaimidae (Filipjev, 1934) Wieser, 1953
Subfamily Hoplolaiminae Filipjev, 1934
Genus *Hoplolaimus* Daday, 1905
Hoplolaimus pararobustus (Schuurmans Stekhoven and Teunissen, 1938) Sher in Coomans, 1963[c]
Genus *Rotylenchus* Filipjev, 1936
Rotylenchus devonensis Van den Berg, 1976[c]
Rotylenchus gracilidens (Sauer, 1958) Sauer, 1958
Rotylenchus unisexus Sher, 1965
Genus *Helicotylenchus* Steiner, 1945
Helicotylenchus sp.[b]
Helicotylenchus dihystera (Cobb, 1893) Sher, 1961
Helicotylenchus digonicus Perry in Perry, Darling and Thorne, 1959[c]
Helicotylenchus martini Sher, 1966
Helicotylenchus paraplatyurus Siddiqi, 1972
Helicotylenchus pseudorobustus (Steiner, 1914) Golden, 1956
Genus *Scutellonema* Andrássy, 1958
Scutellonema bizanae Van den Berg and Heyns, 1973
Scutellonema brachyurus (Steiner, 1938) Andrássy, 1958
Scutellonema truncatum Sher, 1964
Subfamily Rotylenchulinae Husain and Khan, 1967
Genus *Rotylenchulus* Linford and Oliveira, 1940
Rotylenchulus parvus (Williams, 1960) Sher, 1961
Family Heteroderidae Filipjev and Schuurmans Stekhoven, 1941
Subfamily Meloidogyninae Skarbilovich, 1959
Genus *Meloidogyne* Göldi, 1892
Meloidogyne javanica (Treub, 1885) Chitwood, 1949[c]

[a] Species not identified because not enough high quality specimens for morphometric characterization.

[b] Indicates possible new species and needs further investigation.

[c] Species identified but not enough high quality specimens for morphometric characterization.

Table 2.1 (continued): Checklist of the plant–parasitic nematodes found at the Telperion Nature Reserve.

Superfamily Criconematoidea Taylor, 1936
Family Criconematidae (Taylor, 1936) Thorne, 1949
Subfamily Criconematinae Taylor, 1936
Genus *Criconema* Hofmänner and Menzel, 1914
Criconema corbetti (De Grisse, 1967) Raski and Luc, 1985
Criconema sirgeli Van den Berg and Meyer, 1987
Criconema zeae (Van den Berg and Heyns, 1977) Raski and Luc, 1985
Genus *Criconemoides* Taylor, 1936
Criconemoides parvus Raski, 1952
Criconemoides sphaerocephalus (Taylor, 1936) De Grisse and Loof, 1965
Criconemoides xenoplax Raski, 1952
Genus *Hemicriconemoides* Chitwood and Birchfield, 1957
Hemicriconemoides brachyurus (Loos, 1949) Chitwood and Birchfield, 1957
Subfamily Hemicycliophorinae Skarbilovich, 1959
Genus *Hemicycliophora* De Man, 1921
Hemicycliophora sp.[b]
Hemicycliophora typica De Man, 1921
Hemicycliophora demani Edward and Rai, 1971
Hemicycliophora poranga Monteiro and Lordello, 1978
Family Tylenchulidae (Skarbilovich, 1947) Kirjanova, 1955
Genus *Meloidoderita* Pogosyan, 1966[a]
Meloidoderita spp.[a]
Genus *Tylenchulus* Cobb, 1913[a]
Tylenchulus spp.[a]

[a] Species not identified because not enough high quality specimens for morphometric characterization.

[b] Indicates possible new species and needs further investigation.

[c] Species identified but not enough high quality specimens for morphometric characterization.

Table 2.1 (continued): Checklist of the plant–parasitic nematodes found at the Telperion Nature Reserve.

Class Penetrantia Andrássy, 1974
Subclass Enoplia Pearse, 1942
Order Triplonchida Cobb, 1920
Suborder Diphtherophorina Coomans and Loof, 1970
Superfamily Trichodoroidea (Thorne, 1935) Siddiqi, 1974
Family Trichodoridae (Thorne, 1935) Clark, 1961
Genus *Nanidorus* Siddiqi, 1974
Nanidorus minor (Colbran, 1956) Siddiqi, 1974
Genus *Trichodorus* Cobb, 1913
Trichodorus sp.[b]
Genus *Paratrichodorus* Siddiqi, 1974
Paratrichodorus allius (Jensen, 1963) Siddiqi, 1974
Paratrichodorus porosus (Allen, 1957) Siddiqi, 1974
Subclass Dorylaimia Inglis, 1983
Order Dorylaimida Pearse, 1942
Suborder Dorylaimina Pearse, 1942
Super family Dorylaimoidea De Man, 1876
Family Nordiidae Jairajpuri and Siddiqi, 1964
Subfamily Pungentinae Siddiqi, 1969
Genus *Lenonchium* Siddiqi, 1965
Lenonchium fimbricaudatum Swart and Heyns, 1991[c]
Subfamily Nordiinae Jairajpuri and Siddiqi, 1964
Genus *Longidorella* Thorne, 1939[a]
Longidorella spp.[a]
Superfamily Longidoroidea Thorne, 1935
Family Longidoridae Thorne, 1935
Subfamily Longidorinae Thorne, 1935
Genus *Longidorus* Micoletzky, 1922
Longidorus moniloides Heyns, 1966
Longidorus rotundicaudatus Jacobs and Heyns, 1987

[a] Species not identified because not enough high quality specimens for morphometric characterization.

[b] Indicates possible new species and needs further investigation.

[c] Species identified but not enough high quality specimens for morphometric characterization.

Table 2.1 (continued): Checklist of the plant–parasitic nematodes found at the Telperion Nature Reserve.

Subfamily Xiphinematinae Dalmasso, 1969

Genus *Xiphinema* Cobb, 1913

Xiphinema sp.[b]

Xiphinema diannae Kruger and Heyns, 1987

Xiphinema dimorphicaudatum Heyns, 1966

Xiphinema elongatum Schuurmans Stekhoven and Teunissen, 1938

Xiphinema italiae Meyl, 1953

Xiphinema mampara Heyns, 1979

Xiphinema mluci Heyns, 1976

Xiphinema ornativulvatum Kruger and Heyns, 1987

Xiphinema pongolense Hutsebaut, 1990(1989)

Xiphinema ripogranum Hutsebaut, Heyns and Coomans, 1987

Xiphinema swartae Stocker and Kruger, 1988

Xiphinema vanderlindei Heyns, 1962

Xiphinema xenovariabile Kruger and Heyns, 1984

Xiphinema zyzy Heyns and Swart, 2002

Superfamily Belondiroidea Thorne, 1939

Family Belondiridae Thorne, 1939

Subfamily Axonchiinae Thorne, 1964

Genus *Axonchium* Cobb, 1920[a]

Axonchium spp.[a]

Family Swangeriidae Jairajpuri, 1964

Subfamily Roqueinae Thorne, 1964

Genus *Lindseyus* Ferris and Ferris, 1973

Lindseyus sp. [b]

Family Dorylaimellidae Jairajpuri, 1964

Subfamily Dorylaimellinae Jairajpuri, 1964

Genus *Axodorylaimellus* Jairajpuri and Ahmad, 1980[a]

Axodorylaimellus spp.[a]

Genus *Dorylaimellus* Cobb, 1913[a]

Dorylaimellus spp.[a]

[a] Species not identified because not enough high quality specimens for morphometric characterization.

[b] Indicates possible new species and needs further investigation.

[c] Species identified but not enough high quality specimens for morphometric characterization.

Table 2.1 (continued): Checklist of the plant–parasitic nematodes found at the Telperion Nature Reserve.

Superfamily Tylencholaimoidea Filipjev, 1934
Family Tylencholaimidae Filipjev, 1934
Subfamily Tylencholaiminae Filipjev, 1934
Genus *Chitwoodius* Furstenberg and Heyns, 1966[a]
Chitwoodius spp.[a]
Family Leptonchidae Thorne, 1935
Subfamily Xiphinemellinae Jairajpuri, 1964
Genus *Xiphinemella* Loos, 1950
Xiphinemella sp. [b]

[a] Species not identified because not enough high quality specimens for morphometric characterization.

[b] Indicates possible new species and needs further investigation.

[c] Species identified but not enough high quality specimens for morphometric characterization.

2.4.1 Family Pratylenchidae Thorne, 1949

= Nacobbidae Chitwood, 1950

= Radopholidae Allen and Sher, 1967

Subfamily Pratylenchinae Thorne, 1949

Genus *Pratylenchus* Filipjev, 1936

(*Pratylenchus* Filipjev, 1934 = *nomen nudum*)

***Pratylenchus brachyurus* (Godfrey, 1929) Filipjev and Schuurmans Stekhoven, 1941**

= *Tylenchus brachyurus* Godfrey, 1929

= *Anguillulina brachyurus* (Godfrey, 1929) Goodey, 1932

= *Pratylenchus leiocephalus* Steiner, 1949

= *Pratylenchus pratensis* apud Thorne, 1940

= *Pratylenchus steineri* Lordello, Zamith and Boock, 1954

Figure 2.2 A–E

Measurements: Table 2.2

Pratylenchus brachyurus (Godfrey, 1929) Filipjev and Schuurmans Stekhoven, 1941 was described from *Ananas comosus* var. *comosus* (L.) Merr. in Hawaii (Godfrey, 1929). This species of *Pratylenchus* is cosmopolitan and polyphagous (Castillo and Vovlas, 2007). In South Africa *P. brachyurus* has been recorded in all nine provinces from six of the seven biomes, however not recorded in the Thicket Biome (SAPPNS). This species has been mostly recorded from the Grassland (30 % of records) and Savanna (65 % of records) biomes (SAPPNS). The current specimens were identified as *P. brachyurus* based on the labial region bearing two annuli, angular contour of first lip annulus, posterior position of the vulva, short, undifferentiated post vulval uterine branch, and conoid tail with broadly rounded, smooth terminus. The specimens from the Telperion Nature Reserve correspond well to the type description, as well as other populations described (Sher and Allen, 1953; Loof, 1960; Williams, 1960; Thorne, 1961; Román and Hirschmann, 1969; Van den Berg, 1971; Corbett, 1976; Boonduang and Pliansinchai, 1980; Rashid et al., 1987; Ryss, 1988; Loof, 1991; Sakwe and Geraert, 1994; Crozzoli et al., 1998).

***Pratylenchus dellatrei* Luc, 1958**

= *Pratylenchus singhi* Das and Sultana, 1979
= *Pratylenchus portulacus* Zarina and Maqbool, 1998
= *Pratylenchus graminis* Subramaniyan and Sivakumar, 1991

Figure 2.2 F–J

Measurements: Table 2.2

Pratylenchus dellatrei Luc, 1958 was described from the roots of *Gossypium* L. in southern Madagascar (Luc, 1958). The species has also been reported from India, Korea, Oman and Sudan (Castillo and Vovlas, 2007). *Pratylenchus dellatrei* has been recorded in South Africa from all nine provinces and from all biomes, excluding the Thicket Biome. Most of these records are from the Savanna Biome (45 %), with only 18 % from the Grassland Biome (SAPPNS). The current specimens

were identified as *P. dellatrei* due to the following characteristics: labial region with three annuli, undifferentiated post vulval uterine branch, one or more than one times the corresponding body diameter, subcylindrical tail with smooth conical terminus, less than 20 annuli on the ventral surface of tail. The specimens collected from Telperion Nature Reserve fit the description of the type material and correspond well to other descriptions of the species (Das and Sultana, 1979; Subramaniyan and Sivakumar, 1991; Zarina and Maqbool, 1998; Castillo and Vovlas, 2007). This is the first population of the species from South Africa to be characterized by morphometrics.

***Pratylenchus pseudopratensis* Seinhorst, 1968**

= *Pratylenchus sefaensis* Fortuner, 1973

Figure 2.2 K–P

Measurements: Table 2.2

Pratylenchus pseudopratensis was described from a *Malus* Mill. orchard in the Netherlands and is regarded as a cosmopolitan and polyphagous species (Castillo and Vovlas, 2007). This is the first report of the species from South Africa and was found in natural vegetation close to a waterhole in the Telperion Nature Reserve. The current specimen was identified as *P. pseudopratensis* based on labial region with three annuli, lateral field with four lines, with an additional line in middle band, rectangular spermatheca filled with sperm cells, and conoid tail with rounded, annulated tip. The specimen from Telperion corresponds well to the type description by Seinhorst (1968), as well as other descriptions of the species (Fortuner, 1973; Loof, 1978; Ryss, 1988; Sakwe and Geraert, 1994; Pourjam et al., 1997).

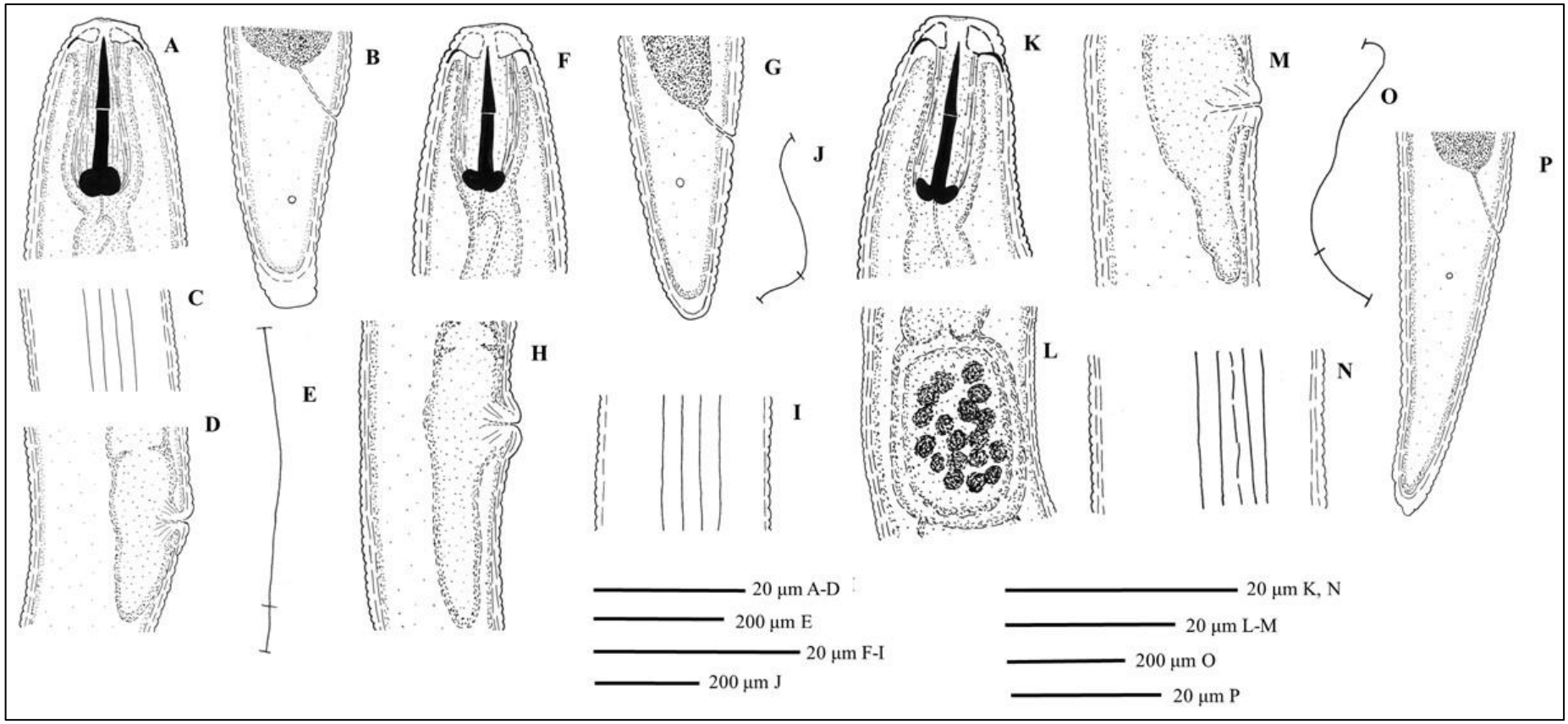


Figure 2.2: A–E**:** *Pratylenchus brachyurus* (Godfrey, 1929) Filipjev and Schuurmans Stekhoven, 1941; F–J: *Pratylenchus dellatrei* Luc, 1958; and K–P: *Pratylenchus pseudopratensis* Seinhorst, 1968 from the Telperion Nature Reserve. A, F, K: Female anterior region; C, I, N: Lateral field at mid body; D, H, M: Vulval region showing post vulval uterine branch; L: Spermatheca; B, G, P: Female posterior region showing tail shape; E, J, O: Whole female specimens indicating position of vulva.

Table 2.2: Morphometrics of *Pratylenchus brachyurus* (Godfrey, 1929) Filipjev and Schuurmans Stekhoven, 1941; *Pratylenchus dellatrei* Luc, 1958 and *Pratylenchus pseudopratensis* Seinhorst, 1968 from the Telperion Nature Reserve. All measurements in µm and in the form: mean ± standard deviation (range).

Species	***Pratylenchus brachyurus* (Godfrey, 1929) Filipjev and Schuurmans Stekhoven, 1941**	***Pratylenchus dellatrei* Luc, 1958**	***Pratylenchus pseudopratensis* Seinhorst, 1968**
n	20 ♀	11 ♀	1 ♀
L	467 ± 52.2 (370–589)	342 ± 28.7 (297–389)	516
a	19.7 ± 1.8 (17.2–22.9)	22.3 ± 2.9 (17.6–28.0)	28.9
b	3.9 ± 0.4 (3.0–4.6)	3.3 ± 0.2 (3.0–3.5)	5.9
b'	5.2 ± 0.6 (3.9–6.0)	4.4 ± 0.2 (4.1–5.8)	9.0
c	18.4 ± 1.7 (15.8–21.2)	18.9 ± 1.9 (15.9–22.0)	13.6
c'	2.0 ± 0.3 (1.6–2.4)	1.9 ± 0.2 (1.6–2.3)	3.0
o	11.4 ± 3.1 (6.1–16.3)	13.6 ± 3.1 (11.4–15.8)	29.6
m	46 ± 2.7 (40–50)	43 ± 4.2 (33–47)	38.6
V	85 ± 0.9 (84–87)	75 ± 0.8 (74–77)	77
OV1	36 ± 5.0 (28–49)	31 ± 3.2 (26–35)	29
Body width at excretory pore	21 ± 1.7 (18–24)	16 ± 1.6 (14–17)	18
Body width at midbody	24 ± 2.4 (18–27)	16 ± 2.1 (11–18)	18
Body width at vulva	19 ± 1.7 (15–21)	15 ± 1.8 (12–18)	18
Body width at anus	13 ± 1.6 (10–16)	10 ± 0.9 (9–11)	13
Labial region height	2 ± 0.5 (1–3)	2 ± 0.4 (2–3)	2
Labial region width	9 ± 0.5 (7–10)	8 ± 0.5 (7–8)	6
Lateral field width	7 ± 1.5 (5–11)	5 ± 0.8 (4–6)	5
Stylet length	19 ± 0.9 (17–21)	13 ± 1.0 (12–15)	12
Metenchium length	9 ± 0.7 (7–10)	6 ± 0.8 (4–7)	4
Telenchium length	10 ± 0.7 (9–12)	8 ± 0.5 (7–8)	7
Stylet knob height	3 ± 0.5 (2–43)	2 ± 0.4 (1–3)	2
Median bulb length	12 ± 1.0 (10–14)	11 ± 0.8 (10–12)	12
Median bulb width	11 ± 1.2 (7–13)	9 ± 0.8 (8–10)	7

Table 2.2 (continued): Morphometrics of *Pratylenchus brachyurus* (Godfrey, 1929) Filipjev and Schuurmans Stekhoven, 1941; *Pratylenchus dellatrei* Luc, 1958 and *Pratylenchus pseudopratensis* Seinhorst, 1968 from the Telperion Nature Reserve. All measurements in µm and in the form: mean ± standard deviation (range).

Species	***Pratylenchus brachyurus* (Godfrey, 1929) Filipjev and Schuurmans Stekhoven, 1941**	***Pratylenchus dellatrei* Luc, 1958**	***Pratylenchus pseudopratensis* Seinhorst, 1968**
n	20 ♀	11 ♀	1 ♀
Median bulb valve from anterior end	55 ± 3.9 (49–62)	40 ± 2.7 (36–43)	48
Nerve ring length	6 ± 1.0 (5–8)	–	7
Nerve ring from anterior end	61 ± 3.4 (56–65)	–	56
Excretory pore from anterior end	75 ± 11.0 (59–97)	62 ± 0.6 (54–74)	73
Length of oesophageal overlap	31 ± 7.2 (19–48)	26 ± 6.9 (14–36)	30
Oesophagus length	119 ± 12.3 (103–153)	102 ± 10.7 (85–121)	87
Anterior genital tract length	165 ± 32.4 (118–242)	106 ± 16.6 (83–138)	148
Vulva from anterior end	398 ± 44.8 (318–507)	257 ± 21.5 (227–292)	396
Post vulval uterine branch length	17 ± 7.7 (9–39)	17 ± 3.3 (11–21)	15
Post vulval uterine branch length/width at vulva	0.9 ± 0.4 (0.5–1.9)	1.1 ± 0.3 (0.7–1.6)	1
Vagina length	7 ± 0.9 (5–9)	4[a]	7
Spermatheca length	15 ± 7.6 (7–27)	–	29
Spermatheca width	11 ± 2.9 (7–14)	–	15
Hyaline portion length	4 ± 0.5 (3–5)	–	–
Ventral tail annuli	16 ± 1.8 (14–20)	16 ± 1.7 (14–20)	–
Phasmid from anus (µm)	10 ± 3.0 (5–15)	3[a]	–
Tail length	26 ± 3.3 (21–32)	18 ± 1.1 (16–20)	38
Excretory pore from anterior as percentage of body length	16.2 ± 1.6 (12.4–18.7)	18 ± 0.7 (18–19)	14
Excretory pore from anterior as percentage of oesophagus length	63.5 ± 7.4 (48.8–79.4)	61 ± 3.6 (54–64)	84
Vagina length as percentage of corresponding body width	36.1 ± 4.1 (30.8–44.4)	29[a]	37
Phasmid location as percentage of tail length	40 ± 9.0 (24–53)	17[a]	–

[a] Only measured in one specimen.

– Indicates structure not present or unable to measure accurately.

2.4.2 Family Hoplomaimidae Filipjev, 1934

= Nemonchidae Skarbilovich, 1959

= Aphasmatylenchidae Sher, 1965

= Rotylenchulidae Husain and Khan, 1965

= Pararotylenchidae Baldwin and Bell, 1981

= Interrotylenchidae Eroshenko, 1984

Subfamily Hoplolaiminae Filipjev, 1934

= Rotylenchoidinae Whitehead, 1958

= Nemonchinae Skarbilovich, 1959

= Aphasmatylenchinae Sher, 1965

= Rotylenchinae Golden, 1971

= Pararotylenchinae Baldwin and Bell, 1981

= Interrotylenchinae Eroshenko, 1984

= Scutellonemoidinae Eroshenko, 1984

Genus *Rotylenchus* Filipjev, 1936

= *Anguillulina* (*Rotylenchus*) Filipjev, 1936

= *Gottholdsteineria* Andrássy, 1958

= *Orientylus* Jairajpuri and Siddiqi, 1977

= *Calvatylus* Jairajpuri and Siddiqi, 1977

= *Interrotylenchus* Eroshenko, 1984

= *Scutellonemoides* Eroshenko, 1984

= *Varotylus* Siddiqi, 1986

***Rotylenchus gracilidens* (Sauer, 1958) Sauer, 1958**

= *Hoplolaimus gracilidens* Sauer, 1958

Figure 2.3 A–D

Measurements: Table 2.3

Described from soil around the roots of *Olearia lepidophylla* (Pers.) Benth., *Rotylenchus gracilidens* (Sauer, 1958) Sauer, 1958 has been reported from Australia (type locality), Bulgaria, New

Zealand, South Africa and Russia (Castillo and Vovlas, 2005). In South Africa this species has been recorded from the Free State, Gauteng, KwaZulu–Natal and North West provinces, from the Grassland Biome and one record form the Savanna Biome (SAPPNS). The presence of this species in the Telperion Nature Reserve is the first report of the species from the Mpumalanga Province. The current specimens were identified as *R. gracilidens* based on a hemispherical labial region set off from body contour bearing four annuli, irregular longitudinal striations in oesophageal region, hemispherical tail and presence of males. The population from the Telperion Nature Reserve were compared to the type population, redescribed by Sher (1965), and the South African population by De Waele and Van den Berg (1988). The specimens from Telperion correspond well to these populations apart from the following differences. From the type population (Sher, 1965) specimens from Telperion are shorter in body length (729–1000 µm *vs* 970–1230 µm in females; 679–1076 µm *vs* 830–1030 µm in males); shorter stylet (27–32 µm *vs* 31–35 in females; 25–29 µm *vs* 28–31 µm in males); and longer gubernaculum (15–19 µm *vs* 12–16 µm). From the South African population (De Waele and Van den Berg, 1988) the current specimens differ in shorter in body length (729–1000 µm *vs* 980–1320 µm in females; 679–1076 µm *vs* 950–1050 µm in males); shorter stylet in males (25–29 µm *vs* 27–30 µm) and shorter spicule length (29–35 µm *vs* 35–41 µm). These difference however are minor and the specimens from Telperion are therefore regarded as conspecific with *R. gracilidens*.

***Rotylenchus unisexus* Sher, 1965**

Figure 2.3 E–G

Measurements: Table 2.3

Rotylenchus unisexus Sher, 1965 was described from soil around the roots of *Chloris* SW. in Kenya (Sher, 1965). The species has since been reported from Democratic Republic of Congo, China, Nambia, New Zealand, South Africa and Spain (Castillo and Vovlas, 2005; SAPPNS). In South Africa

R. unisexus is one of the most wide–spread and frequently recorded species of the genus with records from all nine provinces in all seven biomes (most frequently from the Grassland [50 %] and Savanna [33 %] biomes) (SAPPNS). The current specimens were identified as *R. unisexus* by a hemispherical labial region not set off from body contour, bearing four annuli, no longitudinal striations on the cuticle apart from the lateral field, hemispherical tail, inconspicuous spermatheca and the absence of males. The population from Telperion Nature Reserve were compared to the type population as well as subsequent populations (Ali et al., 1973; Van den Berg and Heyns, 1974; Van den Berg, 1986; Mekete et al., 2008). The current population corresponds well to all the descriptions apart from the following differences from the type population: shorter stylet (22–26 µm *vs* 25–29 µm) and a more anterior situated phasmid (9–14 annulli anterior to anus *vs* 6–7 annuli). These measurements do, however correspond to those in subsequent descriptions and therefore the speciems from Telerion Nature Reserve are considered conspecific with *R. unisexus*.

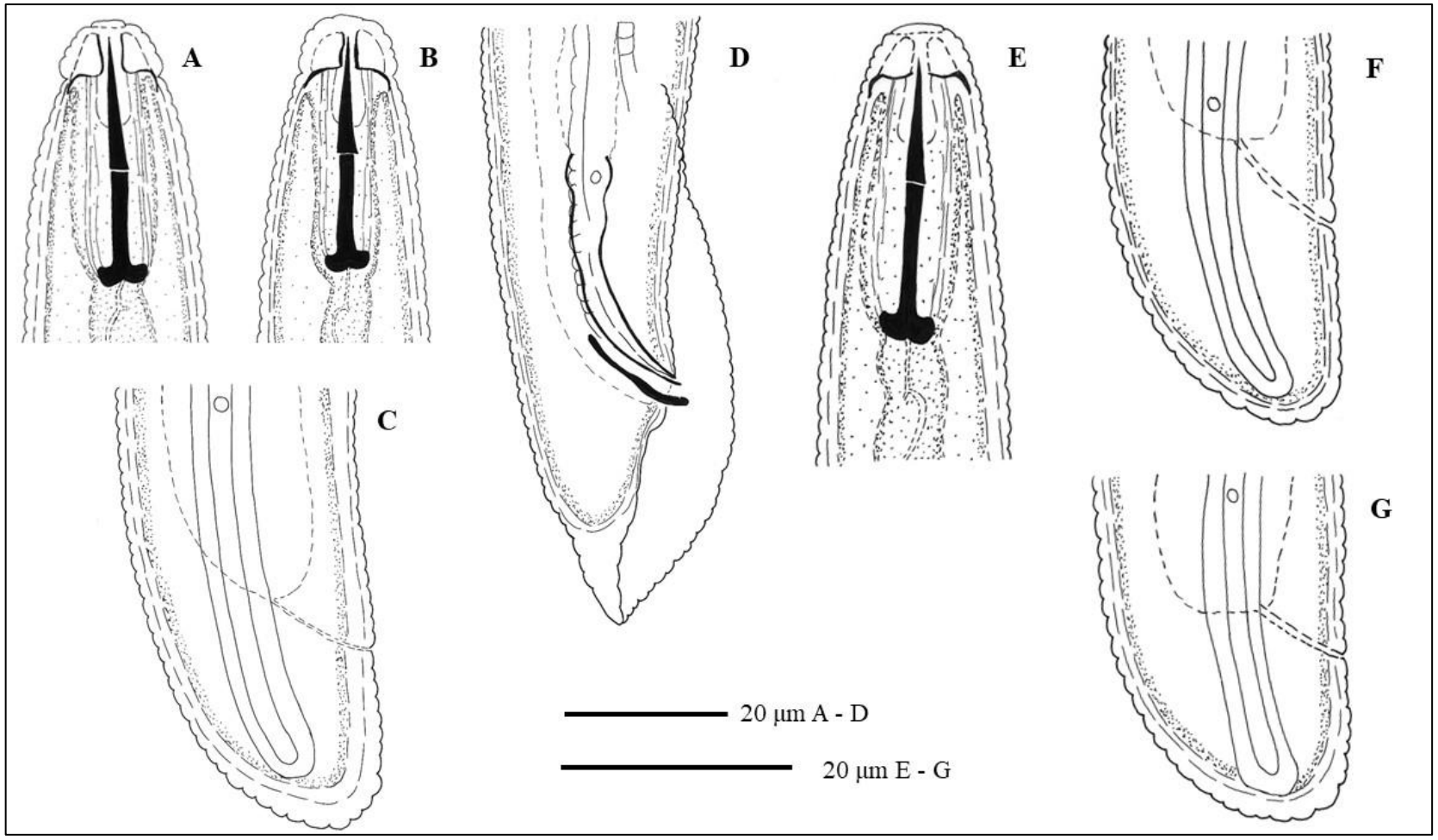


Figure 2.3: A–D: *Rotylenchus gracilidens* (Sauer, 1958) Sauer, 1958 and E–G: *Rotylenchus unisexus* Sher, 1965 from the Telperion Nature Reserve. A, E: Female anterior region; B: Male anterior region; C, F–G: Female posterior region showing tail shape, position of phasmids and central lines of lateral field; D: Male posterior region.

Table 2.3: Morphometrics of *Rotylenchus gracilidens* (Sauer, 1958) Sauer, 1958 and *Rotylenchus unisexus* Sher, 1965 from the Telperion Nature Reserve. All measurements in μm and in the form: mean ± standard deviation (range).

Species	***Rotylenchus gracilidens* (Sauer, 1958) Sauer, 1958**		***Rotylenchus unisexus* Sher, 1965**
n	16 ♀	17 ♂	20 ♀
L	914 ± 65.6 (729-1000)	888 ± 102.1 (679-1076)	705 ± 93.4 (578-928)
a	27. ± 1.6 (24.8-30.0)	29.1 ± 2.0 (25.5-32.4)	27.2 ± 2.8 (21.2-31.8)
b	6.0 ± 0.8 (5.2-7.6)	6.1 ± 0.6 (4.6-7.0)	5.3 ± 0.7 (4.3-6.3)
b'	7.4 ± 0.8 (6.4-9.3)	7.1 ± 0.7 (5.6-8.3)	6.4 ± 0.9 (5.1-7.9)
c	52.1 ± 10.3 (39.5-77.1)	33.9 ± 4.7 (29.0-46.5)	53.2 ± 10.3 (36.2-71.5)
c'	0.7 ± 0.1 (0.5-1.0)	1.5 ± 0.2 (1.2-1.9)	0.7 ± 0.2 (0.5-1.1)
o	17.6 ± 2.4 (14.0-21.7)	20.0 ± 4.4 (14.9-28.6)	18.7 ± 5.7 (10.9-26.0)
m	49 ± 1.9 (45-52)	48 ± 1.8 (45-52)	45 ± 2.0 (42-49)
V \ T	57 ± 1.6 (54-59)	46 ± 4.9 (34-52)	57 ± 1.6 (52-59)
OV1	26 ± 5.2 (17-35)	-	22 ± 3.9 (15-29)
OV2	23 ± 5.1 (17-30)	-	21 ± 3.7 (13-29)
Width at mid body	34 ± 2.3 (32-39)	31 ± 3.0 (25-38)	26 ± 2.5 (22-30)
Width at anus \ cloaca	25 ± 1.7 (22-28)	18 ± 2.2 (15-24)	19 ± 1.5 (15-21)
Annulus width	2 ± 0.2 (2-3)	2 ± 0.2 (2-3)	2*
Lateral field width	6 ± 0.5 (6-7)	6 ± 0.7 (5-7)	6 ± 1.0 (4-7)
Labial region widht	13 ± 0.8 (11-14)	12 ± 0.8 (10-13)	9 ± 0.6 (7-10)
Labial region height	6 ± 1.2 (3-9)	7 ± 0.7 (6-8)	4 ± 0.4 (3-5)
Number of lip annuli	4*	4*	4*
Stylet length	30 ± 1.6 (27-32)	28 ± 1.3 (25-29)	25 ± 1.1 (22-26)
Metenchium length	14 ± 1.0 (13-16)	13 ± 0.8 (12-15)	11 ± 0.7 (10-12)
Telenchium length	15 ± 1.0 (13-17)	14 ± 0.9 (13-16)	14 ± 0.8 (13-15)
Stylet knob heigth	3 ± 0.4 (2-4)	3 ± 0.5 (2-4)	3 ± 0.5 (2-4)

[a] Indicates measurements are the same in all specimens.

– Indicates the structure not measured or structure not present.

Table 2.3 (continued): Morphometrics of *Rotylenchus gracilidens* (Sauer, 1958) Sauer, 1958 and *Rotylenchus unisexus* Sher, 1965 from the Telperion Nature Reserve. All measurements in μm and in the form: mean ± standard deviation (range).

Species	***Rotylenchus gracilidens* (Sauer, 1958) Sauer, 1958**		***Rotylenchus unisexus* Sher, 1965**
n	16 ♀	17 ♂	20 ♀
Stylet knob width	6 ± 0.6 (5-7)	5 ± 0.6 (4-7)	5 ± 0.4 (4-6)
Distance of dorsal gland opening from base of stylet knobs	5 ± 0.8 (4-7)	5 ± 1.1 (4-7)	5 ± 1.3 (3-7)
Median bulb length	16 ± 1.8 (13-18)	14 ± 1.0 (12-16)	13 ± 1.4 (11-16)
Median bulb width	12 ± 2.1 (9-16)	11 ± 1.3 (9-13)	11 ± 2.1 (5-14)
Excretory pore from anterior end	127 ± 7.2 (115-138)	122 ± 13.9 (97-153)	103 ± 12.0 (88-135)
Oesophageal gland overlap length	27 ± 5.9 (21-41)	21 ± 7.2 (9-37)	23 ± 10.1 (11-41)
Oesophagus length	150 ± 17.4 (121-174)	147 ± 11.0 (132-167)	133 ± 13.8 (111-153)
Anterior to vulva	518 ± 40.1 (414-568)	-	397 ± 63.5 (252-523)
Anterior genital tract length	230 ± 45.1 (152-317)	-	159 ± 28.1 (105-208)
Posterior genital tract length	205 ± 33.1 (153-252)	-	153 ± 27.1 (118-208)
Spermatheca width	18 ± 3.7 (14-24)	-	-
Spermatheca length	21 ± 5.1 (14-27)	-	-
Spicule length	-	32 ± 1.2 (29-35)	-
Gubernaculum length	-	17 ± 1.3 (15-19)	-
Testis length	-	408 ± 55.3 (317-522)	-
Number of annuli from phasmid to anus	15 ± 2.7 (10-19)	-	11 ± 2.0 (9-14)
Annuli on ventral side of tail	10 ± 2.0 (7-14)	-	10 ± 1.8 (8-14)
Tail length	18 ± 3.7 (10-24)	26 ± 3.1 (20-31)	14 ± 3.4 (9-22)
Lateral field as percentage of corresponding body length	19 ± 1.9 (17-23)	21 ± 1.7 (17-25)	21 ± 3.4 (15-26)
Anterior to excretory pore as percentage of body length	14 ± 0.9 (12-16)	14 ± 1.1 (12-15)	15 ± 1.0 (14-17)
Anterior to excretory pore as percentage of oesophagus length	86 ± 10.6 (70-109)	83 ± 8.3 (66-94)	77 ± 16.2 (28-96)
Posterior genital tract lengthas percentage of anterior genital tract length	90 ± 20.4 (48-113)	-	99 ± 21.7 (69-156)

[a] Indicates measurements are the same in all specimens.

– Indicates the structure not measured or structure not present.

Genus *Helicotylenchus* Steiner, 1945

= *Rotylenchoides* Whitehead, 1958

= *Zimmermannia* Shamsi, 1973

Helicotylenchus dihystera (Cobb, 1893) Sher, 1961

= *Tylenchus dihystera* Cobb, 1893

= *Tylenchus olaae* Cobb, 1906

= *Tylenchorhynchus olaae* (Cobb, 1906) Micoletzky, 1922

= *Helicotylenchus olaae* (Cobb, 1906) Sher, 1961

= *Aphelenchus dubius* var. *peruensis* Steiner, 1920

= *Tylenchus spiralis* Cassidy, 1930

= *Helicotylenchus spiralis* (Cassidy, 1930) Sher, 1961

= *Helicotylenchus nannus* Steiner, 1945

= *Helicotylenchus crenatus* Das, 1960

= *Helicotylenchus dihysteroides* Siddiqi, 1972

= *Helicotylenchus flatus* Román, 1965

= *Helicotylenchus glissus* Thorne and Malek, 1968

= *Helicotylenchus rotundicauda* Sher, 1966

= *Helicotylenchus punicae* Swarup and Sethi, 1968

= *Helicotylenchus teleductus* Anderson, 1974

Figure 2.4

Measurements: Table 2.4

Helicotylenchus dihystera (Cobb, 1893) Sher, 1961 was described from *Saccharum officinarum* L. in Australia (Cobb, 1893). The species is cosmopolitan and has been reported from several plant species (Marais, 2001). In South Africa *H. dihystera* is one of the most common species in the country, recorded from all nine provinces of South Africa and is commonly recorded from the Savanna (49 %) and Grassland (30 %) biomes (SAPPNS). The current specimens were identified as *H. dihystera* based on the labial region with four to six annuli, anterior position of the phasmids, inner lines of lateral field on tail generally end in a y–shape, tail dorsally curved generally with a ventral rounded projection, and

the absence of males and fasciculi. The population from Telperion compares well with the type and other known descriptions of the species (Cobb, 1893; Carvalho, 1956; Das, 1960; Williams, 1960; Román, 1965; Timm, 1965; Sher, 1966; Thorne and Malek, 1968; Siddiqi, 1972a, 1972b; Edward and Rai, 1970; Ali et al., 1973; Geraert et al., 1975; Ratanaprapa and Boonduang, 1975; Sauer and Winoto, 1975; Van den Berg and Heyns, 1975; Van den Berg and Kirby, 1979; Zavaleta–Mejia and Sosa–Moss, 1979; Eroshenko, 1981a; Eroshenko and Nguen Vu Tkhan; 1981; Baqri and Ahmad, 1983; Brzeski, 1985; Mizukubo et al., 1992; Hui and Zhixin, 1993; Wouts and Yeates, 1994; Hui and Zhixin, 1996a; Kazi, 1996; Marais and Quénéhervé, 1996; Crozzoli et al., 1998; Zheng et al., 1998; Marais et al., 2000; Pudasaini and Bert, 2000; Marais, 2001; Bracamonte et al., 2004; Maggiorani et al., 2004; Mekete et al., 2008; Trinh et al., 2009).

***Helicotylenchus martini* Sher, 1966**

= *Helicotylenchus krugeri* Van den Berg and Heyns, 1975

Figure 2.5

Measurements: Table 2.4

Helicotylenchus martini Sher, 1966 was described from the soil around the roots of *Camellia sinensis* (L.) Kuntze in Zimbabwe (Sher, 1966). The species has since been reported from the Democratic Republic of the Congo, Georgia, and South Africa (Marais, 2001). In South Africa, *H. martini* has been recorded from all nine provinces in all the biomes especially the Grassland Biome with 52 % of the records from this biome (SAPPNS). The specimens from Telperion were identified as *H. martini* based on the following: truncate labial region without annuli, presence of fasciculi, phasmids situated near mid tail, presence of males. The current population was compared to the type population by Sher (1966) and consequent populations of the species (Ali et al., 1973; Van den Berg and Heyns,

1975; Van den Berg, 1978a; Marais, 1993; Marais, 1998; Marais, 2001) and it was found that the population from Telperion matches these descriptions.

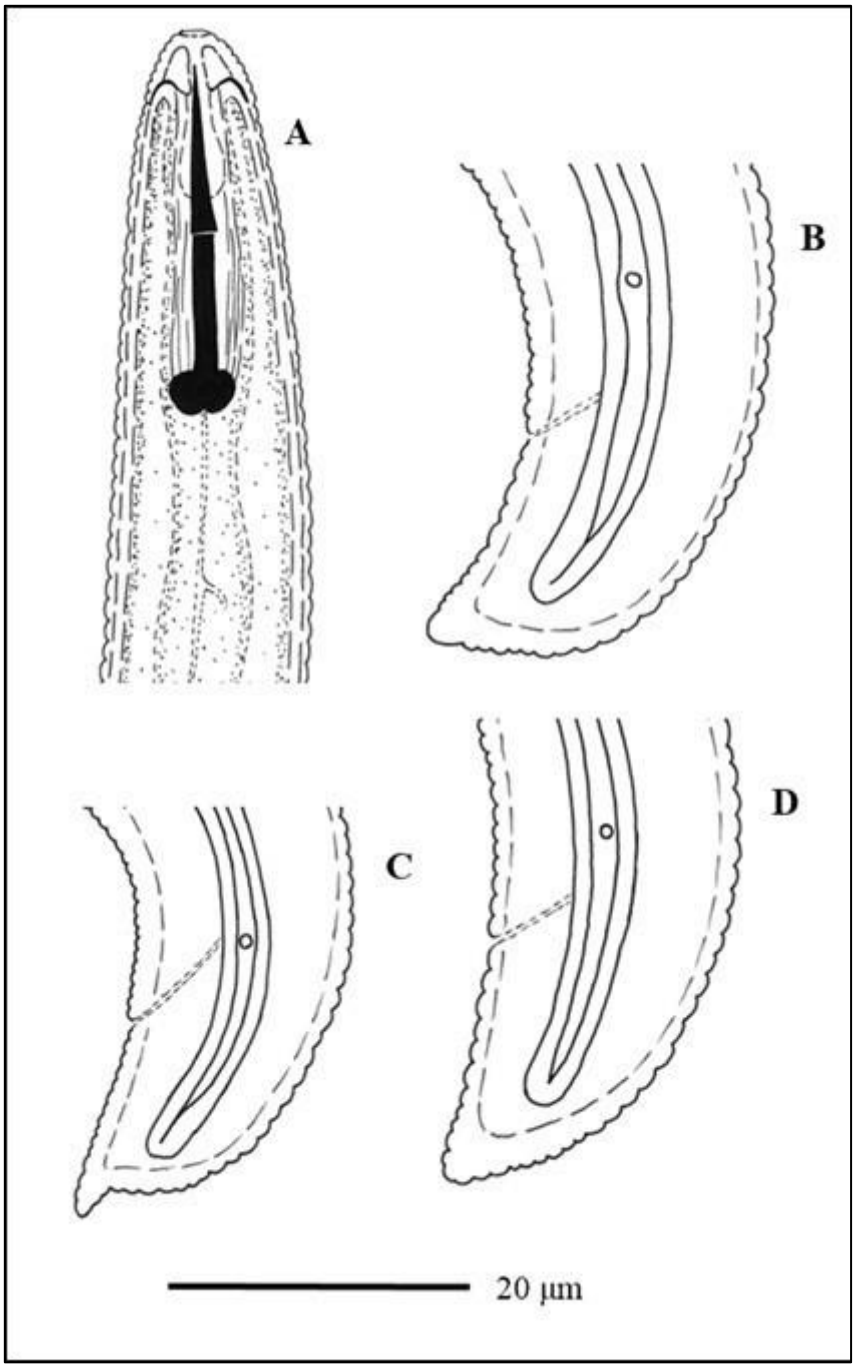


Figure 2.4: *Helicotylenchus dihystera* (Cobb, 1893) Sher, 1961 from the Telperion Nature Reserve. A: Anterior region; B–D: Posterior region showing variation in tail shape and central lines of lateral field.

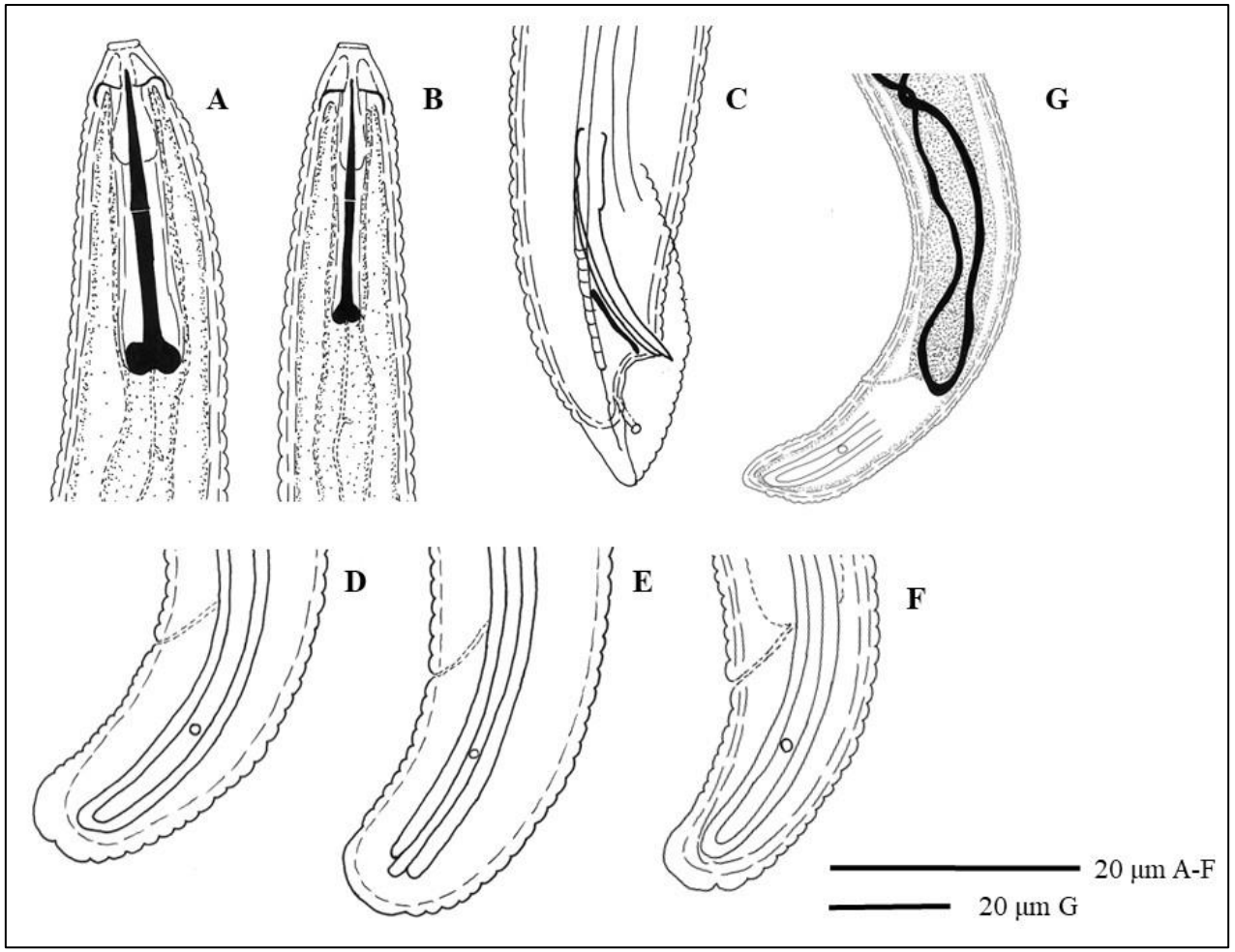


Figure 2.5: *Helicotylenchus martini* Sher, 1966 from the Telperion Nature Reserve. A–B: Anterior region of female (A) and male (B); C–F: Posterior region of female (D–F) and male (C) showing variation in tail shape and central lines of lateral field; G: Posterior region of female showing fasciculi.

***Helicotylenchus paraplatyurus* Siddiqi, 1972**

Figure 2.6 A–C

Measurements: Table 2.4

Described from cultivated soil in Portugal, *Helicotylenchus paraplatyurus* Siddiqi, 1972 has since then reported from India, Kenya, Korea, Senegal, South Africa, and the United States of America (Marais, 2001). In South Africa this species has been recorded from all nine provinces, in six biomes (excluding the Succulent Karoo Biome). Most of these records are in the Savanna (53 %) and Grassland (29 %) biomes (SAPPNS). The current specimens were identified as *H. paraplatyurus* based on spiral habitus, body length, rounded labial region, stylet length, absence of fasciculi, phasmid situated four annuli anterior to five annuli posterior to anus, tail dorsally curved with few annuli at tail terminus smaller than annuli on rest of tail. The population form Telperion compares well to the type population of the species by Siddiqi (1972) as well as descriptions by Van den Berg and Heyns (1975); Van den Berg (1978a); Baqri and Ahmad (1983); Bae and Choi (1997); Marais (2001).

***Helicotylenchus pseudorobustus* (Steiner, 1914) Golden, 1956**

= *Tylenchus pseudorobustus* Steiner, 1914
= *Helicotylenchus microlobus* Perry in Perry, Darling and Thorne, 1959
= *Helicotylenchus phalerus* Anderson, 1974

Figure 2.6 D–F

Measurements: Table 2.4

Helicotylenchus pseudorobustus (Steiner, 1914) Golden, 1956 was described from the soil under moss in Switzerland (Steiner, 1914) and is one of the species in the genus with a cosmopolitan distribution (Marais, 2001). *Helicotylenchus microlobus* Perry in Perry, Darling and Thorne, 1959 was

made a junior synonym of *H. pseudorobustus* by Sher (1966), because he could not distinguish between the paratypes. Siddiqi (1972b) recognized both species while Sauer and Winoto (1975) accepted the synonymization of Sher (1966) and thereby dismissed the conclusion of Siddiqi (1972b). Fortuner et al. (1984), Fortuner (1985), Brzeski (1998) and Marais (2001) regarded *H. microlobus* as a junior synonym of *H. pseudorobustus*. Subbotin et al. (2015) regarded both species as valid on grounds of both molecular and morphological characters. In a paper on the identity of populations of *H. pseudorobustus* Fortuner et al. (2018) confirmed that *H. microlobus* is a junior synonym of *H. pseudorobustus* but proposed that the morphological species includes two molecular operational taxonomic units (MOTU) (Floyd et al., 2002) namely *H. pseudorobustus* A and *H. pseudorobustus* B. In the present paper the synonymization of Sher (1966) is accepted. *Helicotylenchus pseudorobustus* has been recorded from all nine provinces in South Africa and in all the biomes, especially the Grassland (44 %) and Savanna (39 %) biomes (SAPPNS).

The specimens from Telperion were identified as *H. pseudorobustus* based on the rounded labial region, anterior position of phasmid to anus, absence of males and fasciculi, tail dorsally curved with a ventral terminal projection bearing a pointed terminus. The current specimens correspond well to the type population as well as various other populations of the species described (Steiner, 1914; Sher, 1966; Thorne and Malek, 1968; Siddiqi, 1972b; Ali et al., 1973; Sauer and Winoto, 1975; Van den Berg and Heyns, 1975; Mancini and Moretti, 1976; Van den Berg and Kirby, 1979; Fortuner et al., 1984; Brzeski, 1985; Fortuner, 1985; Lal and Khan, 1989; Castillo et al., 1991; Yeates and Wouts, 1992; Kazi, 1996; Kornobis and Dobosz, 1996; Marais and Quénéhervé, 1996; Pudasaini and Bert, 2000; Maggiorani et al., 2004; Walters et al., 2004; Ramaji et al., 2006; Subbotin et al., 2015; Fortuner et al., 2018; Shokoohi, et al., 2018).

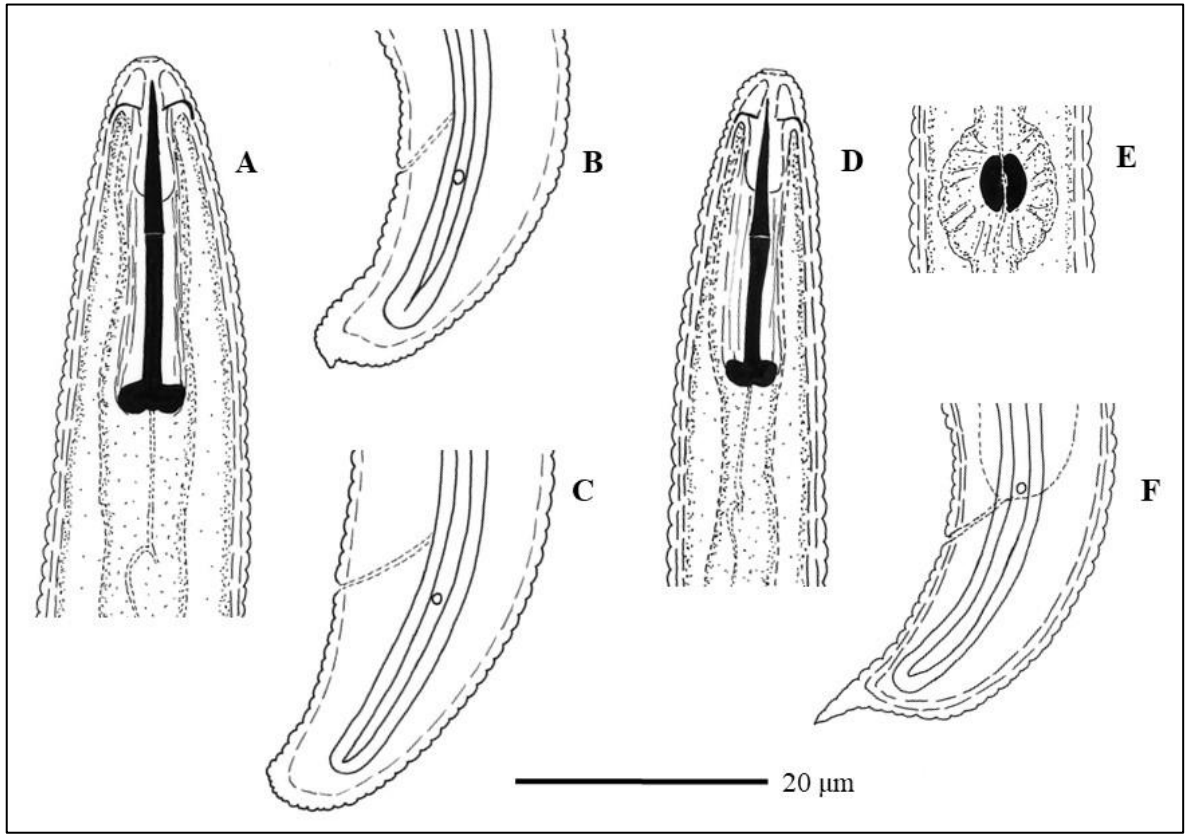


Figure 2.6: A–C: *Helicotylenchus paraplatyurus* Siddiqi, 1972; and D–F: *Helicotylenchus pseudorobustus* (Steiner, 1914) Golden, 1956 from the Telperion Nature Reserve. A+D: Anterior region; B–C+F: Posterior region showing variation in tail shape and central lines of lateral field; E: Median bulb indicating median bulb valve.

Table 2.4: Morphometrics of *Helicotylenchus dihystera* (Cobb, 1893) Sher, 1961; *Helicotylenchus martini* Sher, 1966; *Helicotylenchus paraplatyurus* Siddiqi, 1972; and *Helicotylenchus pseudorobustus* (Steiner, 1914) Golden, 1956 populations in the Telperion Nature Reserve. All measurements in µm and in the form: mean ± standard deviation (range).

Species	***Helicotylenchus dihystera* (Cobb, 1893) Sher, 1961**	***Helicotylenchus martini* Sher, 1966**		***Helicotylenchus paraplatyurus* Siddiqi, 1972**	***Helicotylenchus pseudorobustus* (Steiner, 1914) Golden, 1956**
n	20 ♀	20 ♀	14 ♂	20 ♀	7 ♀
L	594 ± 56.9 (503–701)	548 ± 77.7 (453–708)	535 ± 37.8 (478–588)	624 ± 64.1 (451–707)	578 ± 48.7 (521–677)
a	26.8 ± 2.6 (21.0–31.4)	25.9 ± 1.9 (23.1–29.3)	32.5 ± 2.8 (28.4–37.1)	26.7 ± 4.0 (19.6–34.1)	29.8 ± 2.0 (27.2–31.7)
b	5.0 ± 0.4 (4.3–5.8)	5.3 ± 0.3 (4.8–6.0)	5.0 ± 0.6 (4.4–5.5)	5.0 ± 0.5 (4.2–5.9)	–
b'	4.3 ± 0.3 (3.9–5.1)	4.7 ± 0.7 (3.9–7.2)	4.1 ± 0.5 (3.3–4.7)	4.1 ± 0.5 (3.2–4.8)	4.1 ± 0.3 (3.8–4.4)
c	41.5 ± 10.1 (30.5–73.4)	25.4 ± 4.2 (20.1–35.4)	33.2 ± 4.7 (28.2–43.4)	37.4 ± 4.4 (26.7–44.2)	31.2 ± 3.1 (26.9–36.2)
c'	1.1 ± 0.2 (0.6–1.4)	1.8 ± 0.3 (1.1–2.3)	1.5 ± 0.2 (1.2–1.9)	1.2 ± 0.2 (0.9–1.6)	1.6 ± 0.1 (1.4–1.8)
o	45.1 ± 9.9 (31.0–64.7)	45.5 ± 9.8 (32.3–69.0)	48.2 ± 3.8 (42.2–53.3)	47.3 ± 6.9 (36.8–63.6)	50.1 ± 3.9 (47.3–52.8)
m	46 ± 1.6 (43–49)	46 ± 2.1 (42–49)	50 ± 4.0 (44–55)	45 ± 1.7 (41–47)	43 ± 3.4 (38–47)
V / T	64 ± 2.2 (61–72)	58 ± 2.9 (52–64)	52 ± 4.3 (43–59)	62 ± 1.3 (60–66)	65± 0.9 (63–66)
OV1	24 ± 3.1 (19–29)	22 ± 3.7 (15–32)	–	24 ± 3.6 (17–32)	24 ± 4.2 (20–31)
OV2	20 ± 2.3 (17–25)	20 ± 2.3 (15–24)	–	21 ± 2.1 (17–24)	20 ± 1.4 (17–21)
Body width at mid body	22 ± 2.2 (19–29)	21 ± 3.1 (16–26)	16 ± 1.0 (14–18)	24 ± 2.7 (19–29)	20 ± 1.4 (18–22)
Body width at anus / cloaca	13 ± 1.3 (11–16)	12 ± 1.2 (10–15)	11 ± 1.0 (9–12)	14 ± 1.0 (12–16)	12 ± 0.8 (10–12)
Annulus width	1 ± 0.2 (1–2)	1 ± 0.2 (1–2)	1 ± 0.3 (1–2)	1 ± 0.2 (1–2)	1 ± 0.1 (1–2)
Lateral field width	5 ± 0.6 (4–6)	6 ± 1.1 (5–8)	4 ± 0.3 (4–5)	5 ± 0.5 (4–6)	5 ± 1.1 (4–5)
Labial region height	4 ± 0.4 (3–5)	3 ± 0.4 (3–4)	4 ± 0.3 (4–5)	4 ± 0.3 (3–4)	3 ± 0.3 (3–4)
Labial region width	7 ± 0.7 (5–8)	6 ± 0.5 (5–7)	6 ± 0.3 (6–7)	7 ± 0.6 (5–7)	6 ± 0.7 (4–6)
Stylet length	25 ± 1.2 (22–26)	24 ± 1.5 (21–26)	20 ± 1.2 (18–22)	27 ± 1.3 (24–29)	22 ± 0.9 (20–23)

[a] Indicates values were the same for all specimens.

[b] Indicates structure was measured in only one specimen.

– Indicates structure not present or unable to measure accurately.

Table 2.4 (continued): Morphometrics of *Helicotylenchus dihystera* (Cobb, 1893) Sher, 1961; *Helicotylenchus martini* Sher, 1966; *Helicotylenchus paraplatyurus* Siddiqi, 1972; and *Helicotylenchus pseudorobustus* (Steiner, 1914) Golden, 1956 populations in the Telperion Nature Reserve. All measurements in µm and in the form: mean ± standard deviation (range).

Species	*Helicotylenchus dihystera* (Cobb, 1893) Sher, 1961	*Helicotylenchus martini* Sher, 1966		*Helicotylenchus paraplatyurus* Siddiqi, 1972	*Helicotylenchus pseudorobustus* (Steiner, 1914) Golden, 1956
n	20 ♀	20 ♀	14 ♂	20 ♀	7 ♀
Metenchium length	11 ± 0.7 (10–13)	11 ± 1.0 (9–13)	10 ± 1.4 (8–12)	12 ± 0.9 (10–14)	9 ± 1.0 (8–10)
Telenchium length	13 ± 0.8 (12–14)	13 ± 0.7 (12–14)	10 ± 0.4 (9–11)	15 ± 0.6 (14–16)	12 ± 0.6 (12–13)
Stylet knob height	3 ± 0.3 (2–3)	2 ± 0.3 (2–3)	1 ± 0.2 (1–2)	3 ± 0.2 (2–3)	2[a]
Stylet knob width	4 ± 0.4 (3–5)	4 ± 0.3 (4–5)	2 ± 0.4 (2–3)	5 ± 0.6 (4–7)	4 ± 0.3 (3–4)
Distance of dorsal gland opening from base of stylet knobs	11 ± 2.7 (8–16)	11 ± 2.6 (8–18)	10 ± 1.1 (8–12)	12 ± 2–3 (8–17)	11 ± 1.7 (10–12)
Median bulb length	11 ± 0.9 (10–13)	11 ± 0.6 (10–12)	9 ± 0.9 (8–10)	13 ± 1.0 (11–14)	12 ± 0.8 (11–13)
Median bulb width	9 ± 0.8 (8–11)	9 ± 0.7 (8–10)	7 ± 0.6 (6–8)	11 ± 2.0 (9–19)	10 ± 0.8 (9–11)
Median bulb valve length	3 ± 0.4 (2–4)	2 ± 0.5 (1–4)	2 ± 0.3 (1–2)	3 ± 0.4 (3–4)	4 ± 0.4 (4–5)
Median bulb valve width	2 ± 0.3 (2–3)	2 ± 0.2 (1–2)	1[a]	2 ± 0.3 (2–3)	3 ± 0.4 (3–4)
Excretory pore from anterior end	101 ± 8.8 (78–115)	97 ± 10.9 (82–111)	92 ± 4.2 (84–98)	101 ± 8.6 (84–114)	101 ± 6.7 (92–109)
Oesophageal gland overlap length	19 ± 3.9 (12–24)	17 ± 7.1 (8–31)	24 ± 13.0 (13–39)	24 ± 7.9 (10–41)	–
Oesophagus length	139 ± 7.2 (128–152)	120 ± 15.9 (70–138)	136 ± 11.8 (124–159)	155 ± 13.8 (138–195)	139 ± 11.6 (127–151)
Anterior genital tract length	141 ± 23.9 (100–186)	124 ± 31.1 (91–191)	–	154 ± 18.7 (119–180)	143 ± 19.9 (121–173)
Anterior end to vulva	378 ± 34.0 (325–440)	316 ± 44.8 (255–435)	–	393 ± 33.2 (330–446)	374 ± 34.2 (335–443)
Posterior genital tract length	120 ± 21.4 (87–175)	112 ± 24.6 (79–159)	–	129 ± 15.7 (101–150)	114 ± 6.6 (101–118)
Spermatheca length	12 ± 1.6 (10–14)	12 ± 2.5 (9–18)	–	16 ± 3.0 (11–19)	11 ± 2.9 (9–14)
Spermatheca width	11 ± 1.1 (10–13)	12 ± 1.6 (9–14)	–	12 ± 1.5 (9–13)	12 ± 2.6 (9–14)
Testis length	–	–	276 ± 32.4 (212–317)	–	–

[a] Indicates values were the same for all specimens.

[b] Indicates structure was measured in only one specimen.

– Indicates structure not present or unable to measure accurately.

Table 2.4 (continued): Morphometrics of *Helicotylenchus dihystera* (Cobb, 1893) Sher, 1961; *Helicotylenchus martini* Sher, 1966; *Helicotylenchus paraplatyurus* Siddiqi, 1972; and *Helicotylenchus pseudorobustus* (Steiner, 1914) Golden, 1956 populations in the Telperion Nature Reserve. All measurements in µm and in the form: mean ± standard deviation (range).

Species	***Helicotylenchus dihystera* (Cobb, 1893) Sher, 1961**	***Helicotylenchus martini* Sher, 1966**		***Helicotylenchus paraplatyurus* Siddiqi, 1972**	***Helicotylenchus pseudorobustus* (Steiner, 1914) Golden, 1956**
n	20 ♀	20 ♀	14 ♂	20 ♀	7 ♀
Spicule length	–	–	20 ± 1.3 (18–23)	–	–
Gubernaculum length	–	–	6 ± 0.6 (5–7)	–	–
Position of phasmid (number of annuli)	Six to 11 annuli anterior to anus	Two to 15 annuli posterior to anus	Four to 10 annuli posterior to anus	Four annuli anterior to five annuli posterior to anus	Five to 10 annuli anterior to anus
Phasmid diameter	0.9 ± 0.2 (0.7–1.6)	1.2 ± 0.2 (0.7–1.6)	0.7[b]	1.0 ± 0.2 (0.7–1.4)	1[a]
Phasmid diameter / annulus opposite phasmid width	0.7 ± 0.2 (0.4–1.1)	0.9 ± 0.3 (0.4–1.3)	–	0.8 ± 0.2 (0.6–1.2)	0.7 ± 0.1 (0.6–0.8)
Ventral tail annuli	8 ± 1.5 (5–10)	13 ± 3.1 (7–18)	–	15 ± 2.2 (11–19)	9 ± 1.7 (7–12)
Tail length	15 ± 3.8 (8–22)	22 ± 5.0 (13–30)	16 ± 2.0 (12–19)	17 ± 2.4 (13–21)	19 ± 1.6 (17–21)
Lateral field as percentage of corresponding body width	23 ± 2.9 (19–27)	28 ± 3.4 (22–33)	25 ± 2.0 (23–28)	22 ± 3.0 (17–26)	24 ± 3.8 (21–26)
Anterior to excretory pore as percentage of body length	17 ± 0.9 (15–18)	18 ± 1.3 (16–20)	17 ± 0.8 (16–19)	16 ± 1.4 (15–21)	17 ± 1.2 (16–19)
Anterior to excretory pore as percentage of oesophagus length	84 ± 5.8 (75–94)	92 ± 4.8 (88–104)	83 ± 5.6 (77–88)	80 ± 9.3 (6.7–9.5)	82 ± 10.0 (61–85)
Posterior genital tract length as percentage of anterior genital tract length	86 ± 11.4 (57–108)	90 ± 11.9 (63–114)	–	88.5 ± 12.9 (65–107)	81 ± 14.1 (58.4–95.5)

[a] Indicates values were the same for all specimens.

[b] Indicates structure was measured in only one specimen.

– Indicates structure not present or unable to measure accurately.

Genus *Scutellonema* Andrássy, 1958

Scutellonema bizanae Van den Berg and Heyns, 1973

= *Scutellonema multistriatum* Van den Berg and Heyns, 1973

Figure 2.7

Measurements: Table 2.5

Scutellonema bizanae Van den Berg and Heyns, 1973 was described from unidentified ferns in Port St. Johns, Eastern Cape Province. Apart from several localities in South Africa, this species has since been recorded from China (Vovlas and Li, 1988). In South Africa the species has been recorded in all nine provinces and is evenly distributed amoung the biomes. The Nama Karoo with two records and Succulent Karoo with only one being the exception (SAPPNS). The current specimens were identified as *S. bizanae* based on the labial region set off from the body contour bearing four distinct annuli, well defined spermatheca filled with sperm cells, the posterior position of the scutellum (on tail at end of lateral field), areolation of the lateral field at level of scutellum, short segment of central band of the lateral field visible posterior to scutellum, and the presence of males. The population of *S. bizanae* from Telperion Nature Reserve was compared to the type population, as well as populations reported by Van den Berg and Heyns (1973), as *S. multistriatum* Van den Berg and Heyns, 1973, Germani et al. (1985); and Vovlas and Li (1988). The current population corresponds well to these descriptions apart from the following differences to the type population: longer stylet in females (27–40 *vs* 26–30 µm); and shorter gubernaculum (12–14 µm *vs* 14–16 µm). These measurements do however correspond to those in subsequent descriptions and the current specimens are therefore considered conspecific to *S. bizanae.*

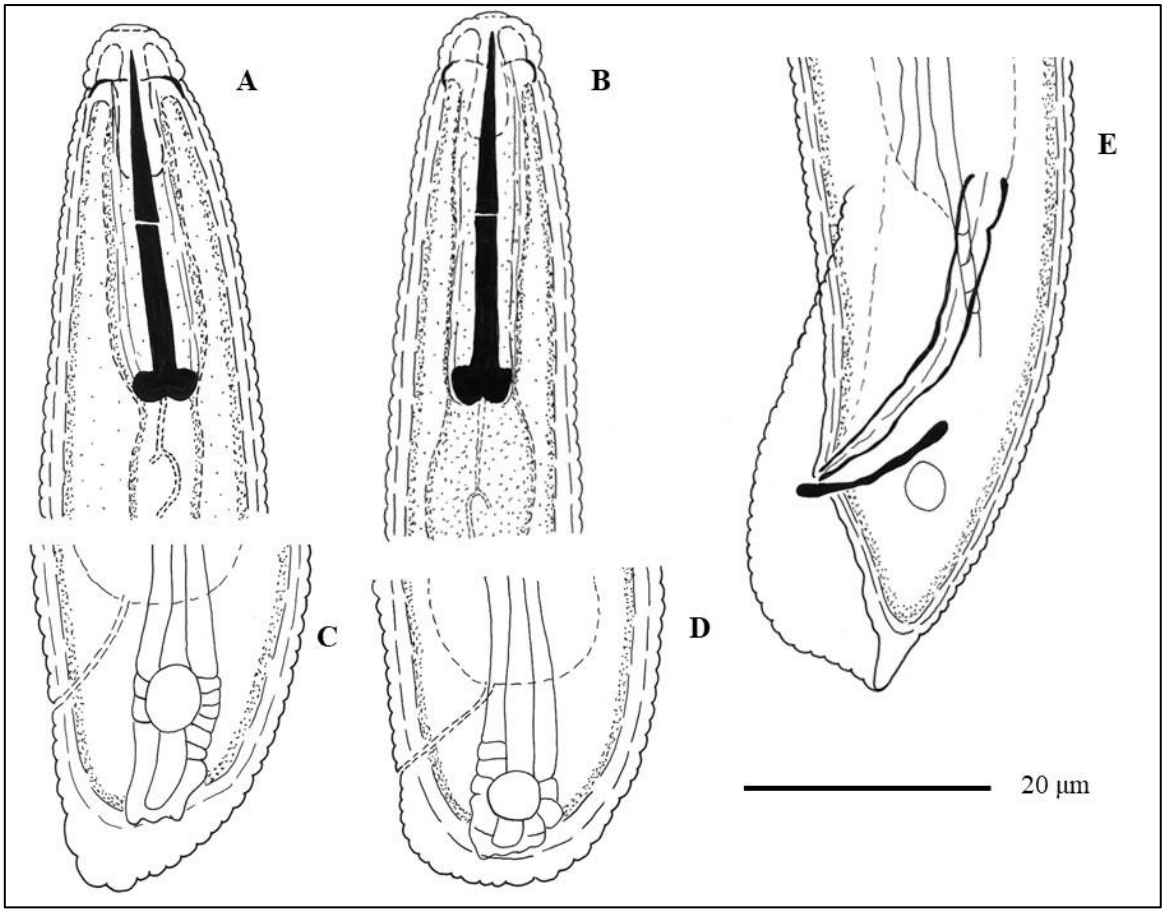


Figure 2.7: *Scutellonema bizanae* Van den Berg and Heyns, 1973 from the Telperion Nature Reserve. A: Female anterior region; B: Male anterior region; C–D: Female posterior region showing variation in tail shape and aerolation of lateral field at level of scutellum; E: Male posterior region.

***Scutellonema brachyurus* (Steiner, 1938) Andrássy, 1958**

= *Rotylenchus brachyurus* Steiner, 1938
= *Rotylenchus coheni* Goodey, 1952
= *Scutellonema coheni* (Goodey, 1952) Andrássy, 1958
= *Rotylenchus boocki* Lordello, 1957
= *Scutellonema boocki* (Lordello, 1957) Andrássy, 1958
= *Scutellonema bangalorense* Khan and Nanjappa, 1972
= *Scutellonema conicaudatum* Sivakumar and Selvasekaran, 1982
= *Scutellonema orientale* Rashid and Khan, 1974
= *Scutellonema ramai* Verma, 1970
= *Scutellonema sheri* Edward and Rai, 1970

Figure 2.8 A–C

Measurements: Table 2.5

Scutellonema brachyurus (Steiner, 1938) Andrássy, 1958 was described from *Lycoris radiata* (L'Hér) Herb. in United States of America (Steiner, 1938). This polyphagous species has a cosmopolitan distribution (CABI, 2006) and is the most frequently recorded species in South Africa, recorded in all seven biomes, mostly from the Savanna (42 %) and Grassland (40 %) biomes, from all nine provinces (SAPPNS). The current specimens were identified as *S. brachyurus* based on the labial region not set off from body contour bearing four to five annuli, inconspicuous spermatheca, scutellum at level of anus, areolation at level of scutellum, and the absence of males. The population of *S. branchyurus* corresponds well to all descriptions in available literature (Steiner, 1938; Lordello, 1957; Williams, 1960; Sher, 1964; Edward and Rai, 1970; Khan and Nanjappa, 1972; Rashid and Khan, 1974; Van den Berg and Heyns, 1973; Ali et al., 1973; Siddiqi, 1974b; Ratanaprapa and Boonduang, 1975; Sivakumar and Selvasekoran, 1982; Brzeski, 1985; Germani et al., 1985; Melillo and Troccoli, 1993; Botha and Heyns, 1993b; Crozzoli et al., 1998; Van den Berg, 1998).

***Scutellonema truncatum* Sher, 1964**

Figure 2.8 D–E

Measurements: Table 2.5

Described from virgin clay soil in KwaZulu–Natal, South Africa, *Scutellonema truncatum* Sher, 1964 has been reported from Mozambique, South Africa and Zimbabwe (Sher, 1964; Van den Berg and Van den Oever, 1993). In South Africa the species has often been recorded in the Savanna (78 %) and Grassland (12 %) biomes, with some records also from the Forest (4 %) and Thicket (4 %) biomes in all the provinces (excluding the Western Cape Province) (SAPPNS). The population of *Scutellonema* from the Telperion Nature Reserve was identified as *S. truncatum* based on the smooth, truncate labial

region, areolation of the lateral field at level of scutellum and the absence of males. The current population was compared to the type population as well as subsequent populations (Van den Berg and Heyns, 1973; Van den Berg and Van den Oever, 1993). Apart from the slightly more posterior vulva in the current population as compared to the type population (56–66 % *vs* 55–60 %), the population from Telperion corresponds well with the descriptions available in literature and is therefore considered conspecific to the species.

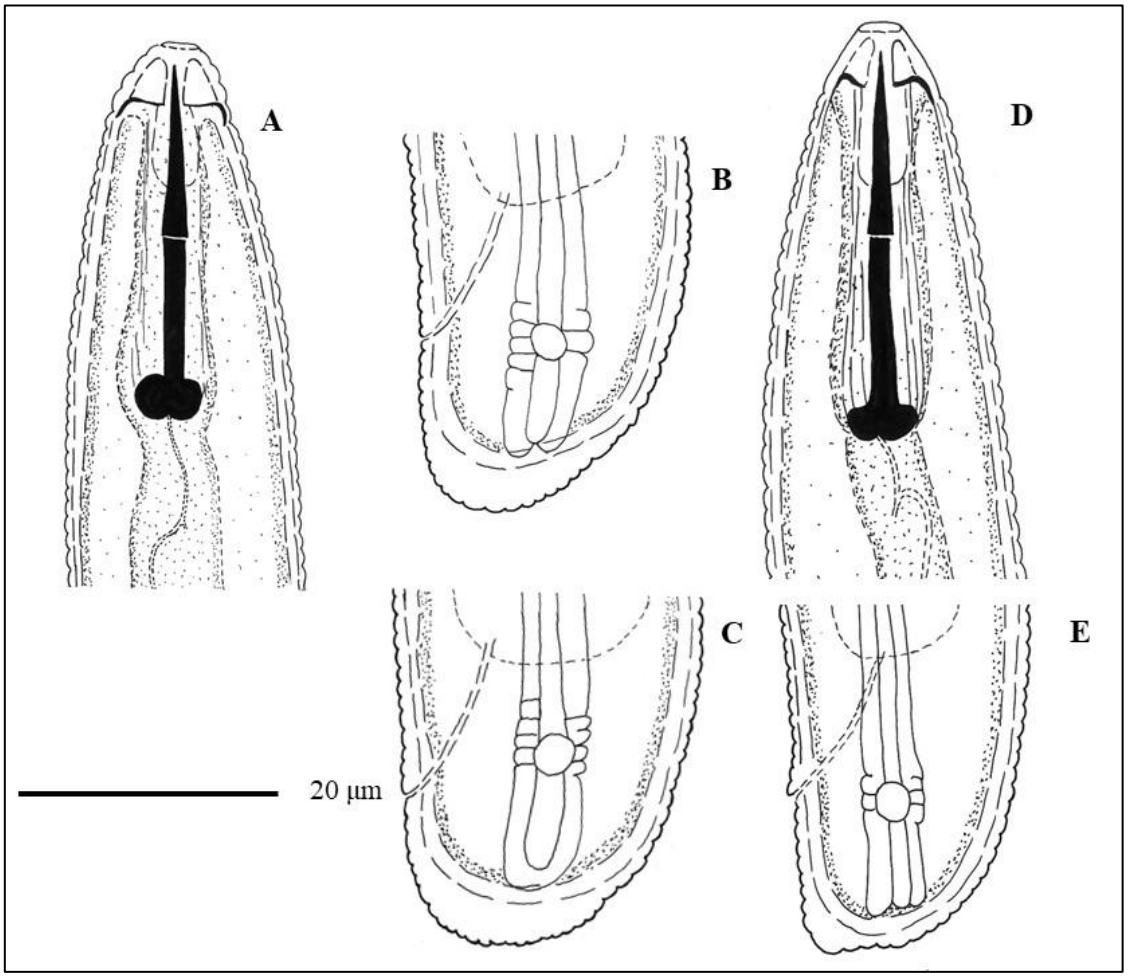


Figure 2.8: A–C: *Scutellonema brachyurus* (Steiner, 1938) Andrássy, 1958 and D–E: *Scutellonema truncatum* Sher, 1964 from the Telperion Nature Reserve. A, D: Female anterior region; B–C, E: Female posterior region showing variation in tail shape and aerolation of lateral field at level of scutellum.

Table 2.5: Morphometrics of *Scutellonema bizanae* Van den Berg and Heyns, 1973; *Scutellonema brachyurus* (Steiner, 1938) Andrássy, 1958 and *Scutellonema truncatum* Sher, 1964 populations in the Telperion Nature Reserve. All measurements in µm and in the form: mean ± standard deviation (range).

Species	***Scutellonema bizanae* Van den Berg and Heyns, 1973**		***Scutellonema brachyurus* (Steiner, 1938) Andrássy, 1958**	***Scutellonema truncatum* Sher, 1964**
n	18 ♀	11 ♂	20 ♀	20 ♀
L	712 ± 45.8 (654–793)	634 ± 77.2 (506–785)	643 ± 94.1 (434–799)	284 ± 60.9 (571–790)
a	24.0 ± 1.9 (19.6–26.4)	27.5 ± 2.6 (23.4–32.3)	26.2 ± 2.4 (22.7–30.7)	24.4 ± 2.3 (21.3–30.4)
b	5.2 ± 0.4 (4.5–5.9)	5.1 ± 0.4 (4.6–5.6)	4.7 ± 0.5 (3.9–5.5)	4.9 ± 0.5 (4.1–6.4)
b'	6.1 ± 0.7 (4.7–6.9)	5.9 ± 0.5 (5.4–6.3)	5.5 ± 0.7 (4.2–6.5)	5.7 ± 0.6 (4.8–6.9)
c	59.2 ± 11–7 (41.4–83.3)	41.5 ± 4.0 (33.1–48.9)	56.3 ± 7.1 (46.2–70.9)	4.9 ± 10.4 (36.6–82.0)
c'	0.7 ± 0.1 (0.5–0.9)	1.1 ± 0.1 (0.9–1.1)	0.7 ± 0.1 (0.5–0.8)	0.8 ± 0.1 (0.6–1.0)
o	16.3 ± 4.4 (8.8–21.8)	17.9 ± 5.3 (10.6–23.9)	15.5 ± 3.5 (9.5–20.4)	20.4 ± 6.4 (4.9–28.6)
m	48 ± 1.3 (45–50)	48 ± 1.7 (45–50)	48 ± 3.0 (41–53)	47 ± 1.7 (41–50)
V \ T	57 ± 0.9 (55–60)	40 ± 9.5 (26–52)	62 ± 2.4 (57–66)	60 ± 2.4 (56–66)
OV1	21 ± 2.4 (17–24)	–	22 ± 3.6 (18–30)	21 ± 4.7 (15–29)
OV2	21 ± 4.4 (17–33)	–	21 ± 3.9 (16–28)	19 ± 3.6 (13–25)
Body width at mid body	30 ± 2.8 (26–37)	23 ± 2.2 (20–27)	25 ± 3.7 (19–31)	28 ± 2.5 (23–33)
Body width at anus \ cloaca	20 ± 1.4 (18–24)	14 ± 1.1 (13–17)	18 ± 2.3 (13–21)	17 ± 1.4 (13–18)
Annulus width	2 ± 0.2 (1–2)	1 ± 0.2 (1–2)	1 ± 0.2 (1–2)	1 ± 0.2 (1–2)
Lateral field width	5 ± 0.7 (4–7)	5 ± 0.4 (4–5)	5 ± 0.8 (4–6)	6 ± 0.7 (5–7)
Labial region height	5 ± 0.5 (4–6)	5 ± 0.3 (4–5)	4 ± 0.8 (3–6)	5 ± 0.4 (4–5)
Labial region widht	9 ±0.4 (8–9)	8[a]	8 ± 1.0 (6–10)	8 ± 0.4 (7–9)
Labial framework length	2 ± 0.2 (1–2)	2 ± 0.2 (1–2)	2 ± 0.4 (1–3)	2 ± 0.3 (2–3)
Stylet length	30 ± 2.8 (27–40)	27 ± 1.5 (25–30)	26 ± 2.2 (22–30)	28 ± 1.1 (24–30)
Metenchium length	14 ± 1.5 (12–19)	13 ± 1.1 (11–15)	12 ± 1.1 (11–15)	13 ± 0.8 (10–14)
Telenchium length	16 ± 1.4 (14–21)	14 ± 0.6 (13–15)	13 ± 1.6 (11–16)	15 ± 0.5 (14–16)
Stylet knob heigth	3 ± 0.3 (2–3)	3 ± 0.4 (2–3)	3 ± 0.4 (2–3)	3 ± 0.3 (2–4)

[a] Indicates values were the same for all specimens.

– Indicates structure not present or unable to measure accurately.

Table 2.5 (continued): Morphometrics of *Scutellonema bizanae* Van den Berg and Heyns, 1973; *Scutellonema brachyurus* (Steiner, 1938) Andrássy, 1958 and *Scutellonema truncatum* Sher, 1964 populations in the Telperion Nature Reserve. All measurements in µm and in the form: mean ± standard deviation (range).

Species	***Scutellonema bizanae* Van den Berg and Heyns, 1973**		***Scutellonema brachyurus* (Steiner, 1938) Andrássy, 1958**	***Scutellonema truncatum* Sher, 1964**
n	18 ♀	11 ♂	20 ♀	20 ♀
Stylet knob width	5 ± 0.5 (5–6)	5 ± 0.5 (4–5)	5 ± 0.6 (4–6)	5 ± 0.4 (5–6)
Distance of dorsal gland opening from base of stylet knobs	5 ± 1.4 (2–6)	5 ± 1.2 (3–6)	4 ± 0.9 (3–5)	6 ± 1.8 (1–8)
Median bulb length	12 ± 1.2 (9–14)	11 ± 0.9 (10–12)	12 ± 0.6 (10–13)	12 ± 0.6 (11–13)
Median bulb width	11 ± 1.1 (10–14)	9 ± 0.5 (9–10)	11 ± 0.8 (9–13)	11 ± 0.6 (10–12)
Excretory pore from anterior end	106 ± 7.9 (91–125)	96 ± 7.8 (86–110)	108 ± 13.9 (83–132)	125 ± 10.6 (96–145)
Oesophageal gland overlap length	19 ± 7.8 (4–26)	15 ± 8.5 (7–24)	20 ± 4.4 (14–29)	22 ± 8.3 (7–37)
Oesophagus length	138 ± 9.6 (118–152)	130 ± 12.1 (116–155)	137 ± 11.4 (112–157)	141 ± 6.6 (123–153)
Anterior genital tract length	148 ± 18.3 (113–175)	–	138 ± 34.2 (101–204)	140 ± 31.4 (95–203)
Anterior to vulva	407 ± 25.7 (373–453)	–	395 ± 55.3 (285–494)	410 ± 33.2 (339–477)
Vagina length	12 ± 0.6 (11–13)	–	11 ± 1.2 (9–14)	10 ± 1.4 (9–15)
Posterior genital tract length	147 ± 29.6 (121–227)	–	133 ± 37.6 (95–201)	128 ± 29.9 (78–178)
Spermatheca length	17 ± 2.1 (14–19)	–	–	–
Spermatheca width	15 ± 2.4 (13–17)	–	–	–
Testis length	–	260 ± 64.5 (166–326)	–	–
Spicule length	–	28 ± 1.4 (26–30)	–	–
Gubernaculum length	–	13 ± 0.5 (12–14)	–	–
Diameter of scutellum	5 ± 0.5 (4–6)	4 ± 0.3 (3–4)	3 ± 0.4 (2–4)	3 ± 0.5 (2–4)
Ventral tail annuli	11 ± 2.1 (8–16)	–	11 ± 2.4 (9–15)	11 ± 1.8 (8–12)
Tail length	13 ± 2.9 (8–19)	15 ± 1.5 (13–19)	11 ± 2.8 (6–16)	14 ± 2.4 (10–19)
Anterior to excretory pore as percentage of body length	15 ± 0.9 (14–17)	14 ± 0.8 (14–16)	17 ± 1.5 (15–20)	18 ± 1.9 (14–21)
Anterior to excretory pore as percentage of oesophagus length	77 ± 7.1 (66–93)	74 ± 6.4 (65–82)	79 ± 5.7 (69–86)	89 ± 7.3 ()69–100
Posterior genital tract length as percentage of anterior genital tract length	100 ± 17.0 (82–134)	–	92 ± 6.9 (82–100)	97 ± 14.9 (61–115)

[a] Indicates values were the same for all specimens.

– Indicates structure not present or unable to measure accurately.

Subfamily Rotylenchulinae Husain and Khan, 1967

= Acontylinae Fotedar and Handoo, 1978

Genus *Rotylenchulus* Linford and Oliveira, 1940

= *Leiperotylenchus* Das, 1960

= *Spyrotylenchus (=Spirotylenchus)* Lordello and Cesnik, 1958

***Rotylenchulus parvus* (Williams, 1960) Sher, 1961**

= *Helicotylenchus parvus* Williams, 1960

Figure 2.9

Measurements: Table 2.6

Rotylenchulus parvus (Williams, 1960) Sher, 1961 was described from Mauritius from soil around the roots of *S. officinarum* (Williams, 1960). The species has since been reported from several localities in Africa, Asia, Central America, Caribbean Islands, Europe, North America and Oceania associated with various plants (CABI, 2005). According to the SAPPNS, *R. parvus* is widely distributed in South Africa with records from all nine provinces mainly from the Savanna (51 %) and Grassland (31 %) biomes, but has been recorded from all seven biomes in South Africa (SAPPNS). The present specimens were identified as *R. parvus* based on the hemispherical lip region, hyaline portion of the tail less than 6 µm, and c' value less than 3. The specimens collected at Telperion compare well with the type and other populations of the species (Dasgupta et al., 1968; Heyns, 1976a; Germani, 1978; Van den Berg, 1978b; Jatala, 1991; Robinson et al., 1997). A longer hyaline portion of the tail region was observed in the present specimens as compared to the type specimens (2–6 µm *vs* less than 3 µm). However, these measurements fit those reported in subsequent descriptions. The population from Telperion is therefore considered conspecific to *R. parvus*.

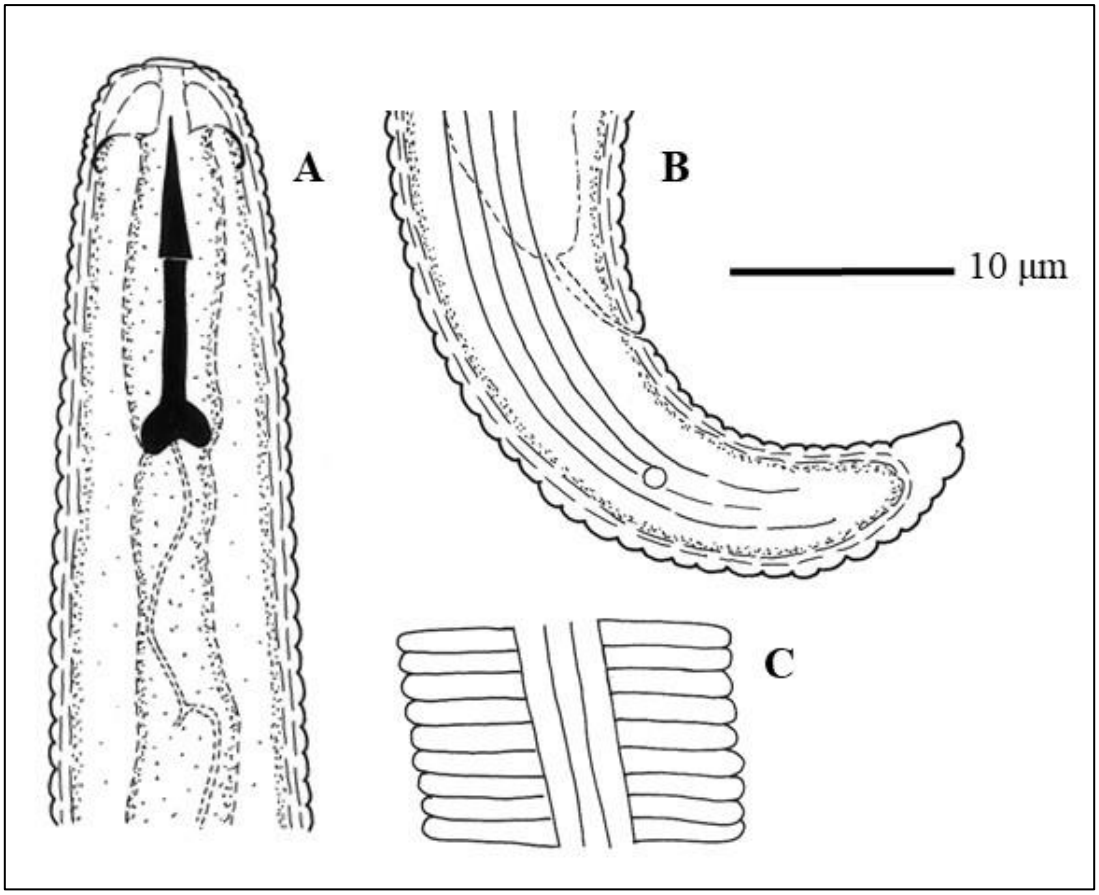


Figure 2.9: *Rotylenchulus parvus* (Williams, 1960) Sher, 1961 from the Telperion Nature Reserve. A: Anterior region; B: Posterior region; C: Lateral field at mid body.

Table 2.6: Morphometrics of *Rotylenchulus parvus* (Williams, 1960) Sher, 1961 population in the Telperion Nature Reserve. All measurements in μm and in the form: mean ± standard deviation (range).

n	20 ♀
L	338 ± 58.5 (226–421)
a	22.9 ± 0.3 (18.7–27.8)
b	2.7 ± 0.3 (2.3–3.3)
b'	3.7 ± 0.3 (3.4–4.1)
c	16.8 ± 1.4 (14.5–19.0)
c'	2.3 ± 0.3 (1.8–2.8)
o	79.6 ± 9.8 (64.0–98.6)
m	44 ± 3.5 (36–50)
V	62 ± 2.6 (59–67)
OV1	11 ± 2.3 (8–15)
OV2	11 ± 2.0 (6–14)
Body width at mid body	15 ± 1.9 (11–17)
Body width at anus	9 ± 1.0 (7–10)
Annulus width	1.1 ± 0.2 (0.7–1.4)
Lateral field width	3 ± 0.5 (2–4)
Labial region height	3 ± 0.4 (2–3)
Labial region width	7 ± 0.5 (6–8)
Stylet length	14 ± 1.4 (9–15)
Metenchium length	6 ± 0.9 (4–7)
Telenchium length	8 ± 0.7 (5–8)
Stylet knob height	2 ± 0.2 (1–2)
Stylet knob width	3 ± 0.3 (2–4)
Distance of dorsal gland opening from base of stylet knobs	11 ± 1.4 (9–13)
Median bulb length	11 ± 0.8 (9–12)
Median bulb width	8 ± 0.7 (6–9)
Median bulb valve length	4 ± 0.5 (3–4)
Median bulb valve width	3 ± 0.5 (2–3)
Excretory pore from anterior end	76 ± 9.5 (55–98)
Oesophageal gland overlap length	32 ± 6.5 (25–44)
Oesophagus length	123 ± 15.7 (91–150)
Anterior genital tract length	36 ± 5.8 (29–48)
Anterior to vulva	207 ± 30.6 (147–250)
Posterior genital tract length	35 ± 6.0 (21–44)
Ventral tail annuli	15 ± 1.4 (13–17)
Length of hyaline portion of tail	4 ± 1.1 (2–6)
Tail length	20 ± 3.9 (13–27)
Anterior to excretory pore as percentage of body length	23 ± 2.2 (20–27)
Anterior to excretory pore as percentage of oesophagus length	62 ± 6.1 (53–79)
Posterior genital tract length as percentage of anterior genital tract length	99 ± 24.1 (45–140)

2.4.3 Family Criconematidae (Taylor, 1936) Thorne, 1949

= Ogmidae Southern, 1914

= Macroposthoniidae Skarbilovich, 1959

= Madinematidae Khan, Chawla and Saha, 1976

Subfamily Criconematinae Taylor, 1936

= Ogminae (Southern, 1914) Filipjev and Schuurmans Stekhoven, 1941

Genus *Criconema* Hofmänner and Menzel, 1914

= *Lobocriconema* De Grisse and Loof, 1965

= *Nothocriconema* De Grisse and Loof, 1965

= *Mesocriconema* Raski and Pinochet, 1976

= *Nenocriconema* Darekar and Khan, 1981

= *Notholetus* Ebsary, 1981

= *Nothocriconemella* Ebsary, 1981

= *Paracriconema* Ebsary, 1981

= *Amphisbaenema* Orton Williams, 1982

= *Cerchnotocriconema* Bernard, 1982

Criconema corbetti (De Grisse, 1967) Raski and Luc, 1985

= *Nothocriconema corbetti* De Grisse, 1967

= *Criconemoides corbetti* (De Grisse, 1967) Luc, 1970

= *Notholetus corbetti* (De Grisse, 1967) Ebsary, 1981

Figure 2.10

Measurements: Table 2.7

Criconema corbetti (De Grisse, 1967) Raski and Luc, 1985 was described from Malawi from soil around the roots of *Fimbristylis* Vahl. (De Grisse, 1967). This species has since been recorded from eight provinces within South Africa (Northern Cape only province not recorded) (Van den Berg, 1984;

1992; SAPPNS). This species has mainly been recorded from the Grassland (61 %) and Savanna (32 %) biomes however, a few records are also from the Forest, Fynbos and Thicket biomes (SAPPNS). The current specimens were identified as *C. corbetti* based on one annulus in labial region, set off from body contour and distinctly wider than first body annuli, labial plate with pseudolips protruding beyond lip annulus, number of body annuli, and tail annuli with lobes varying in number. The specimens from Telperion correspond well with the type description, as well as additional descriptions (De Grisse, 1967; Van den Berg, 1984, 1992).

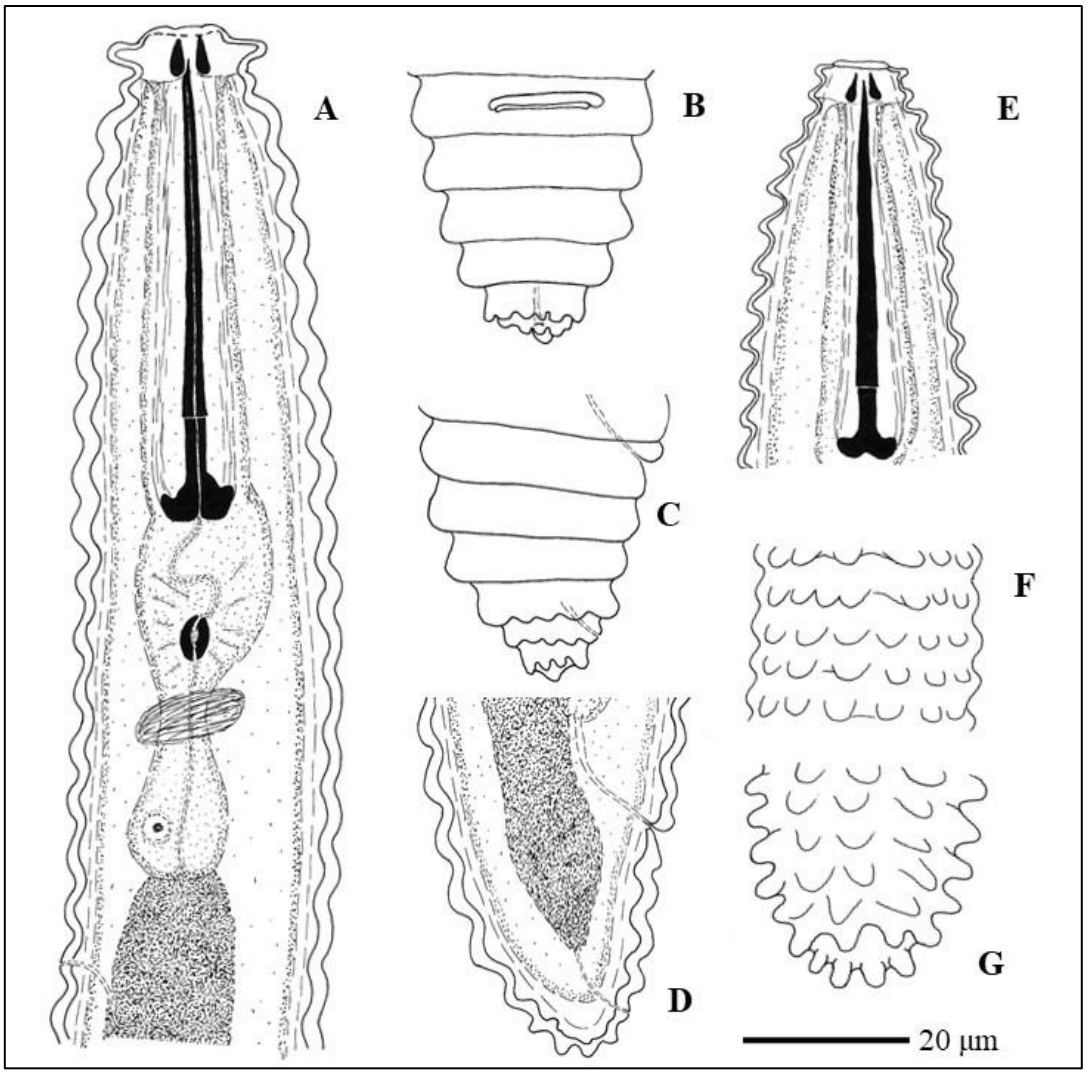


Figure 2.10: A–C: *Criconema corbetti* (De Grisse, 1967) Raski and Luc, 1985 from the Telperion Nature Reserve. A: Anterior region of female; B–C: Tail region of female; E: Anterior region of juvenile; F: Cuticular morphology of juvenile; G: Tail region juvenile.

***Criconema sirgeli* Van den Berg and Meyer, 1987**

= *Notholetus sirgeli* (Van den Berg and Meyer, 1987) Siddiqi, 2000

Figure 2.11 A–C

Measurements: Table 2.7

Criconema sirgeli Van den Berg and Meyer, 1987 was described from fynbos vegetation in Landdroskloof, Western Cape Province (Van den Berg and Meyer, 1987). Since then the species has only been recorded five times from the Eastern Cape, KwaZulu–Natal and Limpopo provinces in the Forest, Fynbos, Grassland and Thicket biomes (SAPPNS). This is the first report of the species form the Mpumalanga Province, from natural vegetation close to a waterhole in the Telperion Nature Reserve. The current specimen was identified as *C. sirgeli* based on the labial region with one annulus, wider than the first body annulus, tail long with sharply pointed terminus. The current species corresponds well with the description by Van den Berg and Meyer (1987) and Van den Berg et al. (2000).

***Criconema zeae* (Van den Berg and Heyns, 1977) Raski and Luc, 1985**

= *Lobocriconema zeae* Van den Berg and Heyns, 1977

= *Notholetus zeae* (Van den Berg and Heyns, 1977) Ebsary, 1981

Figure 2.11 D–F

Measurements: Table 2.7

Criconema zeae (Van den Berg and Heyns, 1977) Raski and Luc, 1985 was described from soil around the roots of *Zea mays* L. in the North West Province in the Grassland Biome (Van den Berg and Heyns, 1977). The species has since been recorded from a second locality in the North West Province (Schweizer Reneke in the Savanna Biome) from *Z. mays* as well as from the Limpopo Province

(Magoebaskloof) from natural veld in the Grassland Biome (SAPPNS). This is the first report of the species from Mpumalanga in the Telperion Nature reserve. The current specimens were identified as *C. zeae* based on the lip region with one annulus, wider than the first body annulus and the appearance of the tail annuli bearing eight rounded scales. The specimens from Telperion corresponds well to the type and only description of the species by Van den Berg and Heyns (1977) except for the following differences: less body annulus (R = 47 and 52 *vs* 53–59).

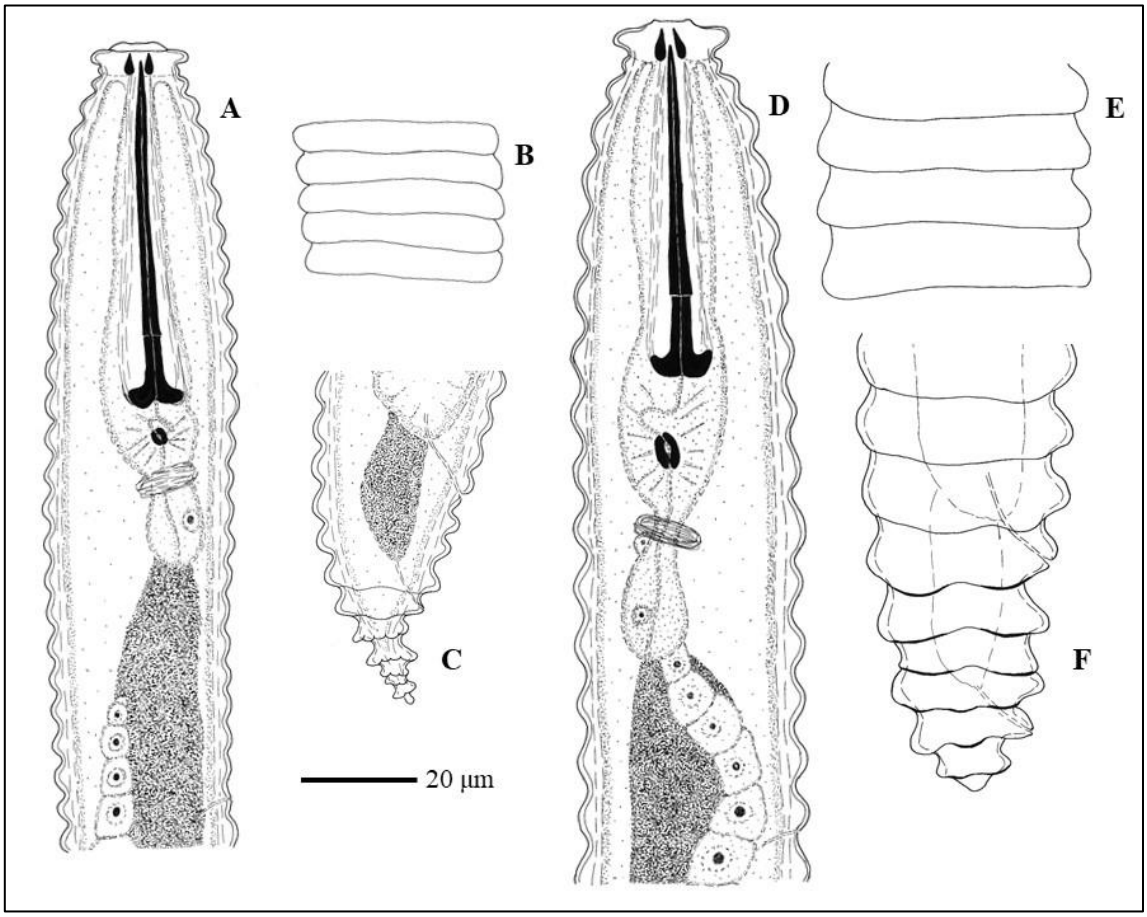


Figure 2.11: A–C: *Criconema sirgeli* Van den Berg and Meyer, 1987; and D–F: *Criconema zeae* (Van den Berg and Heyns, 1977) Raski and Luc, 1985 from the Telperion Nature Reserve. A+D: Anterior region; B+E: Cuticular morphology at mid body; C+F: Tail region.

Table 2.7: Morphometrics of *Criconema corbetti* (De Grisse, 1967) Raski and Luc, 1985; *Criconema sirgeli* Van den Berg and Meyer, 1987 and *Criconema zeae* (Van den Berg and Heyns, 1977) Raski and Luc, 1985 populations in the Telperion Nature Reserve. All measurements in µm and in the form: mean ± standard deviation (range).

Species	***Criconema corbetti*** **(De Grisse, 1967) Raski and Luc, 1985**	***Criconema sirgelli*** **Van den Berg and Meyer, 1987**	***Criconema zeae*** **(Van den Berg and Heyns, 1977) Raski and Luc, 1985**	
n	20 ♀	1 ♀	2 ♀	
L	360 ± 43.9 (271–428)	437	502	506
a	10.4 ± 1.0 (8.6–12.1)	13.0	9.9	11.4
b	3.8 ± 0.3 (3.3–4.3)	–	3.3	4.1
c	78.2 ± 33.9 (15.6–157.8)	22.5	38.6	–
c'	0.4 ± 0.2 (0.3–1.0)	1.4	0.4	–
o	4.1[a]	–	–	–
V	93 ± 0.7 (92–95)	91	93	92
OV1	58 ± 13.6 (29–83)	67	–	–
VL/VB	0.9 ± 0.1 (0.6–1.1)	1.6	1.0	1.2
Body width at mid body	35 ± 3.2 (30–43)	34	51	44
Body width at vulva	26 ± 2.0 (23–30)	25	37	34
Body width at anus	13 ± 3.7 (9–24)	14	31	–
Annule width	6 ± 0.7 (5–7)	6	7	9
R	63 ± 3.8 (56–70)	84	47	52
RSt	10 ± 0.9 (9–13)	13	8	8
ROes	17 ± 1.3 (14–19)	–	13	14
RExp	19 ± 1.1 (17–20)	–	15	–
RV (ant)	58 ± 3.6 (51–64)	9	44	48
RV	5 ± 0.8 (4–7)	–	5	4
RVan	3 ± 1.0 (1–6)	5	3	–
RAn	2 ± 1.0 (1–5)	4	2	–

[a] Only measured in one specimen.

– Indicates structure not present or unable to measure accurately.

Table 2.7 (continued): Morphometrics of *Criconema corbetti* (De Grisse, 1967) Raski and Luc, 1985; *Criconema sirgeli* Van den Berg and Meyer, 1987 and *Criconema zeae* (Van den Berg and Heyns, 1977) Raski and Luc, 1985 populations in the Telperion Nature Reserve. All measurements in µm and in the form: mean ± standard deviation (range).

Species	***Criconema corbetti*** **(De Grisse, 1967) Raski and Luc, 1985**	***Criconema sirgelli*** **Van den Berg and Meyer, 1987**	***Criconema zeae*** **(Van den Berg and Heyns, 1977) Raski and Luc, 1985**	
n	20 ♀	1 ♀	2 ♀	
Width of 1st annulus	18 ± 1.8 (13–21)	7	20	21
Width of 2nd annulus	15 ± 1.5 (13–18)	16	15	17
Width of 3rd annulus	20 ± 2.7 (15–24)	12	24	23
Width of 4th annulus	24 ± 1.9 (20–27)	16	–	–
Stylet length	52 ± 2.7 (47–59)	55	60	57
Metenchium length	39 ± 2.0 (37–43)	45	46	43
Telenchium length	12 ± 1.2 (10–16)	11	14	13
Stylet knobs height	4 ± 0.4 (3–5)	4	3	5
Stylet knobs width	8 ± 0.5 (8–10)	7	11	11
Dorsal gland opening from base of stylet knobs	2[a]	–	–	–
Oesophagus length	95 ± 7.6 (81–108)	–	154	123
Excretory pore from anterior end	109 ± 9.3 (93–123)	–	136	–
Length of anterior genital tract	210 ± 54.6 (124–313)	291	–	–
Vulva from anterior end	336 ± 41.1 (253–403)	398	465	466
Vagina length	14 ± 1.4 (12–15)	–	–	–
Post vulval body length	24 ± 4.1 (17–31)	39	37	40
Tail length	6 ± 4.7 (2–23)	19	13	–
Stylet length as percentage of body length	14.5 ± 1.6 (12.6–17.8)	12.6	12.0	11.2

[a] Only measured in one specimen.

– Indicates structure not present or unable to measure accurately.

Subfamily Macroposthoniinae Skarbilovich, 1959

= Madinematinae Khan, Chawla and Saha, 1976

= Criconemellinae Khan and Saeed, 1985

Genus *Criconemoides* Taylor, 1936

= *Criconemella* De Grisse and Loof, 1965
= *Macroposthonia* De Man, 1880
= *Mesocriconema* Andrássy, 1965
= *Xenocriconemella* De Grisse and Loof, 1965
= *Madinema* Khan, Chawla and Saha, 1976
= *Seshadriella* Darekar and Khan, 1981
= *Neobakernema* Ebsary, 1981
= *Crossonemoides* Eroshenko, 1981
= *Neocriconema* Diab and Jenkins, 1965
= *Pakriconemoides* Shahina and Maqbool, 1993

***Criconemoides parvus* Raski, 1952**

= *Criconemella parva* (Raski, 1952) De Grisse and Loof, 1965
= *Neocriconema adamsi* Diab and Jenkins, 1965
= *Criconemoides adamsi* (Diab and Jenkins, 1965) Tarjan, 1966
= *Criconemella adamsi* (Diab and Jenkins, 1965) Siddiqi, 1986
= *Criconemoides microserratus* Raski and Golden, 1966
= *Criconemella microserrata* (Raski and Golden, 1966) Siddiqi, 2000

Figure 2.12

Measurements: Table 2.8

Criconemoides parvus Raski, 1952 was described from soil around the roots of a species of *Artemisia* L. (Raski, 1952). This polyphagous species has a cosmopolitan distribution (Geraert, 2010) and has been recorded from all nine provinces in South Africa from all seven biomes especially the

Grassland (51 %) and Savanna (28 %) biomes (SAPPNS). The current specimens were identified as *C. parvus* based on the small size, short stylet, number of body annules, anastomoses along the lateral line and bluntly rounded tail. The specimens from Telperion fit the type description from United States of America, as well as populations reported by Raski and Golden (1966); Diab and Jenkins (1965); Heyns (1970a); Van den Berg (1980); Rashid et al. (1986); Loof and Barooti (1991); Eyualem–Abebe and Geraert (1995); Loof et al. (1997); Popovici and Ciabano (2000).

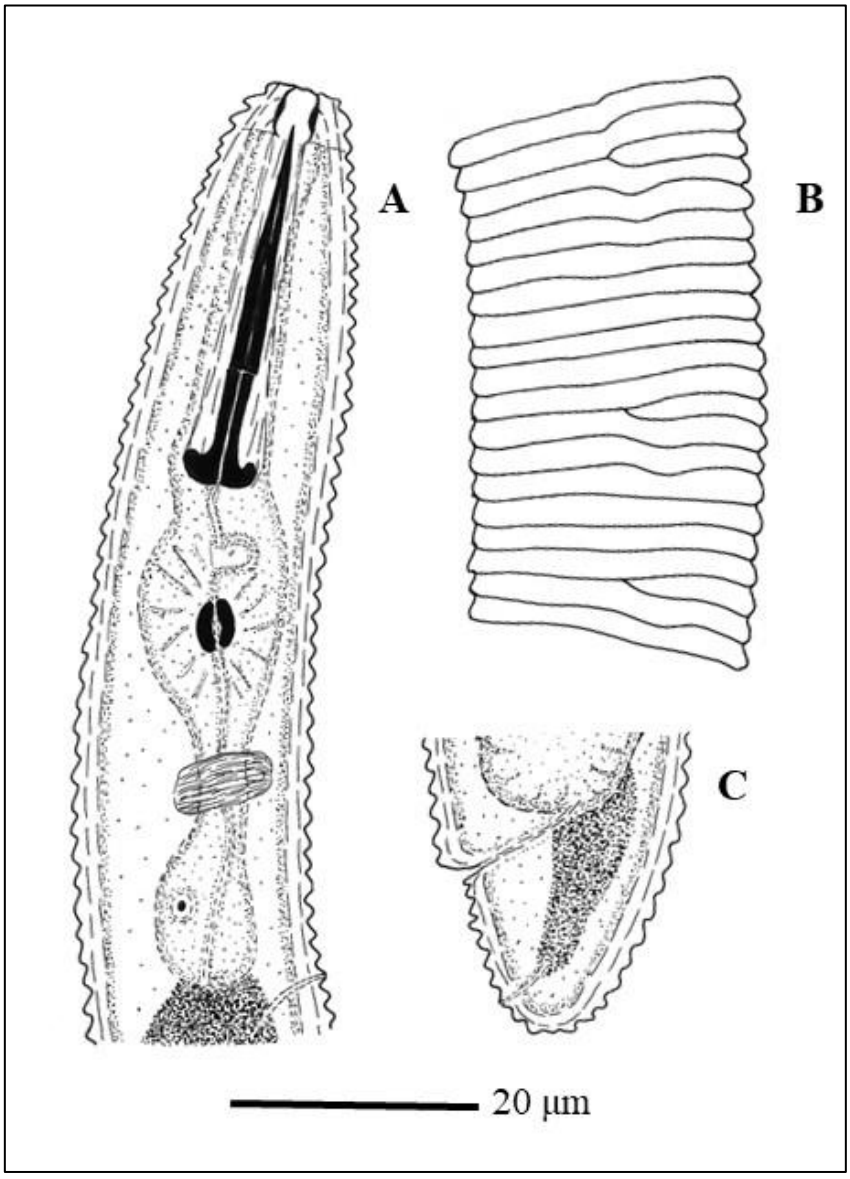


Figure 2.12: A–C: *Criconemoides parvus* Raski, 1952 from the Telperion Nature Reserve. A: Anterior region; B: Cuticular morphology at mid body; C: Tail region.

***Criconemoides sphaerocephalus* (Taylor, 1936) Loof and De Grisse, 1989**

= *Macroposthonia sphaerocephala* (Taylor, 1936) De Grisse and Loof, 1965

= *Criconemella sphaerocephala* (Taylor, 1936) Luc and Raski, 1981

= *Mesocriconema sphaerocephalum* (Taylor, 1936) Loof and De Grisse, 1989

= *Criconemoides citri* Steiner, 1949

= *Macroposthonia citri* (Steiner, 1949) Siddiqi, 1986

= *Criconemoides georgii* Prasad, Khan and Mathur, 1966

= *Macroposthonia georgii* (Prasad, Khan and Mathur, 1966) Siddiqi, 1986

Figure 2.13 A–C

Measurements: Table 2.8

Criconemoides sphaerocephalus (Taylor, 1936) Loof and De Grisse, 1989 was described from soil around the roots of grass in Trinidad (Taylor, 1936). The species has since been reported from Belgium, Cameroon, Democratic Republic of Congo, Eastern Sudan, Hawaii, India, Iran, Italy, Japan, Mauritius, the Netherlands, South Africa, Spain, and Venezuela from various plants (Geraert, 2010). In South Africa, *C. sphaerocephalus* has been recorded from all nine provinces and is one of the most frequently recorded species of the genus, especially in the Savanna (63 %) and Grassland (28 %) biomes (SAPPNS). The current specimens of *Criconemoides* were identified as *C. sphaerocephalus* because of the large and few body annules, robust stylet, lateral field marked only by anastomoses forming a zigzag line, and broadly rounded tail. The specimens from Telperion fit the description by Taylor (1936), as well as other descriptions of the species (Williams, 1960; Siddiqi, 1961; Loof, 1964; Coomans, 1966; Raski and Golden, 1966; Heyns, 1970b; Luc, 1970; Szczygiel, 1974; Van den Berg, 1980; Toida and Momota, 1981; Chaves, 1983; Deswal and Bajaj, 1987; Lopez–Chaves and Salazar–Figueroa, 1987; Gómez Barcina et al., 1989; Zeidan and Geraert, 1989; Gómez Barcina et al., 1991; Renubala et al., 1991; Sakwe and Geraert, 1993; Crozzoli et al., 1998; Crozzoli and Lamberti, 2001).

***Criconemoides xenoplax* Raski, 1952**

= *Macroposthonia xenoplax* (Raski, 1952) De Grisse and Loof, 1965

= *Criconemella xenoplax* (Raski, 1952) Luc and Raski, 1981

= *Mesocriconema xenoplax* (Raski, 1952) Loof and De Grisse, 1989

Figure 2.13 D–F

Measurements: Table 2.8

Criconemoides xenoplax Raski, 1952 was described from soil around the roots of *Vitis vinifera* L. in California, United States of America (Raski, 1952). This species is a cosmopolitan species and has been associated with various plant hosts especially *Vitis* L. (Geraert, 2010). In South Africa *C. xenoplax* has been recorded in all nine provinces from all seven biomes especially the Savanna (46 %) and Fynbos (24 %) biomes with only 6 % of the records from the Grassland Biome (SAPPNS). The current specimen was identified as *C. xenoplax* based on retrose cuticular annuli, two annuli in labial region, smaller and narrower than body annuli, first labial annulus directed laterally and anteriorly indented, lateral field not marked, very few anastomoses present, tail terminus with a single lobe. The female specimen from Telperion corresponds well with the types by Raski (1952), as well as specimens described by Siddiqi (1961); Thorne and Malek (1968); Heyns (1970c); Williams (1972); Szczygiel (1974); Khan et al. (1976); Van den Berg (1980); Toida and Momota (1981); Bernard (1982); Maqbool (1982); Loof and Barooti (1991); Sakwe and Geraert (1993); Loof et al. (1997); Brzeski (1998).

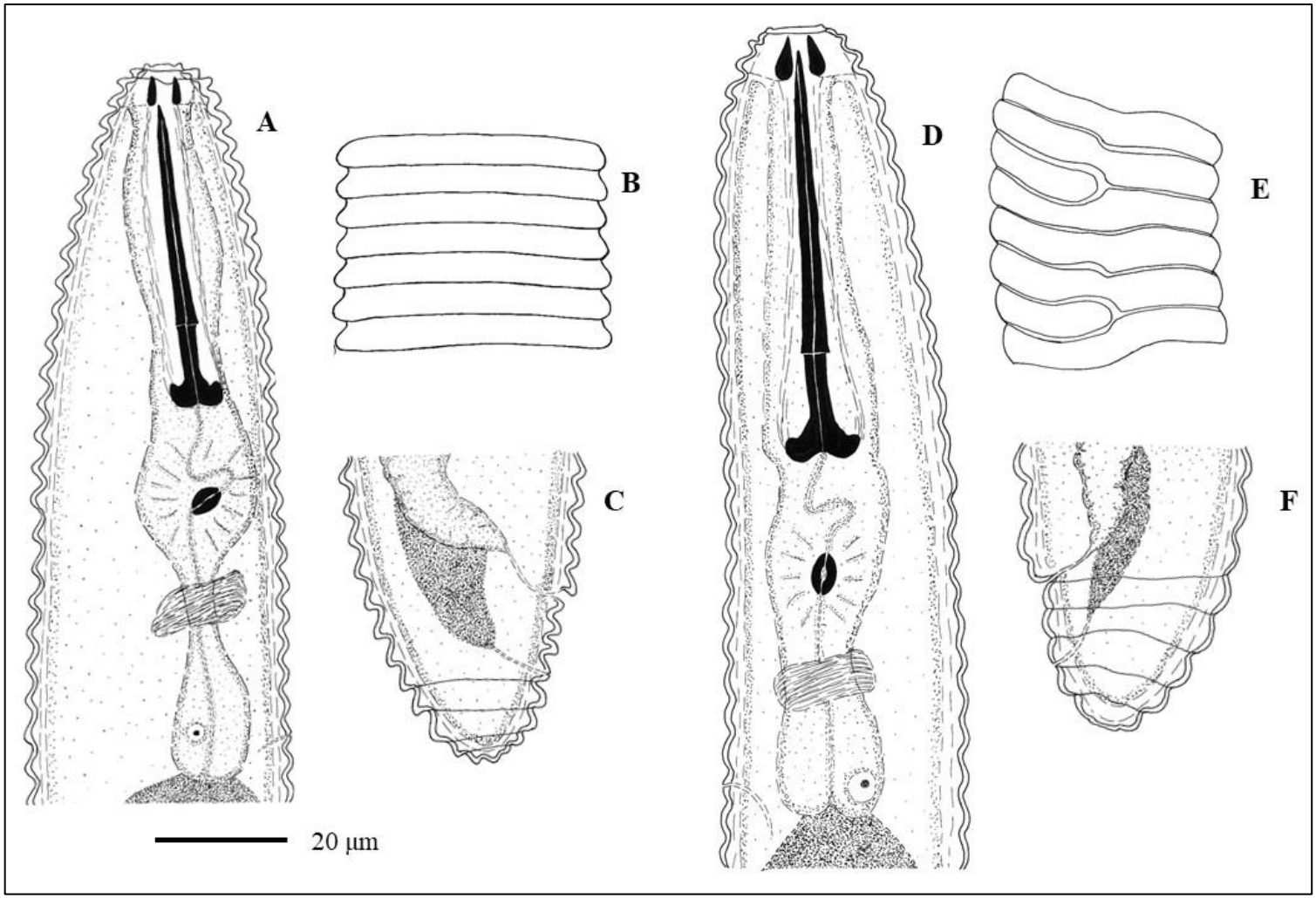


Figure 2.13: A–C: *Criconemoides xenoplax* Raski, 1952; and D–F: *Criconemoides sphaerocephalus* (Taylor, 1936) Loof and De Grisse, 1989 from the Telperion Nature Reserve. A+D: Anterior region; B+E: Cuticular morphology at mid body; C+F: Tail region.

Table 2.8: Morphometrics of *Criconemoides parvus* Raski, 1952*; Criconemoides sphaerocephalus* (Taylor, 1936) Loof and De Grisse, 1989; and *Criconemoides xenoplax* Raski, 1952 populations in the Telperion Nature Reserve. All measurements in µm and in the form: mean ± standard deviation (range).

Species	***Criconemoides parvus* Raski, 1952**	***Criconemoides sphaerocephalus* (Taylor, 1936) Loof and De Grisse, 1989**	***Criconemoides. xenoplax* Raski, 1952**
n	20 ♀	10 ♀	1 ♀
L	273 ± 30.2 (223–323)	335 ± 34.5 (282–369)	384
a	11.8 ± 1.4 (9.1–14.1)	9.1 ± 0.9 (7.7–10.8)	9.3
b	3.5 ± 0.3 (2.8–4.1)	3.1 ± 0.3 (2.6–3.5)	3.4
c	25.1 ± 4.2 (20.1–29.5)	51.9 ± 20.2 (29.1–88.6)	32.3
c'	0.7 ± 0.1 (0.6–0.8)	0.4 ± 0.1 (0.2–0.6)	0.5
o	10.6 ± 1.7 (8.3–12.1)	10.7 ± 3.4 (8.3–13.1)	–
V	92 ± 2.5 (89–96)	95 ± 1.6 (92–96)	93
OV1	58 ± 8.5 (42–74)	54 ± 15.5 (37–76)	69
VL/VB	1.3 ± 0.4 (0.7–1.9)	0.7 ± 0.2 (0.6–1.0)	0.8
Body width at mid body	23 ± 3.0 (17–30)	37 ± 2.7 (33–41)	41
Body width at vulva	17 ± 1.8 (14–20)	25 ± 2.4 (21–28)	32
Body width at anus	11 ± 4.2 (5–19)	18 ± 2.1 (16–22)	23
Annule width	2 ± 0.3 (1–3)	5 ± 0.8 (4–6)	4
R	139 ± 7.3 (122–154)	67 ± 2.8 (63–71)	86
RSt	21 ± 1.9 (18–26)	13 ± 2.7 (10–20)	13
ROes	44 ± 4.2 (31–50)	22 ± 2.7 (17–25)	26
RExp	43 ± 3.3 (36–49)	21 ± 2.1 (18–25)	24
RV(ant)	126 ± 12.4 (83–142)	62 ± 3.5 (57–67)	78
RV	10 ± 0.8 (8–11)	5 ± 1.0 (3–6)	8
RVan	1 ± 0.5 (1–2)	2 ± 0.7 (1–3)	3
RAn	8 ± 0.8 (7–9)	3 ± 0.7 (2–4)	4
Width of 1st annulus	7 ± 0.5 (6–7)	14 ± 2.0 (10–16)	15

– Indicates structure not present or unable to measure accurately.

Table 2.8 (continued): Morphometrics of *Criconemoides parvus* Raski, 1952*; Criconemoides sphaerocephalus* (Taylor, 1936) Loof and De Grisse, 1989; and *Criconemoides xenoplax* Raski, 1952 populations in the Telperion Nature Reserve. All measurements in µm and in the form: mean ± standard deviation (range).

Species	***Criconemoides parvus* Raski, 1952**	***Criconemoides sphaerocephalus* (Taylor, 1936) Loof and De Grisse, 1989**	***Criconemoides. xenoplax* Raski, 1952**
n	20 ♀	10 ♀	1 ♀
Width of 2nd annulus	8 ± 0.8 (6–10)	17 ± 2.1 (14–21)	17
Width of 3rd annulus	10 ± 0.8 (8–11)	21 ± 2.2 (18–24)	20
Width of 4th annulus	11 ± 0.9 (9–13)	23 ± 1.8 (21–26)	23
Stylet length	31 ± 2.0 (27–34)	56 ± 6.8 (44–64)	48
Metenchium length	21 ± 1.8 (18–24)	42 ± 6.0 (31–47)	35
Telenchium length	10 ± 0.9 (9–12)	15 ± 1.6 (13–17)	13
Stylet knobs height	3 ± 0.6 (2–5)	5 ± 0.8 (3–6)	4
Stylet knobs width	6 ± 0.5 (5–7)	10 ± 1.0 (8–11)	8
Dorsal gland opening from base of stylet knobs	3 ± 0.5 (3–4)	7 ± 1.9 (5–8)	–
Oesophagus length	78 ± 4.5 (67–87)	107 ± 10.4 (87–118)	113
Excretory pore from anterior end	75 ± 6.6 (59–84)	104 ± 11.1 (90–117)	104
Length of anterior genital tract	160 ± 33.5 (107–211)	175 ± 53.1 (103–251)	266
Vulva from anterior end	250 ± 29.1 (198–310)	317 ± 34.0 (271–354)	357
Vagina length	9 ± 2.2 (6–13)	13 ± 2.8 (11–18)	14
Post vulval body length	22 ± 7.3 (12–31)	18 ± 4.9 (11–25)	27
Tail length	11 ± 1.4 (9–13)	7 ± 2.4 (4–10)	12
Stylet length as percentage of body length	11.3 ± 1.4 (8.8–14.7)	16.9 ± 1.8 (14.7–20.6)	12.5

– Indicates structure not present or unable to measure accurately.

Subfamily Hemicriconemoidinae Andrássy, 1979

Genus *Hemicriconemoides* Chitwood and Birchfield, 1957

= *Iota* Cobb, 1913

***Hemicriconemoides brachyurus* (Loos, 1949) Chitwood and Birchfield, 1957**

= *Criconemoides brachyurus* Loos, 1949
= *Hemicycliophora brachyurus* (Loos, 1949) Goodey, 1963
= *Hemicycliophora saccharіae* Heyns, 1970
= *Hemicriconemoides intermedius* Dasgupta, Raski and Van Gundy, 1969
= *Hemicriconemoides promissus* Vovlas, 1980

Figure 2.14

Measurements: Table 2.9

Hemicriconemoides brachyurus (Loos, 1949) Chitwood and Birchfield, 1957 was described from Sri Lanka in a *C. sinensis* nursery soil and has since been reported from France, India, Indonesia, Italy, Japan, Korea, Mauritius, Namibia, South Africa, Taiwan, United States of America, Vietnam and Zambia (Geraert, 2010; SAPPNS). In South Africa *H. brachyurus* has been recorded from all nine provinces, most frequently in the Savanna (51 %) and Grassland (29 %) biomes (SAPPNS). The current specimens were identified as *H. brachyurus* based on the following: labial region bearing two rounded annuli, second labial annulus larger than the first, no lateral differentiation, lateral vulval flap present, and a conical tail with a rounded lobe at its terminus. The specimens found in Telperion fit the type and other descriptions of the species (Loos, 1949a; Gotoh, 1965; Dasgupta et al., 1969; Heyns, 1970c; Van den Berg and Heyns, 1977; Rashid et al., 1989).

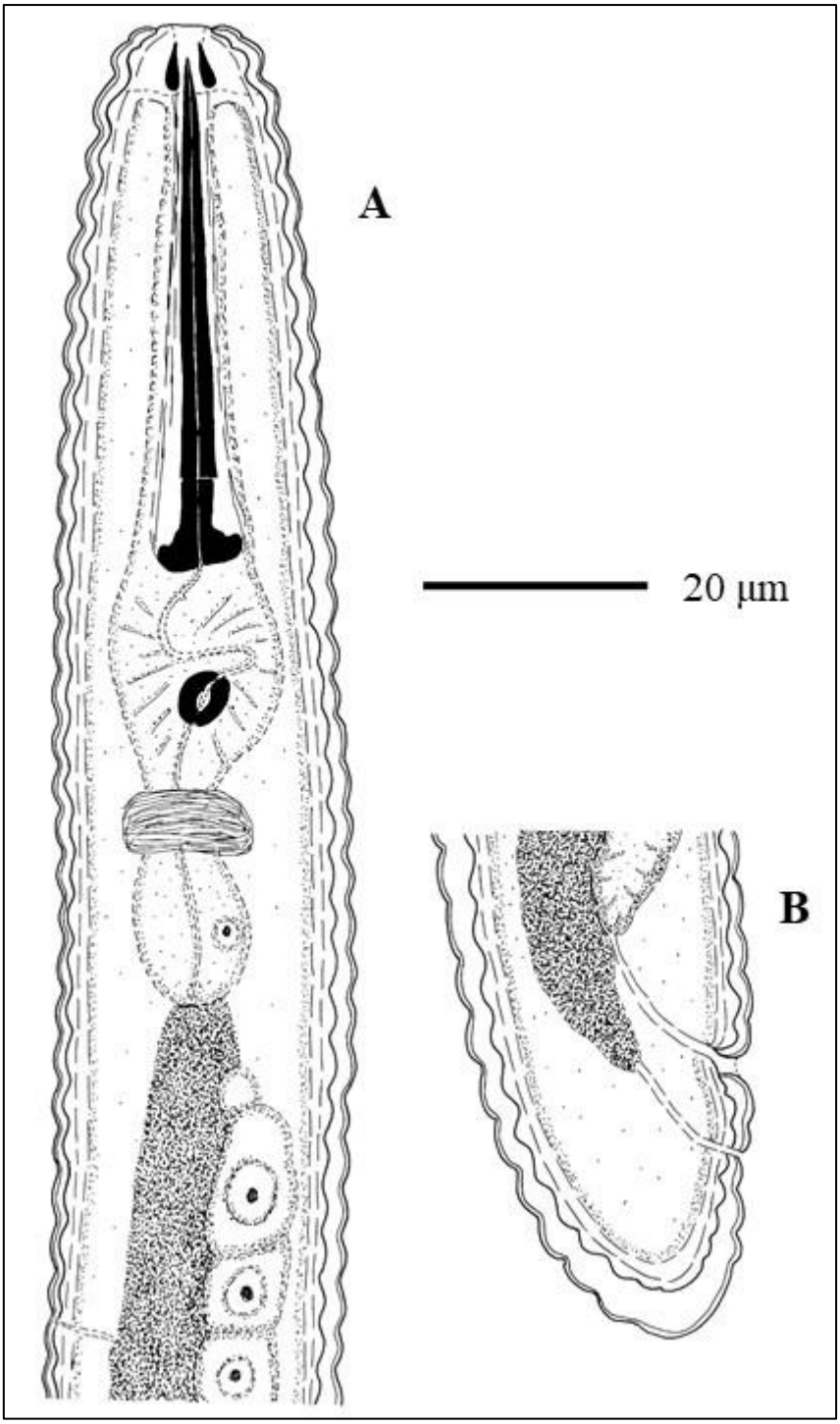


Figure 2.14: *Hemicriconemoides brachyurus* (Loos, 1949) Chitwood and Birchfield, 1957 from the Telperion Nature Reserve. A: Anterior region; B: Tail region.

Table 2.9: Morphometrics of *Hemicriconemoides brachyurus* (Loos, 1949) Chitwood and Birchfield, 1957 species populations in the Telperion Nature Reserve. All measurements in µm and in the form: mean ± standard deviation (range).

n	18 ♀
L	378 ± 28.4 (334–435)
a	13.7 ± 1.3 (11.7–16.6)
b	4.3 ± 0.3 (3.6–4.9)
c	28.2 ± 6.6 (18.2–42.9)
c'	0.8 ± 0.1 (0.6–1.0)
o	7.1 ± 3.4 (4.3–14.7)
V	93 ± 2.8 (83–96)
OV1	60 ± 11.9 (43–76)
VL/VB	1.1 ± 0.7 (0.5–3.7)
Body width at mid body	28 ± 2.4 (21–32)
Body width at vulva	21 ± 1.7 (17–24)
Body width at anus	18 ± 2.1 (15–22)
Annule width	4 ± 0.5 (3–5)
R	99 ± 3.9 ()91–105
RSt	12 ± 1.1 (10–14)
ROes	21 ± 1.7 (18–24)
RExp	28 ± 2.3 (25–31)
RV(ant)	93 ± 4.1 (86–100)
RV	6 ± 1.0 (4–8)
RVan	2 ± 0.9 (1–4)
RAn	4 ± 1.3 (3–7)
Width of 1st annulus	10 ± 2.3 (7–14)
Width of 2nd annulus	14 ± 2.0 (11–17)
Width of 3rd annulus	17 ± 1.4 (14–20)
Width of 4th annulus	19 ± 1.4 (16–21)
Stylet length	46 ± 2.4 (41–50)
Metenchium length	37 ± 2.4 (32–42)
Telenchium length	9 ± 0.6 (8–10)
Stylet knobs height	3 ± 0.4 (2–4)
Stylet knobs width	7 ± 0.4 (7–8)
Dorsal gland opening from base of stylet knobs	3 ± 1.3 (2–6)
Oesophagus length	88 ± 5.6 (80–100)
Excretory pore from anterior end	115 ± 11.4 (89–131)
Length of anterior genital tract	228 ± 50.3 (146–299)
Vulva from anterior end	353 ± 23.0 (314–397)
Vagina length	13 ± 1.7 (9–15)
Post vulval body length	26 ± 14.7 (17–75)
Tail length	14 ± 3.5 (9–22)
Stylet length as percentage of body length	12.2 ± 0.7 (11.2–13.7)

Subfamily Hemicycliophorinae Skarbilovich, 1959

Genus *Hemicycliophora* De Man, 1921

= *Procriconema* (Micoletzky, 1925) Loos, 1948
= *Colbranium* Andrássy, 1979
= *Aulosphora* (Siddiqi, 1980) Loof, 1985
= *Loofia* (Siddiqi, 1980) Loof, 1985

***Hemicycliophora typica* De Man, 1921**

= *Hemicycliophora sculpturata* Loof, 1984
= *Hemicycliophora karachiensis* Maqbool, Shahina and Zarina, 1986
= *Aulophora karachiensis* Maqbool, Shahina and Zarina, 1986
= *Procriconema membranifer* Micoletzky, 1925
= *Hemicycliophora membranifer* (Micoletzky, 1925) Loos, 1948
= *Hemicycliophora nyanzae* Schoemaker, 1967
= *Hemicycliophora transvaalensis* Heyns, 1962
= *Hemicycliophora thienemanni* apud Rashid, Geraert and Sharma, 1986

Figure 2.15

Measurements: Table 2.10

Hemicycliophora typica De Man, 1921 is the type species of the genus and was described from a compost heap in the Netherlands (De Man, 1921). The species has since been reported form several countries from aquatic and terrestrial habitats (Chitambar and Subbotin, 2013). In South Africa, *H. typica* is one of the most commonly recorded species of the genus and has been recorded in all nine provinces and in all the biomes except in the Succulent Karoo Biome. This species is especially common in the Savanna (45 %) and Grassland (41 %) biomes (SAPPNS). Specimens from Telperion were identified as *H. typica* based on the longitudinal lines outside the lateral field forming distinct blocks with transverse striae, labial region with two annuli, labial disc slightly elevated, vulval lips slightly elongated, tail cylindrical, tapering gradually to a distal conoid terminus. The current specimens

correspond well with descriptions of various populations (Taylor, 1936; Tarjan, 1952; Thorne, 1955; Williams, 1960; Heyns, 1962b; Schoemaker, 1967; Loof, 1968; Loof and Heyns, 1969; Brzeski, 1974; Eroshenko, 1976; Van den Berg, 1981; Loof, 1984; Costa Manso, 1996; Brzeski, 1998; Costa Manso, 1998).

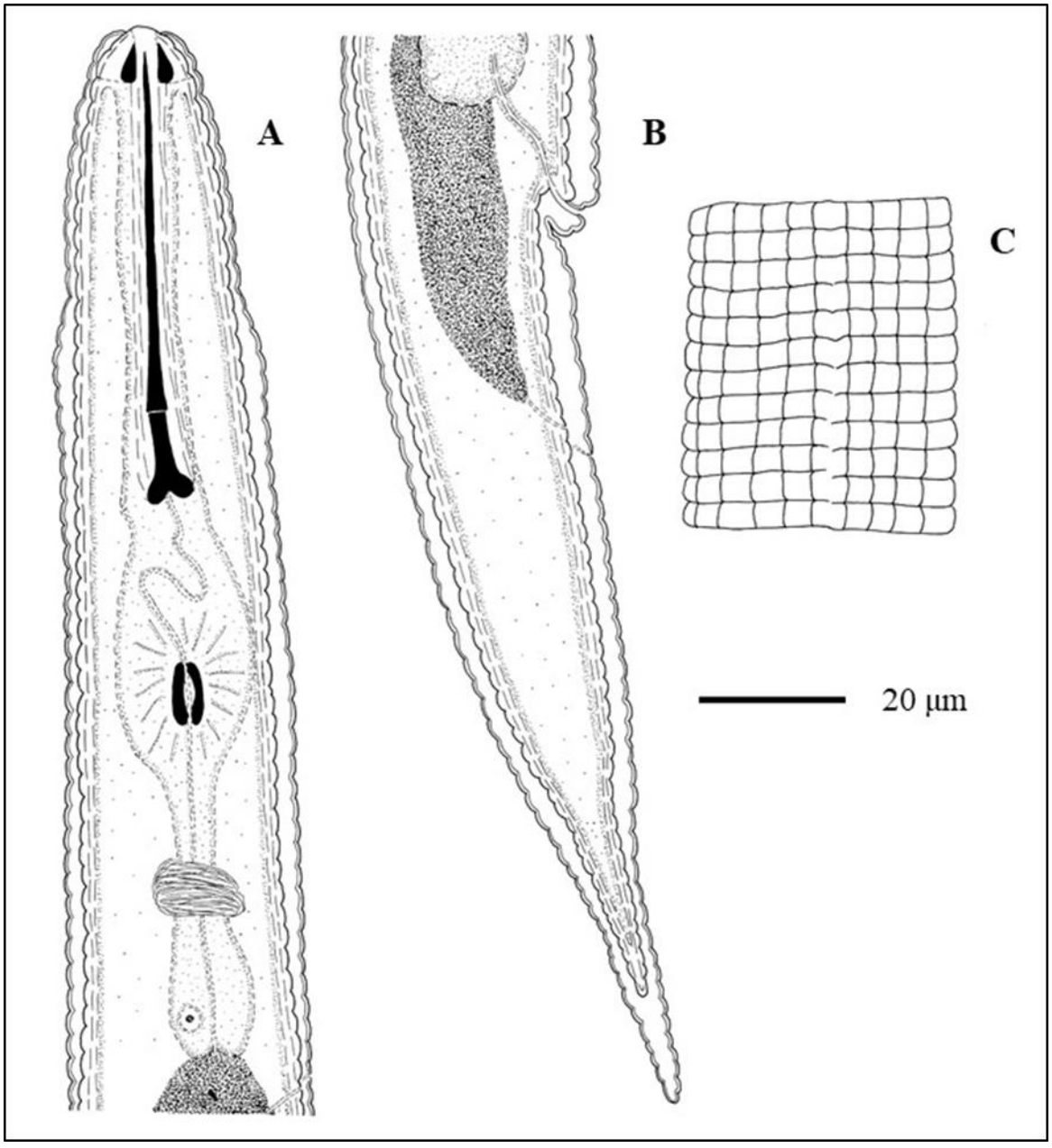


Figure 2.15: *Hemicycliophora typica* De Man, 1921 from the Telperion Nature Reserve. A: Anterior region; B: Tail region; C: Lateral field at mid body.

***Hemicycliophora demani* Edward and Rai, 1971**

= *Hemicycliophora nullinca* Van den Berg, 1987

= *Hemicycliophora mangiferae* Misra and Edward, 1971

= *Hemicycliophora quercea* Mehta and Raski, 1984

Figure 2.16

Measurements: Table 2.10

Hemicycliophora demani Edward and Rai, 1971 was described from soil from a field previously sown to *Cucurbita pepo* L. (Edward and Rai, 1971). The species has since been described from India, Honduras and South Africa (Chitambar and Subbotin, 2013). In South Africa, *H. demani* has been recorded in the Eastern Cape, Free State, Gauteng and Western Cape provinces in the Forest (14 %), Fynbos (58 %), Grassland (21 %) and Nama Karoo (7 %) biomes (SAPPNS). This is the first report of the species from the Mpumalanga Province. The current specimens were identified as *H. demani* based on the lateral field marked by two longitudinal lines and intersected by transverse striation to form blocks, labial region with three annuli, and a short conoid tail with a narrowly rounded terminus. The specimens from Telperion correspond well with the type, as well as other descriptions of the species (Edward and Rai, 1971; Misra and Edward, 1974; Ray and Das, 1981; Mehta and Raski, 1984; Van den Berg, 1987; Van den Berg et al., 2003).

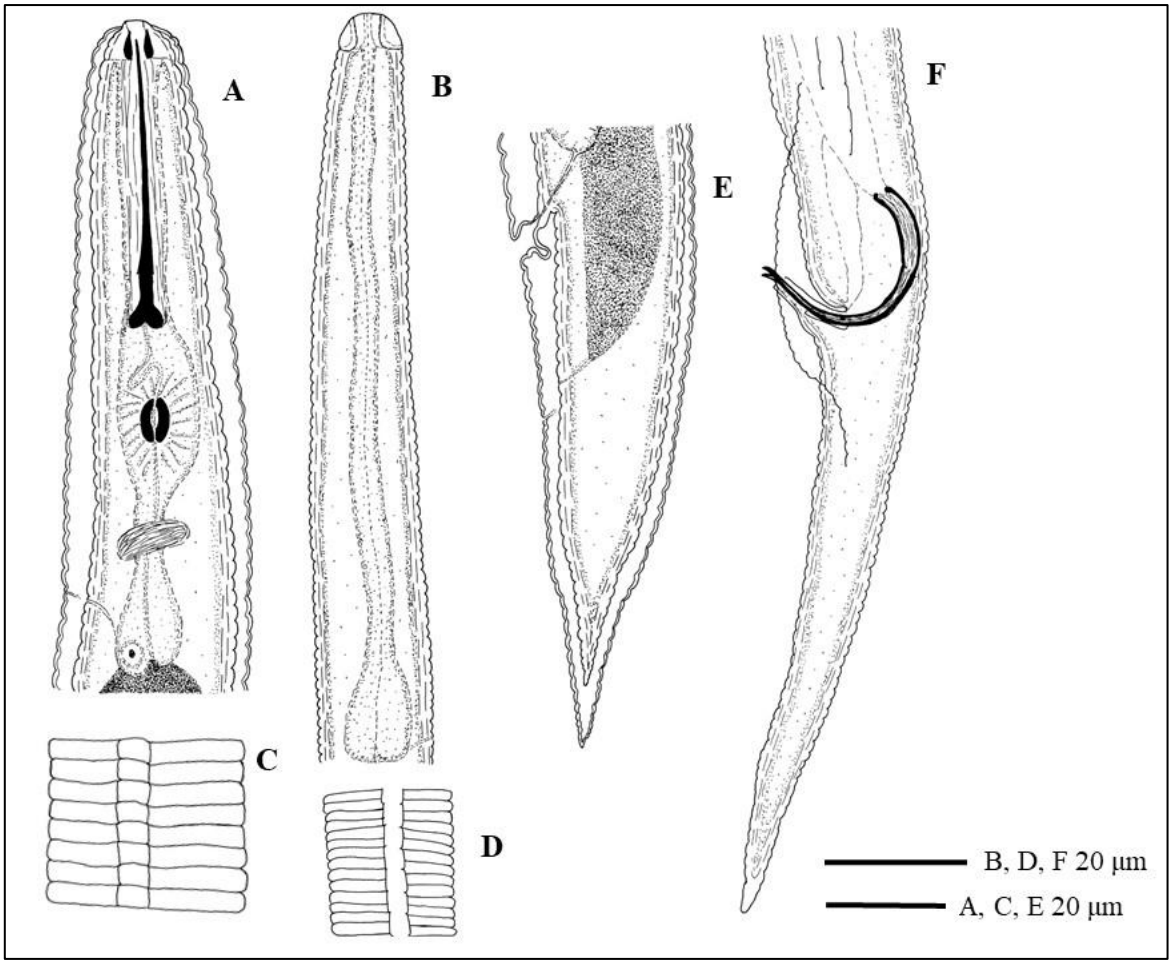


Figure 2.16: *Hemicycliophora demani* Edward and Rai, 1971 from the Telperion Nature Reserve. A: Female anterior region; B: Male anterior region; C: Female lateral field at mid body; D: Male lateral field at mid body; E: Female Tail; F: Male tail.

***Hemicycliophora poranga* Monteiro and Lordello, 1978**

= *Hemicycliophora ripa* Van den Berg, 1981

Figure 2.17

Measurements: Table 2.10

Hemicycliophora poranga Monteiro and Lordello, 1978 was described from Brazil in the rhizosphere of *Brassica oleracea* var. *capitata* L. and has since been reported from Argentina, Brazil,

Iran, Italy, Spain, South Africa, United States of America and Venezuela, (Chitambar and Subbotin, 2013). In South Africa, this species has been recorded from all provinces excluding the Free State Province and most frequently in the Grassland (45 %) and Savanna (40 %) biomes, but has also been recorded from the Forest, Fynbos and Nama Karoo biomes (SAPPNS). The current specimens were identified as *H. poranga* based on a loosely fitting cuticle marked by longitudinal scratches, lateral field marked by few anastomoses, labial region with two annuli, elevated labial disc and a tail tapering gradually to a finely rounded terminus. The current specimens fit into the type description, as well as other description of the species (Monteiro and Lordello, 1978; Van den Berg, 1981; Doucet, 1982; Chaves, 1983; Chitambar, 1994; Costa Manso, 1996; Crozzoli and Lamberti, 2006, Van den Berg et al., 2018).

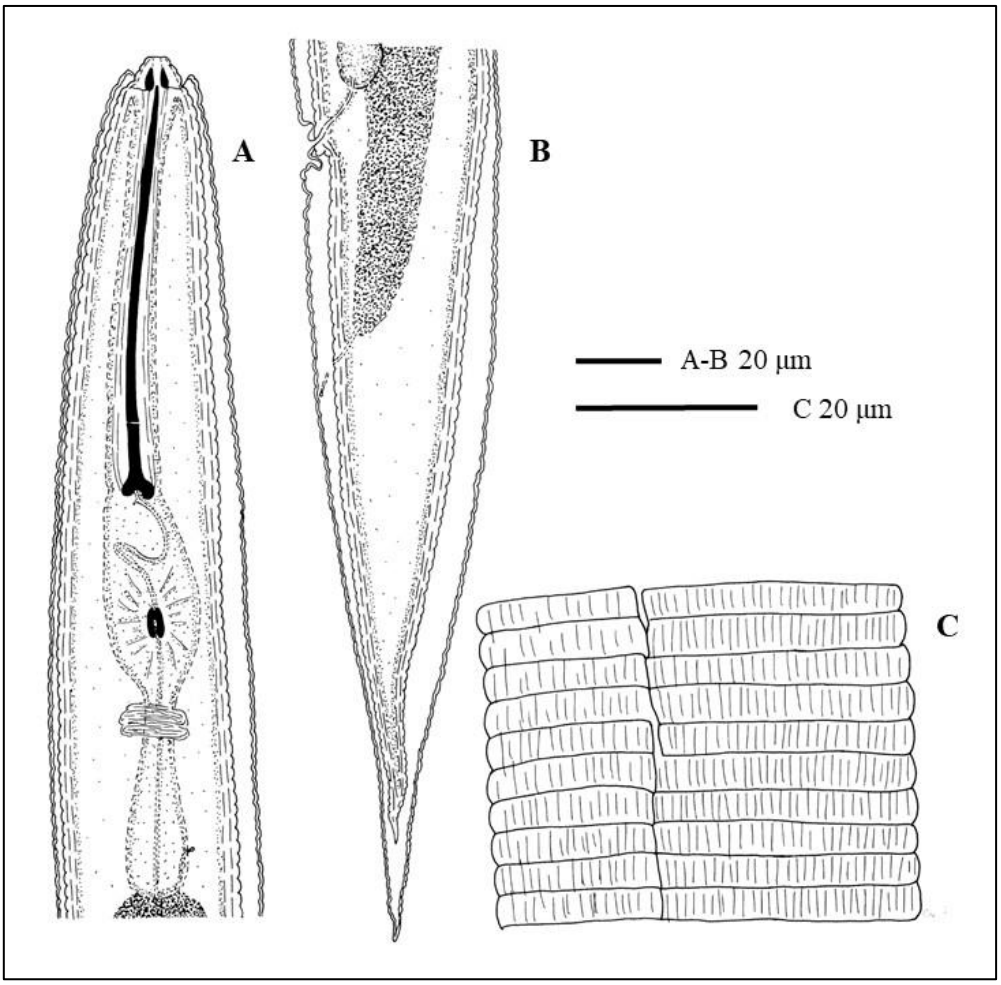


Figure 2.17: *Hemicycliophora poranga* Monteiro and Lordello, 1978 from the Telperion Nature Reserve. A: Anterior region; B: Tail; C: Lateral field at mid body.

Table 2.10: Morphometrics of *Hemicycliophora demani* Edward and Rai, 1971; *Hemicycliophora poranga* Monteiro and Lordello, 1978 and *Hemicycliophora typica* De Man, 1921 populations in the Telperion Nature Reserve. All measurements in µm and in the form: mean ± standard deviation (range).

Species	***Hemicycliophora typica* De Man, 1921**	***Hemicycliophora demani* Edward and Rai, 1971**		***Hemicycliophora poranga* Monteiro and Lordello, 1978**
n	20 ♀	7 ♀	4 ♂	1 ♀
L	741 ± 117.6 (526–873)	557 ± 40.0 (484–588)	539 ± 39.5 (512–598)	988
a	21.6 ± 1.8 (19.0–24.8)	17.3 ± 1.4 (15.5–19.5)	28.9 ± 1.1 (28.1–30.2)	21.1
b	5.4 ± 0.5 (4.6–6.3)	4.7 ± 0.3 (4.4–5.1)	5.0[a]	5.4
c	8.8 ± 0.8 (7.2–11.2)	8.4 ± 0.7 (7.5–9.2)	6.3 ± 0.6 (5.7–6.8)	8.1
c'	3.3 ± 0.3 (3.0–4.0)	2.6 ± 0.3 (2.3–3.3)	5.7 ± 0.3 (5.5–6.1)	–
o	6.8 ± 3.0 (2.9–10.5)	–	–	3.9
V / T	83 ± 1.3 (81–87)	83 ± 0.9 (81–84)	28 ± 9.5 (22–39)	82
OV1	46 ± 7.8 (38–66)	45 ± 11.5 (24–58)	–	37
VL/VB	3.9 ± 0.4 (3.3–4.9)	3.3 ± 0.4 (2.8–3.8)	–	4.3
PV/ABW	1.7 ± 1.0 (1.0–5.3)	1.2 ± 0.3 (0.8–1.6)	–	1.5
T/AWB	3.3 ± 0.3 (2.8–4.0)	2.6 ± 0.3 (2.3–3.3)	5.7 ± 0.3 (5.5–6.1)	3.2
Body width at mid body	34 ± 5.4 (25–42)	32 ± 3.9 (25–36)	19 ± 1.9 (17–21)	47
Body width at vulva	32 ± 4.3 (26–29)	30 ± 3.6 (23–34)	–	42
Body width at anus / cloaca	25 ± 3.5 (19–31)	26 ± 2.6 (24–31)	15 ± 0.8 (14–16)	38
Annule width	4 ± 1.0 (3–4)	4 ± 0.3 (3–4)	2[b]	4
R	206 ± 27.6 (150–242)	178 ± 8.4 (168–189)	337[b]	301
RSt	18 ± 2.6 (13–23)	18 ± 2.7 (15–23)	–	28
ROes	40 ± 4.8 (34–50)	39 ± 3.4 (35–45)	–	58
RExp	41 ± 4.0 (34–47)	36 ± 4.7 (28–43)	65[a]	56
RV(ant)	169 ± 21.3 (137–198)	139 ± 8.5 (126–148)	–	65
RV	40 ± 5.2 (31–53)	39 ± 2.9 (34–42)	48[a]	65
RVan	12 ± 3.4 (8–25)	12 ± 1.2 (10–13)	–	17

[a] Only measured in one specimen. [b] Indicates values were the same for all specimens. – Indicates structure not present or unable to measure accurately.

Table 2.10 (continued): Morphometrics of *Hemicycliophora demani* Edward and Rai, 1971; *Hemicycliophora poranga* Monteiro and Lordello, 1978 and *Hemicycliophora typica* De Man, 1921 populations in the Telperion Nature Reserve. All measurements in µm and in the form: mean ± standard deviation (range).

Species	***Hemicycliophora typica* De Man, 1921**	***Hemicycliophora demani* Edward and Rai, 1971**		***Hemicycliophora poranga* Monteiro and Lordello, 1978**
n	20 ♀	7 ♀	4 ♂	1 ♀
RAn	28 ± 4.6 (20–37)	27 ± 1.8 (24–29)	48[a]	48
Lip region height	8 ± 1.0 (6–9)	6 ± 0.6 (6–7)	5 ± 0.8 (4–6)	6
Lip region width	17 ± 2.1 (14–23)	17 ± 0.9 (169–18)	9 ± 0.6 (9–10)	18
Stylet length	61 ± 4.7 (53–70)	54 ± 2.1 (51–57)	–	94
Metenchium length	48 ± 3.9 (39–53)	43 ± 2.1 (41–16)	–	79
Telenchium length	13 ± 1.9 (11–18)	11 ± 0.9 (10–12)	–	15
Stylet knobs height	4 ± 0.6 (3–5)	3 ± 0.9 (2–4)	–	4
Stylet knobs width	6 ± 0.5 (5–7)	5 ± 0.2 (4–5)	–	6
Dorsal gland opening from base of stylet knobs	4 ± 2.0 (2–7)	–	–	4
Excretory pore from anterior end	136 ± 17.4 (111–163)	115 ± 8.4 (103–126)	109 ± 3.1 (107–111)	176
Oesophagus length	136 ± 13.8 (111–153)	118 ± 6.3 (109–129)	105[b]	182
Length of anterior genital tract	343 ± 91.3 (203–553)	246 ± 54.8 (138–288)	–	370
Vulva from anterior end	619 ± 104.1 (434–737)	461 ± 33.2 (401–484)	–	809
Vagina length	20 ± 3.2 (16–26)	15 ± 1.8 (13–17)	–	19
Distance from vulva to anus	38 ± 8.9 (28–68)	29 ± 5.0 (23–38)	–	57
Post vulval body length	122 ± 15.9 (92–144)	96 ± 8.9 (83–110)	–	179
Spicule length	–	–	40 ± 3.4 (35–43)	–
Gubernaculum length	–	–	6[b]	–
Testis length	–	–	151 ± 46.7 (115–204)	–
Tail length	85 ± 14.9 (62–111)	66 ± 8.1 (56–79)	86 ± 5.9 (77–90)	122
Stylet length as percentage of body length	8.3 ± 0.8 (7.3–9.6)	9.7 ± 0.7 (9.0–10.9)	–	9.5
Distance from vulva to anus as percentage of tail length	46.2 ± 14.3 (21.1–87.7)	44.7 ± 9.7 (32.9–59.5)	–	46.8

[a] Only measured in one specimen. [b] Indicates values were the same for all specimens. – Indicates structure not present or unable to measure accurately.

2.4.4 Family Trichodoridae (Thorne, 1935) Clark, 1961

Genus *Nanidorus* Siddiqi, 1974

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

= *Trichodorus minor* Colbran, 1956
= *Paratrichodorus minor* (Colbran, 1956) Siddiqi, 1974
= *Paratrichodorus (Nanidorus) minor* (Colbran, 1956) Siddiqi, 1974
= *Nanidorus minor* (Colbran, 1956) Siddiqi, 1974
= *Trichodorus christiei* Allen, 1957
= *Paratrichodorus (Nanidorus) christiei* (Allen, 1957) Siddiqi, 1974
= *Nanidorus christiei* (Allen, 1957) Siddiqi, 1974
= *Trichodorus obesus* Razjivin and Penton, 1975
= *Paratrichodorus (Nanidorus) obesus* (Razjivin and Penton, 1975) Rodriguez–M and Bell, 1978

Figure 2.18

Measurements: Table 2.11

Nanidorus minor (Colbran, 1956) Siddiqi, 1974 was described from an unknown host from Queensland, Australia (Colbran, 1956). This species is cosmopolitan and polyphagous (CABI, 2002). In South Africa, *N. minor* is the most commonly recorded trichodorid species with records from all nine provinces most frequently from the Savanna (43 %) and Grassland (36 %) biomes but has been recorded in all seven biomes of the country (SAPPNS). The current specimens were identified as *N. minor* because of the absence of lateral body– or caudal pores, presence of an inner onchium, vulva a transverse slit, vagina quadrangular / barrel shaped, less than 50 % of corresponding body width. No males were observed in the Telperion population. The incidence of *N. minor* males is extremely rare with specimens reported from only Mexico, Namibia, Nicaragua, South Africa and the United States of America (Marais and Botha, 1997). The Telperion specimens fit the type description from Australia as

well as populations reported by Allen (1957); Hooper (1962); Siddiqi (1962); Bird and Mai (1967); Mamiya (1967); Hooper (1972); Heyns (1975); Vermeulen and Heyns (1983); Decraemer (1991); Decraemer (1995); Chau (1997); and Crozzoli et al. (1998).

Genus Paratrichodorus Siddiqi, 1974

= *Paratrichodorus* Siddiqi, 1974

= *Atlantodorus* Siddiqi, 1974

Paratrichodorus allius (Jensen, 1963) Siddiqi, 1974

= *Trichodorus allius* Jensen, 1963

= *Paratrichodorus (Paratrichodorus) allius* (Jensen, 1963) Siddiqi, 1974

= *Paratrichodorus tansaniensis* Siddiqi, 1974

Figure 2.18

Measurements: Table 2.11

Paratrichodorus allius (Jensen, 1963) Siddiqi, 1974 was described from soil around the roots of *Allium cepa* L. in Oregon, United States of America (Jensen, 1963). The species has consequently been reported from Chile, Madeira Archipelaga, Portugal, Italy, Israel and Tanzania (Decraemer, 1995). In South Africa, this species has been recorded only on four occasions from the Grassland Biome (Free State and KwaZulu–Natal provinces) and the Savanna Biome (Gauteng and Mpumalanga provinces) associated with *Musa* L., *Salix babylonica* L., an unidentified shrub in a garden and mountain veld (SAPPNS). As in the case of *N. minor, P. allius* is a vector of the Tobacco Rattle Virus (Mojtahedi et al., 2003). The current population was identified as *P. allius* based on the oval vaginal sclerotizations situated more than 2 µm apart, wide, oval shaped vagina, vulva a longitudinal slit and the absence of lateral body pores. The current specimens fit the type description as well as other descriptions of the species (Siddiqi, 1974a; Vermeulen and Heyns, 1983; Sturhan, 1989; De Waele and Cohn, 1992; Hui and Zhixin, 1996b).

***Paratrichodorus porosus* (Allen, 1957) Siddiqi, 1974**

= *Trichodorus porosus* Allen, 1957

= *Paratrichodorus (Atlantadorus) porosus* (Allen, 1957) Siddiqi, 1974

= *Atlantadorus porosus* (Allen, 1957) Siddiqi, 1974

= *Trichodorus bucrius* Lordello and Zamith, 1958

Figure 2.18

Measurements: Table 2.11

Paratrichodorus porosus (Allen, 1957) Siddiqi, 1974 was described from soil around the roots of *Musa* in California, United States of America (Allen, 1957) and was reported from Australia, Brazil, Chile, China, Hawaii, India, Japan, Korea, Madeira, New Zealand, Papua New Guinea, South Africa, United States of America and Vietnam (CABI, 2009; Chau, 1997; Decraemer, 1995; Knight, 2001). In South Africa, *P. porosus* occurs in all nine provinces. Althought this species has been recorded from all seven biomes it is more frequently recorded from the Savanna Biome (47 %) (SAPPNS). The current specimen from Telperion was identified as *P. porosus* based on presence of pre– and post–advulvar pores and pore–like vulva. The current specimens correspond well to the type population and subsequent reports (Siddiqi, 1962; Vermeulen and Heyns, 1983; Decraemer, 1991; Decraemer, 1995; Wang et al., 1996; Chau, 1997; Zheng et al., 2004).

Table 2.11: Morphometrics of *Nanidorus minor* (Colbran, 1956) Siddiqi, 1974; *Paratrichodorus allius* (Jensen, 1963) Siddiqi, 1974; and *Paratrichodorus porosus* (Allen, 1957) Siddiqi, 1974 populations in the Telperion Nature Reserve. All measurements in µm and in the form: mean ± standard deviation (range).

Species	**Nanidorus minor (Colbran, 1956) Siddiqi, 1974**	***Paratrichodorus allius* Jensen 1963) Siddiqi, 1974**	***Paratrichodorus porosus* (Allen, 1957) Siddiqi, 1974**
n	15 ♀	9 ♀	1 ♀
L	613 ± 41.2 (534–676)	523 ± 36.1 (472–583)	696
a	21.9 ± 3.5 (17.2–29.5)	18.0 ± 1.5 (15.7–20.3)	17.2
b	5.3 ± 0.5 (4.7–6.4)	3.9 ± 0.5 (3.3–4.7)	4.7
c	151.6 ± 28.4 (65.4–187.3)	219.0 ± 61.5 (150.6–319.1)	269.7
c'	0.4 ± 0.1 (0.3–0.9)	0.3 ± 0.1 (0.2–0.4)	0.3
V	55 ± 2.3 (47–58)	54 ± 1.7 (52–58)	54
OV1	17 ± 3.8 (9–23)	19± 2.9 (15–24)	23
OV2	21 ± 4.6 (15–31)	19 ± 3.1 (13–23)	16
Cuticle width at cardia	2 ± 0.3 (2–3)	2[a]	2
Cuticle width at mid body	3 ± 0.4 (2–3)	2 ± 0.4 (2–3)	2
Body width at cardia	27 ± 3.7 (22–34)	27 ± 2.5 (24–32)	34
Body width at mid body	29 ± 4.7 (20–37)	29 ± 3.6 (25–35)	40
Body width at anus	10 ± 1.5 (8–14)	9 ± 1.1 (8–10)	8
Body width at vulva	29 ± 4.7 (20–37)	29 ± 3.9 (25–37)	40
Onchiostyle length	33 ± 2.0 (29–35)	40 ± 1.0 (38–40)	51
Inner onchium length	9 ± 1.2 (7–11)	–	–
Guide ring from anterior end	13 ± 1.7 (10–16)	18 ± 0.8 (16–18)	23
Nerve ring from anterior end	52 ± 3.8 (47–59)	59 ± 4.0 (56–61)	–
Length of oesophageal gland overlap	–	19 ± 5.0 (12–27)	–
Oesophageal bulb length	38 ± 4.3 (32–45)	54 ± 7.3 (40–65)	61
Oesophageal bulb width	15 ± 2.6 (10–20)	16 ± 1.9 (13–18)	22

[a] Indicates values were the same for all specimens.

– Indicates structure not present or unable to measure accurately.

Table 2.11 (continued): Morphometrics of *Nanidorus minor* (Colbran, 1956) Siddiqi, 1974; *Paratrichodorus allius* (Jensen, 1963) Siddiqi, 1974; and *Paratrichodorus porosus* (Allen, 1957) Siddiqi, 1974*;* populations in the Telperion Nature Reserve. All measurements in µm and in the form: mean ± standard deviation (range).

Species	**Nanidorus minor (Colbran, 1956) Siddiqi, 1974**	***Paratrichodorus allius* Jensen 1963) Siddiqi, 1974**	***Paratrichodorus porosus* (Allen, 1957) Siddiqi, 1974**
n	15 ♀	9 ♀	1 ♀
Secretory–excretory pore from anterior end	108 ± 8.3 (91–116)	70 ± 9.7 (61–81)	95
Oesophagus length	117 ± 8.0 (104–134)	136 ± 9.8 (124–152)	147
Anterior to vulva	279 ± 26.0 (279–370)	282 ± 20.5 (246–307)	377
Anterior genital tract length	105 ± 27.8 (52–145)	98 ± 18.3 (73–120)	163
Posterior genital tract length	131 ± 30.4 (99–195)	97 ± 20.2 (67–127)	114
Vagina length	10 ± 1.2 (9–12)	9 ± 1.0 (8–10)	–
Vaginal pieces length	2 ± 0.4 (1–2)	1[a]	–
Distance between vaginal pieces	1 ± 0.4 (1–2)	2[a]	–
First pre–vulvar body pore from vulva	–	–	11
Second pre–vulvar body pore from vulva	–	–	20
First post–vulvar body pore form vulva	–	–	6
Second post–vulvar body pore form vulva	–	–	16
Tail length	–	3 ± 0.7 (2–4)	3
Vagina length as percentage of corresponding body width	36 ± 5.2 (28–43)	29± 1.5 (27–30)	–
Anterior to secretory–excretory pore as percentage of oesophagus length	97 ± 7.8 (85–107)	55 ± 9.9 (43–65)	64
Onchiostyle length as percentage of oesophagus length	28 ± 2.5 (24–32)	31 ± 2.5 (27–32)	35

[a] Indicates values were the same for all specimens.

– Indicates structure not present or unable to measure accurately.

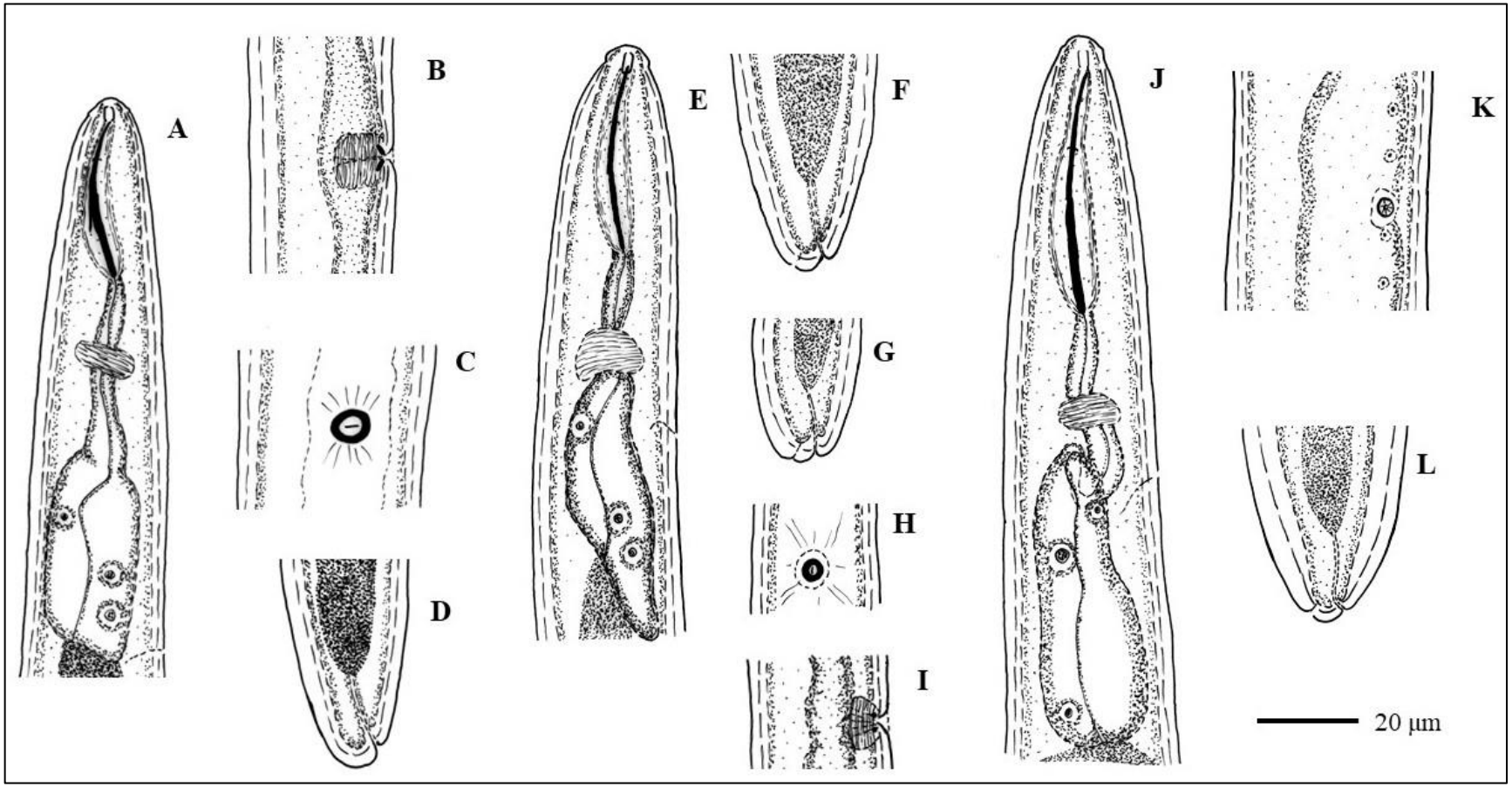


Figure 2.18: A–D: *Nanidorus minor* (Colbran, 1956) Siddiqi, 1974; E–I: *Paratrichodorus allius* (Jensen, 1963) Siddiqi, 1974 and J–L: *Paratrichodorus porosus* (Allen, 1957) Siddiqi, 1974; and from the Telperion Nature Reserve. A, E, J: female anterior region; D,F,G, L: female tail; C, H, K: ventral view of vulval region; and B, I: lateral view of vulval region.

2.4.5 Family Longidoridae Thorne, 1935

Subfamily Longidorinae Thorne, 1935

Genus *Longidorus* Micoletzky, 1922

= *Brevinema* Stegarescu, 1980

= *Neolongidorus* Khan, 1987

Longidorus moniloides Heyns, 1966

Figure 2.19 A + C

Measurements: Table 2.12

Longidorus moniloides Heyns, 1966 was described from several localities in KwaZulu–Natal from virgin veld with grass and *Vachellia* Wight and Arn. (Heyns, 1966). According to the SAPPNS database this species has since been recorded only from the Western Cape Province associated with *Prunus persica* (L.) Batsch in the Fynbos Biome and KwaZulu–Natal associated with *S. officinarum* and unidentified grasses in the Savanna Biome. This is the first report of this species for Mpumalanga as well as the first report from the Grassland Biome. The current specimen from Telperion was identified as *L. moniloides* based on the slightly truncate, expanded lip region with a large depression towards the oral aperture, convex–conoid and bluntly rounded tail and the combination of morphometric measurements in Table 2.12. The specimen from Telperion was compared to the type and only description of the species and the following differences were observed: higher a–value (93.8 *vs* 68–88), slightly higher b–value (15.5 *vs* 10.1–14) and the anterior position of the guide ring (23 *vs* 26–31). As

this specimen of *L. moniloides* is the first to be described from grass veld in Mpumalanga the morphometric differences to the type description are attributed to geographic adaptation.

***Longidorus rotundicaudatus* Jacobs and Heyns, 1987**

Figure 2.19 B + D

Measurements: Table 2.12

Longidorus rotundicaudatus Jacobs and Heyns, 1987 was described from soil around the roots of *Vitis* L. in the Western Cape in the Fynbos biome (Jacobs and Heyns, 1987). The species has since only been recorded once from indigenous forest in KwaZulu–Natal from the Savanna Biome (SAPPNS). This is the first report of this species from Mpumalanga and the Grassland Biome. The current *Longidorus* specimen was identified as *L. rotundicaudatus* based on the slightly set off lip region, tail conoid and dorsally convex with a rounded, hemispherical terminus, and thickened outer layer of the cuticle at the tail terminus. The female specimen from Telperion was morphometrically compared to the type and only description of the species and the following differences were observed: slightly wider lip region (13 µm *vs* 10–12 µm), higher b–value (17.9 *vs* 9.3–11.9) and shorter basal bulb (80 *vs* 95–123). These differences are attributed to geographic adaptation of the present population of *L. rotundicaudatus.*

Table 2.12: Morphometrics of *Longidorus moniloides* Heyns, 1966 and *Longidorus rotundicaudatus* Jacobs and Heyns, 1987 female specimens in the Telperion Nature Reserve. All measurements in µm

Species	***Longidorus moniloides* Heyns, 1966**	***Longidorus rotundicaudatus* Jacobs and Heyns, 1987**
n	1 ♀	1 ♀
L	3825	5134
a	93.8	110.0
b	15.5	17.9
c	102.0	196.7
c'	1.5	0.9
V	47	55
OV1	10	–
OV2	11	–
Body width at mid body	41	47
Body width at vulva	40	45
Body width at anus	25	29
Lip region width	16	13
Anterior to guide ring	23	30
Total spear length	85	134
Odontostyle length	55	77
Odontophore length	29	57
Basal bulb length	80	80
Basal bulb width	15	16
Oesophagus length	247	286
Anterior to vulva	1792	2805
Anterior genital tract length	369	–
Posterior genital tract length	406	–
Tail length	37	26
Hyaline length	7	9
Tail hyaline length as percentage of tail length	19	34

– Indicates structure not present or unable to measure accurately.

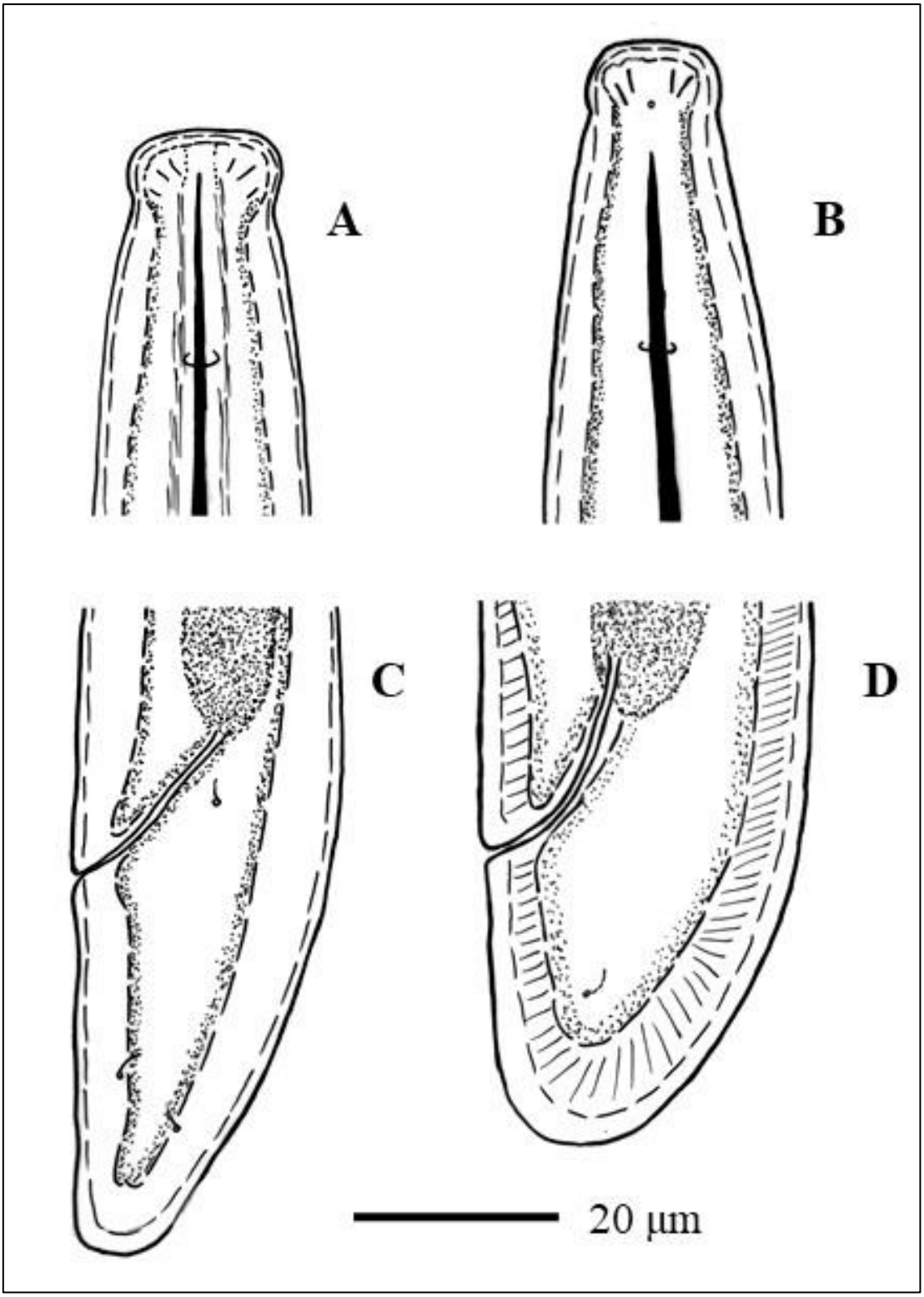


Figure 2.19: A, C: *Longidorus moniloides* Heyns, 1966; and B, D: *Longidorus rotundicaudatus* Jacobs and Heyns, 1987 from the Telperion Nature Reserve. A–B: female anterior region; C–D: female tail.

Subfamily Xiphinematinae Dalmasso, 1969

Genus *Xiphinema* Cobb, 1913

Xiphinema diannae Kruger and Heyns, 1987

Figure 2.20 A–D

Measurements: Table 2.13

Xiphinema diannae Kruger and Heyns, 1987 was described from grasses in natural veld at Bourkes Luck Potholes, in the Blyde River Canyon Nature Reserve, Mpumalanga Province (Kruger and Heyns, 1987). It has since been recorded from the Free State, Gauteng, KwaZulu–Natal, and North West provinces in the Forest (5 %), Grassland (40 %) and Savanna (55 %) biomes. A population of *X. diannae* has also been found associated with *Mangifera indica* L. in Namibia (SAPPNS). The current *Xiphinema* specimens found in Telperion were identified as *X. diannae* based on the short subdigitate–conoid tail, the presence of males, slight sexual dimorphism except for tail length, uteri containing a few scattered spines, as well crystalline structures in the uterus proximally to the *pars dilatata* and the combination of morphometrics observed (Table 2.13). The current specimens correspond well with the morphology and morphometrics of the type and only description by Kruger and Heyns (1987) and is considered conspecific to *X. diannae*. The slightly longer body of the specimens from Telperion (3379–3499 µm *vs* 2660–3190 µm in females and 3181–3283 µm *vs* 2770–3210 µm in males) broadens the morphometric range of this species.

***Xiphinema dimorphicaudatum* Heyns, 1966**

Figure 2.20 E–G

Measurements: Table 2.13

Xiphinema dimorphicaudatum Heyns, 1966 was described from various localities in East London, Eastern Cape and, associated with *Ananas comosus* var. *comosus* (L.) Merr., *Citrus* L., *Gossypium* L. and *Acacia mearnsii* De Wild. (Heyns, 1966). Four more populations were described from the Eastern Cape, KwaZulu Natal, and Mpumalanga provinces (Heyns 1966; 1976b). This species has also been recorded from various localities in all provinces in South Africa (except the Northern Cape Province) from all the biomes, especially the Grassland (33 %) and Savanna (30 %) biomes (SAPPNS). A population of *X. dimorphicaudatum* was also recorded from Namibia from natural veld (SAPPNS). The current specimens were identified as *X. dimorphicaudatum* based on the large size, distinct ventrally curved habitus, sexual dimorphism in the tail region, no uterine differentiation and a combination of other measurements (Table 2.13). The present specimens were compared to the type populations as described by Heyns (1966), as well as further populations of *X. dimorphicaudatum* described by Heyns (1976b). The following differences were observed from the type populations: shorter tail length in females (95 µm *vs* 120–167 µm) and longer spicules (75 µm *vs* 49–63 µm). These measurements also differed from specimens described by Heyns (1976b) (tail length 120 µm and 165 µm; and spicule length 54 µm and 49 µm). Despite these differences the specimens from Telperion are regarded as nearest to *X. dimorphicaudatum*.

***Xiphinema elongatum* Schuurmans Stekhoven and Teunissen, 1938**

= *Xiphinema campinense* Lordello, 1951

= *Xiphinema hydrabadense* Quraishi and Das, 1984

= *Xiphinema nagarjunense* Khan, 1982

= *Xiphinema pratense* Loos, 1949

= *Xiphinema uasi* Edward and Sharma, 1982

Figure 2.20 H–I

Measurements: Table 2.13

Described from an unknown host from the Democratic Republic of the Congo, *X. elongatum* Schuurmans Stekhoven and Teunissen, 1938 is regarded as a cosmopolitan species with reports from various parts of the world (Tarjan and Luc, 1963; Heyns, 1974). This species has an extended distribution in South Africa and has been recorded from various localities in all the provinces from all seven biomes but mainly in the Savanna (58 %) and Fynbos (21 %) biomes with only 13 % of records in the Grassland Biome (SAPPNS). The current specimens were identified as *X. elongatum* based on the position of the vulva and elongate–conoid tail shape. The specimens from Telperion were compared to descriptions by Heyns (1974), Luc and Southey (1980), Heyns and Luc (1987), Coomans et al. (1990), Sakwe and Coomans (1993), Lamberti et al. (1996), Coomans and Heyns (1997) and Razak (1998). The *X. elongatum* specimens from Telperion correspond well to these descriptions and are regarded as conspecific to *X. elongatum*.

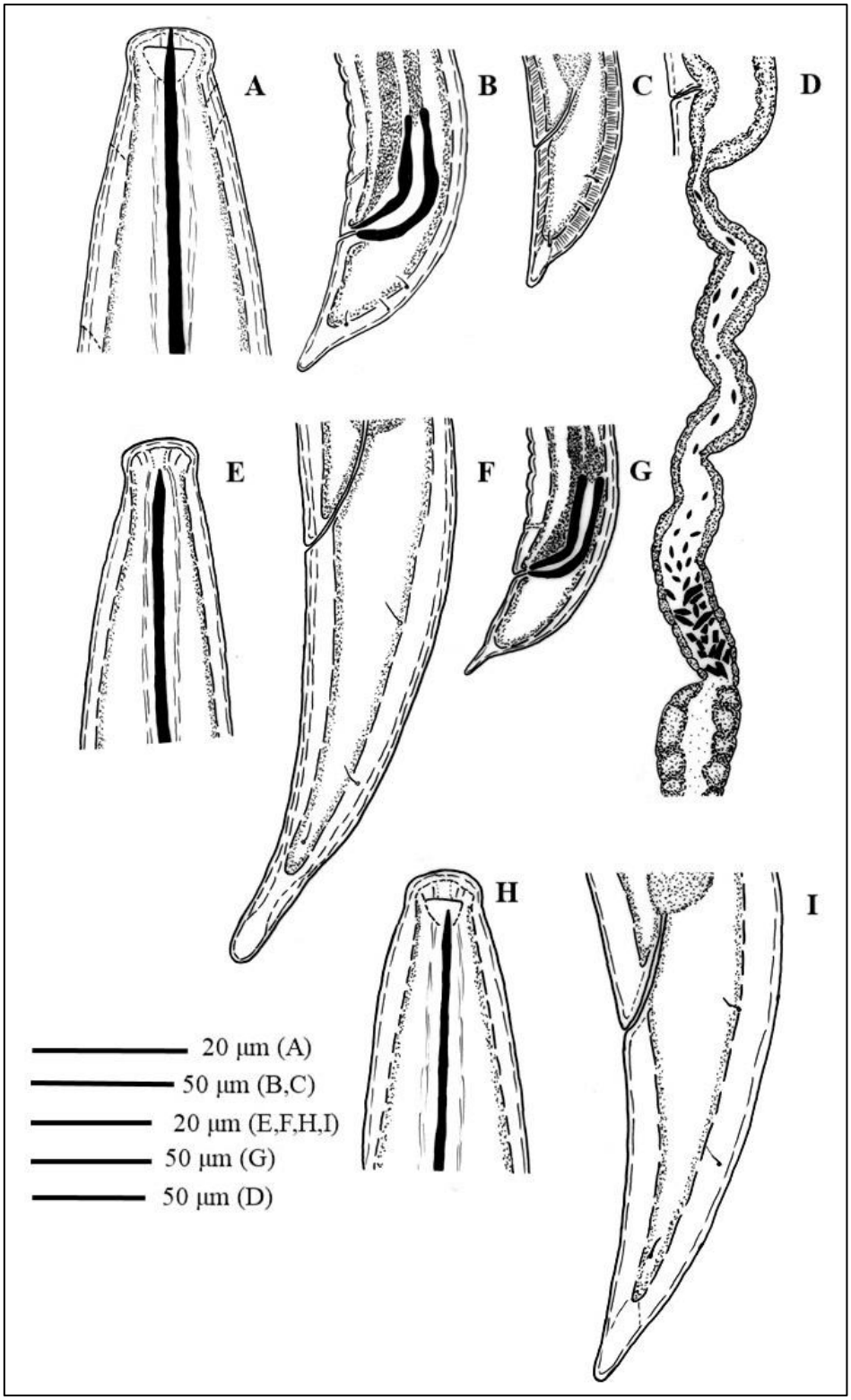


Figure 2.20: A–D: *Xiphinema diannae* Kruger and Heyns, 1987; E–G: *Xiphinema dimorphicaudatum* Heyns, 1966; and H–I: *Xiphinema elongatum* Schuurmans Stekhoven and Teunissen, 1938 from the Telperion Nature Reserve. A, E, H: female anterior region; C, F, I: female tail; B, G: male tail; and D: part of the reproductive system indicating uterine differentiation.

Table 2.13: Morphometrics of *Xiphinema diannae* Kruger and Heyns, 1987*; Xiphinema dimorphicaudatum* Heyns, 1966; and *Xiphinema elongatum* Schuurmans Stekhoven and Teunissen, 1942 populations in the Telperion Nature Reserve. All measurements in µm and in the form: mean ± standard deviation (range).

Species	***Xiphinema diannae* Kruger and Heyns, 1983**				***Xiphinema dimorphicaudatum* Heyns, 1966**		***Xiphinema elongatum* Schuurmans Stekhoven and Teunissen, 1942**
n	1 ♀	1 ♀	1 ♂	1 ♂	1 ♀	1 ♂	3 ♀
L	3499	3379	3181	3283	4873	4405	2326 ± 110.3 (2199–2390)
a	60.8	70.2	68.6	65.7	108.3	90.6	55.0 ± 6.2 (47.8–59.1)
b	9.2	10.0	7.1	–	8.6	8.8	6.6 ± 0.7 (6.1–7.4)
c	71.3	66.4	56.1	57.9	51.1	64.4	36.0 ± 2.3 (34.0–38.5)
c'	1.7	1.8	1.9	1.9	2.9	1.7	2.8 ± 0.3 (2.5–3.0)
V	50	49	–	–	46	–	41 ± 0.6 (40–41)
OV1	13	12	–	–	12	–	11[a]
OV2	14	10	–	–	8	–	10[a]
Body width at mid body	58	48	46	50	45	49	43 ± 6.4 (38–50)
Body width at vulva	58	48	–	–	43	–	42 ± 6.6 (37–50)
Body width at anus	29	28	31	31	32	40	23 ± 1.8 (21–25)
Lip region width	13	13	12	13	17	15	12 ± 1.1 (12–14)
Anterior to guide ring	83	86	81	90	108	105	84 ± 3.6 (80–87)
Total spear length	158	166	155	160	203	194	150 ± 6.1 (143–155)
Odontostyle length	92	101	91	91	118	116	91 ± 3.5 (87–94)
Odontophore length	65	65	64	69	85	79	59 ± 2.6 (56–61)
Flanges width	13	12	10	11	14	14	12 ± 1.1 (11–13)
Basal bulb length	102	73	104	–	154	137	93 ± 7.9 (87–102)
Basal bulb width	21	23	23	–	21	25	19 ± 3.1 (16–22)

[a] Only measured in one specimen.

– Indicates structure not present or unable to measure accurately.

Table 2.13 (continued): Morphometrics of *Xiphinema diannae* Kruger and Heyns, 1987*; Xiphinema dimorphicaudatum* Heyns, 1966; and *Xiphinema elongatum* Schuurmans Stekhoven and Teunissen, 1942 populations in the Telperion Nature Reserve. All measurements in µm and in the form: mean ± standard deviation (range).

Species	***Xiphinema diannae* Kruger and Heyns, 1983**				***Xiphinema dimorphicaudatum* Heyns, 1966**		***Xiphinema elongatum* Schuurmans Stekhoven and Teunissen, 1942**
n	1 ♀	1 ♀	1 ♂	1 ♂	1 ♀	1 ♂	3 ♀
Oesophagus length	380	338	447	–	566	498	356 ± 28.9 (325–382)
Anterior to vulva	1732	1651	–	–	2261	–	948 ± 35.5 (908–977)
Anterior genital tract length	451	390	–	–	560	–	270[a]
Posterior genital tract length	480	329	–	–	370	–	247[a]
Spicule length	–	–	54	60	–	75	–
Number of supplements	–	–	1+6	1+4	–	1+4	–
Tail length	49	51	57	57	95	68	65 ± 2.6 (62–67)
Peg length (if present)	–	–	14	–	–	18	–
Hyaline length	17	18	21	19	22	23	14 ± 1.8 (12–15)
Tail hyaline length as percentage of tail length	35	35	37	33	23	33	22 ± 3.5 (19–24)
Z differentiation	Four to seven distinct, closely–associated, crystalline structures, located, as pseudo–Z–differentiation, adjacent to the *pars dilatata uteri*.		–		No	–	No
Uterine inclusions	Spines irregularly distributed in the uterus from the proximal part of the *pars dilatata uteri* to junction of uterus with ovejector		–		No	–	No

[a] Only measured in one specimen.

– Indicates structure not present or unable to measure accurately.

***Xiphinema italiae* Meyl, 1953**

= *Xiphinema arenarium* Luc and Dalmasso, 1964

= *Xiphinema bulgariense* Stoyanov, 1964

= *nec Xiphinema italiae apud* Chavez and Geraert (1977) (= *Xiphinema savanicola*)

Figure 2.21 A–B

Measurements: Table 2.14

Xiphinema italiae Meyl, 1953 was described from light volcanic sand (fumaroles) in Ischia, Italy (Meyl, 1953). Due to lost type specimens, *X. italiae* was redescribed based on the neotype collected from the type locality and females and males from various localities (Martelli et al., 1966). This species has since been reported from various localities in Africa, Asia and Europe (Basri and Lamberti, 2003). According to the SAPPNS database, *X. italiae* has been recorded from all provinces except the Eastern Cape and Free State provinces. These records are especially from the Savanna (59 %) and Fynbos (35 %) biomes with 5 % of records from the Grassland and 1 % from the Succulent Karoo biomes. The current specimen from Telperion was identified as *X. italiae* based on the almost straight habitus, slender body, nearly median vulva, stylet length and anal body diameter to tail length ratio (c' value). The current specimen of *X. italiae* was compared to the neotype population, as by Martelli et al. (1966), as well as other descriptions by Basri and Lamberti (2003) and Groza et al. (2013). The following differences from the neotype population could be observed: longer body (3461 µm *vs* 1980–2580 µm); higher a–value (91 *vs* 63–73); longer odontostyle (93 µm *vs* 89 µm); and longer odontophore (61 µm *vs* 56 µm). However, these measurements correspond well to the measurements reported by Basri and Lamberti (2003) and Groza et al. (2013). The specimen from Telperion is therefore regarded as *X. italiae.*

***Xiphinema mampara* Heyns, 1979**

Figure 2.21 E, F, H

Measurements: Table 2.14

The type population of *X. mampara* Heyns, 1979 was described from a nursery in Durban, KwaZulu–Natal with several other populations described in the same paper from various localities in South Africa (Heyns, 1979). The species was then redescribed from a number of localities in South Africa into three morphological forms (*X. mampara* f. *bisexuale* Hutsebaut, Heyns, and Coomans, 1988, *X. mampara* f. *major* Hutsebaut, Heyns, and Coomans, 1988 and *X. mampara* f. *minor* Hutsebaut, Heyns, and Coomans, 1988) based on size and reproduction (gonochoristic and parthenogenetic forms) (Hutsebaut et al., 1988). According to the SAPPNS database, this species has since been recorded from all provinces except the Northern Cape and the North West provinces, especially in the Grassland (41 %) and Savanna (47 %) biomes. Other than the reports from South Africa, a population was reported from Lesotho from a potato field (Coomans et al., 1999). The current Telperion specimens were identified as *X. mampara* based on a combination of characteristics (Table 2.14) including absence of uterine differentiation, absence of ornamentation at vulva and strongly ventrally curved tail. The Telperion specimens of *X. mampara* resemble all three forms and morphometrics and morphology correspond well to specimens described by Heyns (1979); Hutsebaut et al., (1988) and Coomans et al. (1999). These specimens are therefore regarded as conspecific to *X. mampara* and more closely resemble *X. mampara* f. *major*.

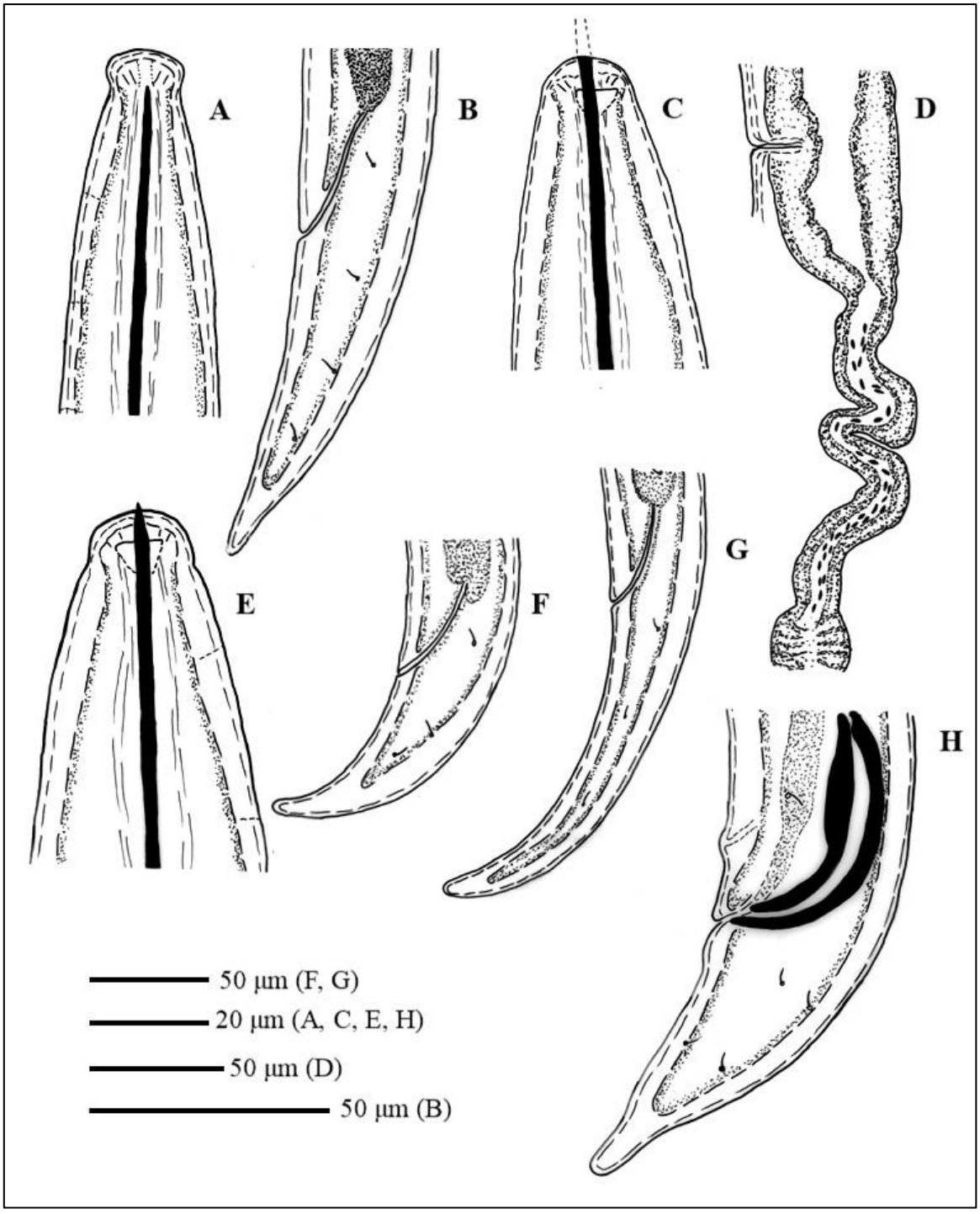


Figure 2.21: A–B: *Xiphinema italiae* Meyl, 1953; E, F, H: *Xiphinema mampara* Heyns, 1979; and C, D, G: *Xiphinema mluci* Heyns, 1976 from the Telperion Nature Reserve. A, C, E: female anterior region; B, F, G: female tail; H: male tail; and D: part of the reproductive system indicating uterine differentiation.

***Xiphinema mluci* Heyns, 1976**

= *Xiphinema hallei apud* Heyns (1962) and (1971)

Figure 2.21 C, D, G

Measurements: Table 2.14

Xiphinema mluci Heyns, 1976 was described from a *Z. mays* field near Ottosdal (North West Province) (Heyns, 1976b). The species was then redescribed by Stocker and Kruger (1987). Since then, this species has been recorded from all provinces in South Africa from various biomes. *Xiphinema mluci* is commonly found in the Grassland Biome (55 % of records). The current specimens were identified as *X. mluci* based on numerous spines in the uterus, long odontostyle, absence of uterine differentiation, absence of males, and a combination of other morphometrics (Table 2.14). The current specimens correspond well to descriptions by Heyns (1976b), Heyns and Luc (1987) and Stocker and Kruger (1987). The only remarkable difference found with these populations were tail length in one of the females *i.e.* 143 µm *vs* 160–195 µm in type population and 170–283 µm in Stocker and Kruger (1987). However in other populations described in Heyns (1976b) tail length of *X. mluci* was indicated as 108–240 µm, which corresponds well to the specimens of Telperion. The two female specimens found in Telperion are therefore regarded as conspecific to *X. mluci.*

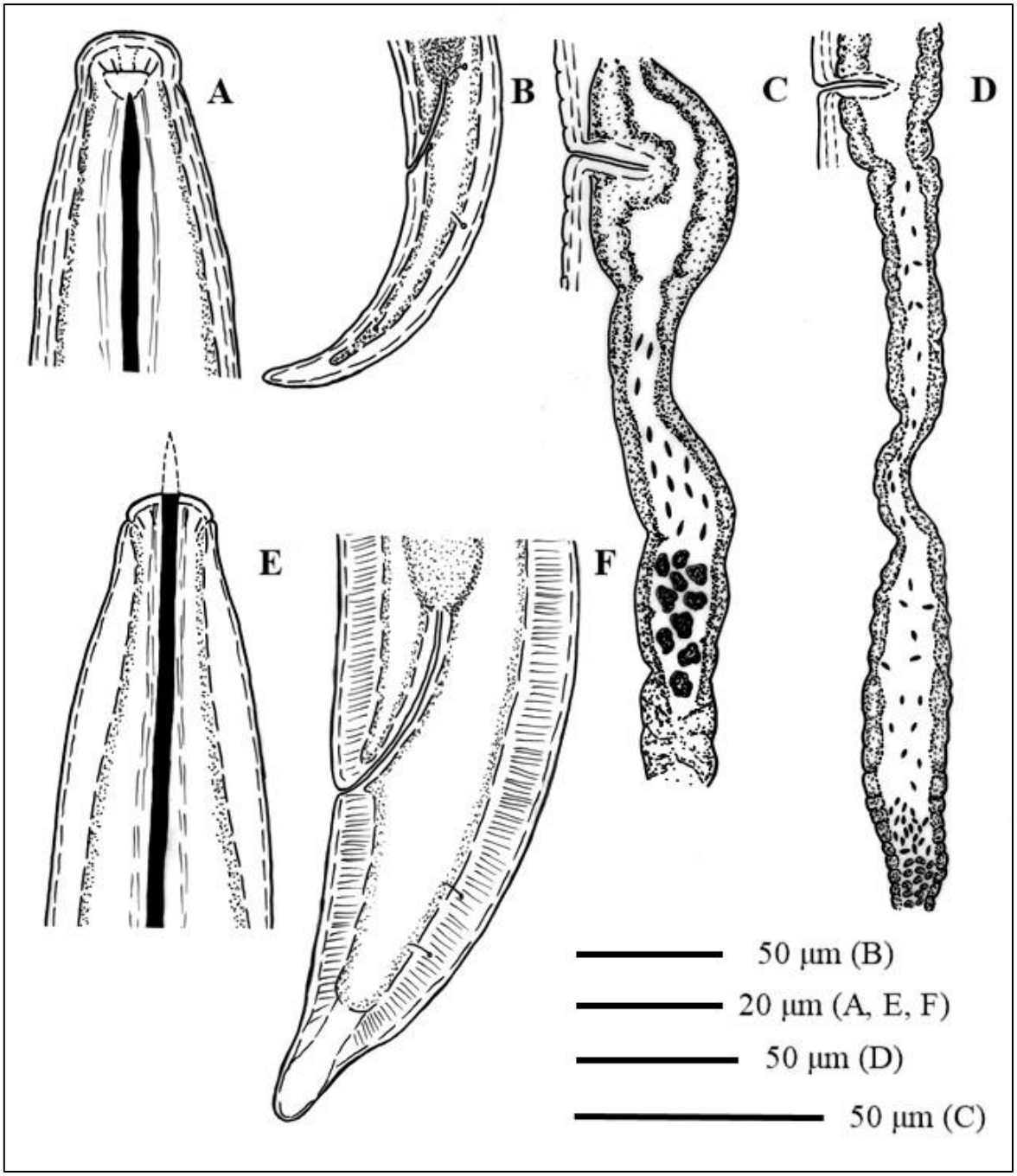


Figure 2.22: A–C: *Xiphinema ornativulvatum* Kruger and Heyns, 1987; and D–F: *Xiphinema pongolense* Hutsebaut, 1990(1989) from the Telperion Nature Reserve. A, E: female anterior region; B, F: female tail; and C, D: part of the reproductive system indicating uterine differentiation.

***Xiphinema ornativulvatum* Kruger and Heyns, 1987**

Figure 2.22 A–C

Measurements: Table 2.14

Xiphinema ornativulvatum Kruger and Heyns, 1987 was described from grasses in a natural veld near Bourkes Luck Potholes in the Blyde River Canyon Nature Reserve, Mpumalanga Province (Kruger and Heyns, 1987). This species has since been recorded from the Eastern Cape, KwaZulu–Natal, Limpopo and Western Cape provinces from the Forest (13 %), Fynbos (6 %), Grassland (62 %) and Savanna (19 %) biomes. There is also a report of this species from Namibia from *Beta vulgaris* L. and *Cucurbita* L. (SAPPNS). The current specimens were identified as *X. ornativulvatum* based on the unique cuticular ornamentation in the vulval region, six to eight globular structures in a pseudo–Z organ and spines distributed irregularly throughout the uterus as well as other morphometrics (Table 2.14). The current specimens of *X. ornativulvatum* correspond well to the type and only description of this species and no notable differences were observed. The specimens from Telperion are therefore regarded as conspecific to *X. ornativulvatum*.

Table 2.14: Morphometrics of *Xiphinema italiae* Meyl, 1953*; Xiphinema mampara* Heyns, 1979*; Xiphinema mluci* Heyns, 1976*; Xiphinema ornativulvatum* Kruger and Heyns, 1987 populations in the Telperion Nature Reserve. All measurements in µm and in the form: mean ± standard deviation (range).

Species	***Xiphinema italiae* Meyl, 1953**	***Xiphinema mampara* Heyns, 1979**		***Xiphinema mluci* Heyns, 1976**		***Xiphinema ornativulvatum* Kruger and Heyns, 1987**
n	1 ♀	6 ♀	1 ♂	1 ♀	1 ♀	3 ♀
L	3461	3536 ± 218.5 (3346–3895)	2324	4469	4759	3390 ± 107.1 (3270–3475)
a	91.4	50.5 ± 6.8 (43.5–63.3)	64.6	65.8	69.1	69.2 ± 0.6 (68.5–69.5)
b	10.0	7.0 ± 0.3 (6.6–7.3)	6.9	11.0	10.4	8.1 ± 1.3 (7.1–9.0)
c	48.0	36.9 ± 2.5 (33.5–40.8)	46.1	31.2	29.8	33.0 ± 1.5 (31.3–34.3)
c'	3.1	2.4 ± 0.1 (2.2–2.5)	1.9	4.8	4.7	3.7 ± 0.2 (3.5–3.9)
V	49	51 ± 0.8 (50–52)	–	45	47	47 ± 1.8 (45–48)
OV1	–	12 ± 1.6 (10–14)	–	9	–	–
OV2	–	12.2 ± 0.5 (11–13)	–	8	–	–
Body width at mid body	38	71 ± 6.8 (58–77)	36	68	69	49 ± 1.7 (47–50)
Body width at vulva	36	69 ± 6.1 (61–78)	–	66	69	48 ± 1.1 (46–49)
Body width at anus	23	40 ± 0.9 (39–41)	27	30	34	28 ± 0.9 (27–29)
Lip region width	12	14 ± 2.2 (11–16)	11	15	15	14[a]
Anterior to guide ring	85	120 ± 3.8 (115–126)	75	98	99	94 ± 0.7 (93–95)
Total spear length	154	212 ± 2.5 (209–215)	132	192	192	172 ± 1.6 (171–173)
Odontostyle length	93	120 ± 1.5 (119–123)	75	115	111	99 ± 1.5 (98–100)
Odontophore length	61	91 ± 1.2 (90–92)	57	77	81	73[a]
Flanges width	12	18 ± 2.1 (14–20)	12	16	14	14 ± 1.1 (12–15)
Oesophagus length	345	502 ± 17.6 (482–531)	338	407	458	423 ± 52.9 (386–460)
Basal bulb length	107	141 ± 3.4 (137–146)	89	115	125	132 ± 4.0 (130–135)
Basal bulb width	17	29 ± 2.0 (26–32)	18	26	–	19 ± 5.6 (15–23)

[a] Indicates values were the same for all specimens.

– Indicates structure not present or unable to measure accurately.

Table 2.14 (continued): Morphometrics of *Xiphinema italiae* Meyl, 1953*; Xiphinema mampara* Heyns, 1979*; Xiphinema mluci* Heyns, 1976*; Xiphinema ornativulvatum* Kruger and Heyns, 1987 populations in the Telperion Nature Reserve. All measurements in µm and in the form: mean ± standard deviation (range).

Species	***Xiphinema italiae* Meyl, 1953**	***Xiphinema mampara* Heyns, 1979**		***Xiphinema mluci* Heyns, 1976**		***Xiphinema ornativulvatum* Kruger and Heyns, 1987**
n	1 ♀	6 ♀	1 ♂	1 ♀	1 ♀	3 ♀
Anterior to vulva	1709	1798 ± 122.2 (1698–2020)	–	2023	2250	1592 ± 70.9 (1539–1673)
Anterior genital tract length	–	424 ± 44.6 (371–465)	–	381	–	–
Posterior genital tract length	–	434 ± 37.1 (382–486)	–	337	–	–
Spicule length	–	–	49	–	–	–
Number of supplements	–	–	1+4	–	–	–
Tail length	72	96 ± 3.2 (91–101)	50	143	160	103 ± 2.5 (100–104)
Peg length (if present)	–	–	–	–	–	–
Hyaline length	15	43 ± 9.3 (36–54)	14	11	17	20 ± 2.6 (17–22)
Tail hyaline length as percentage of tail length	20	45 ± 12.2 (36–59)	27	8	10	19 ± 2.9 (16–22)
Z differentiation	No	No	–	No		Conspicuous pseudo–Z organ consisting of six to eight irregularly shaped and closely associated, globular inclusions or granular bodies
Uterine inclusions	No	No	–	Numerous spines evenly distributed throughout the entire uterus		Long uterus containing spines

[a] Indicates values were the same for all specimens.

– Indicates structure not present or unable to measure accurately.

***Xiphinema pongolense* Hutsebaut, 1990 (1989)**

Figure 2.22 D–F

Measurements: Table 2.15

Xiphinema pongolense Hutsebaut, 1990 (1989) was described from soil collected under a *Vachellia xanthophloea* (Benth.) P.J.H. Hurter in Nhlanjani, KwaZulu–Natal Province (Hutsebaut, 1989). This species has since been recorded from the Free State and Limpopo provinces from unidentified grasses and natural veld in the Grassland (50 %) and Savanna (50 %) biomes (SAPPNS). This is the first report of this species in the Mpumalanga Province and also the first report since 2000. The current *Xiphinema* specimen is provisionally identified as *X. pongolense* based on the uterus with granular structures, crystals and spines in the pseudo–Z–organ and sparsely distributed spines in the tubular part of the uterus, a slightly offset lip region, amphid aperture situated on the lip region and a subdigitate convex–conoid tail. However, the specimen from Telperion also shows some similarities to *X. rarum* (Heyns, 1979) Kruger and Heyns, 1987 in the short odontostyle, short odontophore and short body. In the present study the specimen from Telperion is guardedly identified as a smaller variant of *X. pongolense*. More specimens will be collected to confirm the identity

***Xiphinema ripogranum* Hutsebaut, Heyns and Coomans, 1987**

Figure 2.23 A–D

Measurements: Table 2.15

Xiphinema ripogranum Hutsebaut, Heyns and Coomans, 1987 was described from dune vegetation in a holiday resort near Port Elizabeth, Eastern Cape Province (Hutsebaut et al., 1987). This

species has also been recorded from the Northern Cape and Western Cape provinces, most commonly from indigenous vegetation in the Fynbos Biome (71 %) but has also been recorded from the Forest (8 %), Thicket (13 %), Grassland (4 %) and Nama Karoo (4 %) biomes (SAPPNS). There is one report from the Grassland Biome from dune vegetation in the Eastern Cape (SAPPNS). This report is the first report of this species in the Mpumalanga Province and the second report from the Grassland Biome. The current specimens from Telperion were identified as *X. ripogranum* based on large number of granular structures arranged in longitudinal rows and a pseudo–Z organ, a convex–conoid tail shape with a terminally situated peg, and other morphometric characteristics (Table 2.15). The current specimens correspond well to the morphometric and morphological description of the type population as well as the two other populations described by Hutsebaut et al. (1987), and is therefore regarded as conspecific to *X. ripogranum*.

***Xiphinema swartae* Stocker and Kruger, 1988**

Figure 2.23 E–F

Measurements: Table 2.15

Xiphinema swartae Stocker and Kruger, 1988 was described from soil around the root of *Z. mays* and *Vigna unguiculata* (L.) Walp. near Bothaville, Free State Province (Stocker and Kruger, 1988). According to the SAPPNS, this species has since been recorded from the Eastern Cape, Gauteng, KwaZulu–Natal, Mpumalanga and North West provinces from various crops and natural vegetation in the Grassland (63 %), Savanna (25 %), Nama Karoo (6 %) and Forest (6 %) biomes (SAPPNS). The current specimen from Telperion has been identified as *X. swartae* based on the elongate–conoid tail shape, no uterine differentiation, and the combination of morphometrics in Table 2.15. The specimen from Telperion corresponds well to the type population as well as other populations described by Stocker and Kruger, (1988). However the following differences to the type population were observed:

shorter body length (3622 µm *vs* 4060–4340 µm); smaller lip region (11 µm *vs* 16–19 µm); and shorter total spear length (169 µm *vs* 180–187 µm). Apart from the small lip region (11 µm *vs* 13–17 µm), the specimen from Telperion fits well into the other populations described by Stocker and Kruger, (1988) and is therefore regarded as conspecific to *X. swartae*.

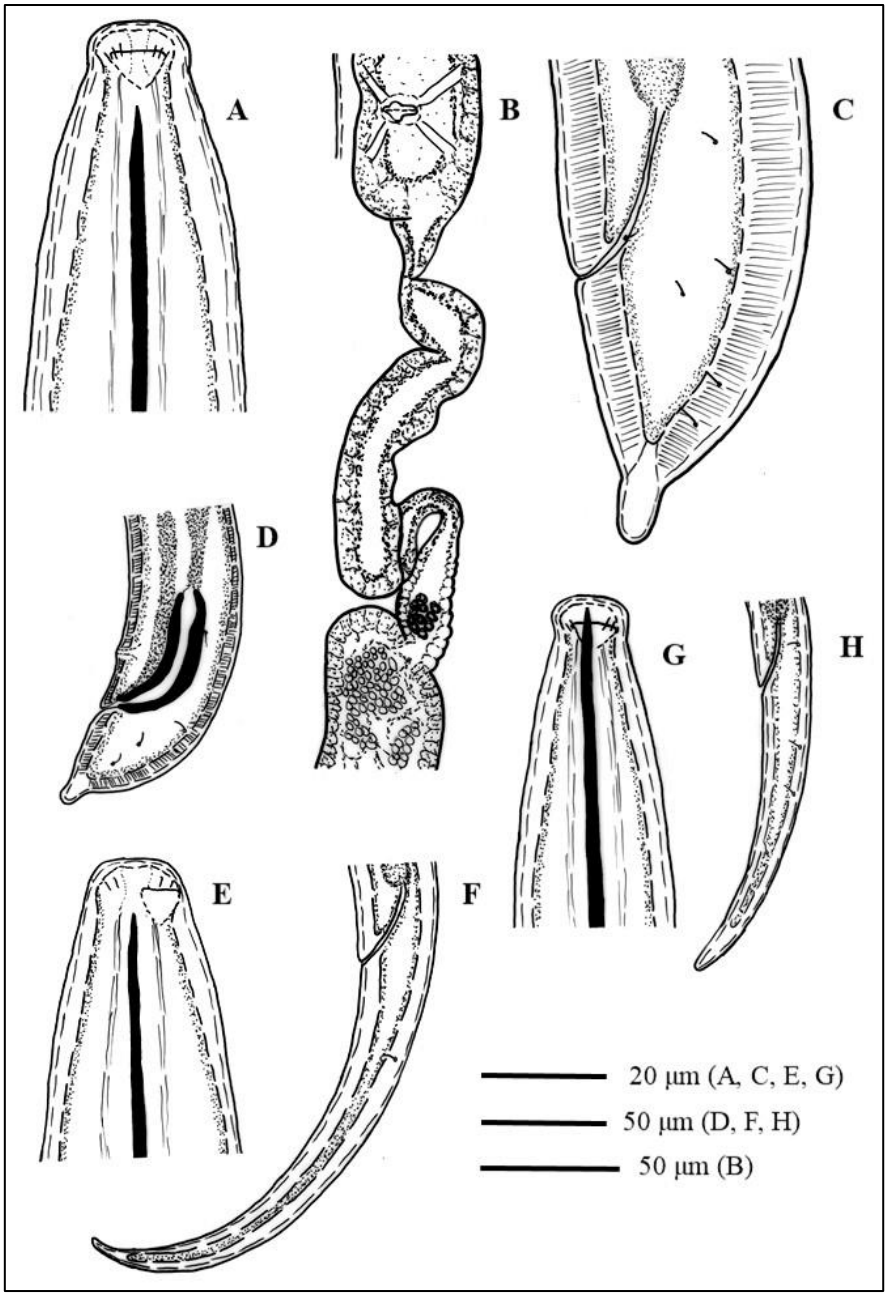


Figure 2.23: A–D: *Xiphinema ripogranum* Hutsebaut, Heyns and Coomans, 1987; E–F: *Xiphinema swartae* Stocker and Kruger, 1988; and G–H: *Xiphinema vanderlindei* Heyns, 1962 from the Telperion Nature Reserve. A, E, G: female anterior region; C, F, H: female tail; D: male tail; and B: part of the reproductive system indicating uterine differentiation.

Table 2.15: Morphometrics of *Xiphinema pongolense* Hutsebaut, 1990(1989); *Xiphinema ripogranum* Hutsebaut, Heyns and Coomans, 1987; *Xiphinema swartae* Stocker and Kruger, 1988 populations in the Telperion Nature Reserve. All measurements in µm and in the form: mean ± standard deviation (range).

Species	***Xiphinema pongolense*** **Hutsebaut, 1990(1989)**	***Xiphinema ripogranum*** **Hutsebaut, Heyns and Coomans, 1994**		***Xiphinema swartae*** **Stocker and Kruger, 1988**
n	1 ♀	3 ♀	1 ♂	1 ♂	1 ♀
L	3044	3348 ± 336.0 (3089–3728)	3686	3607	3622
a	75.2	62.0 ± 2.5 (59.2–64.1)	71.2	74.9	80.5
b	9.0	7.7 ± 0.9 (7.1–8.7)	8.6	8.3	8.5
c	69.0	78.7 ± 5.1 (75.0–84.5)	87.1	85.3	20.0
c'	1.6	1.2 ± 0.1 (1.2–1.3)	1.1	1.1	6.4
V	49	46 ± 2.0 (44–48)	–	–	47
OV1	–	15 ± 2.6 (13–18)	–	–	–
OV2	–	13 ± 1.1 (12–14)	–	–	–
Body width at mid body	41	54 ± 7.6 (49–63)	52	48	45
Body width at vulva	40	55 ± 8.0 (50–64)	–	–	48
Body width at anus	27	35 ± 0.8 (34–36)	38	37	28
Lip region width	12	16 ± 0.7 (15–16)	16	17	11
Anterior to guide ring	83	99 ± 2.9 (96–102)	97	96	90
Total spear length	162	180 ± 2.6 (178–183)	178	187	169
Odontostyle length	96	103 ± 4.0 (100–108)	104	111	101
Odontophore length	67	77 ± 2.7 (75–80)	75	76	68
Flanges width	11	13 ± 2.8 (10–15)	14	14	13
Oesophagus length	338	436 ± 16.7 (427–456)	428	433	427
Basal bulb length	99	115 ± 11.1 (105–127)	119	115	120
Basal bulb width	18	23 ± 2.8 (21–26)	26	23	23
Anterior to vulva	1489	1527 ± 163.2 (1402–1712)	–	–	1710

– Indicates structure not present or unable to measure accurately.

Table 2.15 (continued): Morphometrics of *Xiphinema pongolense* Hutsebaut, 1990(1989); *Xiphinema ripogranum* Hutsebaut, Heyns and Coomans, 1987; *Xiphinema swartae* Stocker and Kruger, 1988 populations in the Telperion Nature Reserve. All measurements in µm and in the form: mean ± standard deviation (range).

Species	***Xiphinema pongolense*** **Hutsebaut, 1990(1989)**	***Xiphinema ripogranum*** **Hutsebaut, Heyns and Coomans, 1994**			***Xiphinema swartae*** **Stocker and Kruger, 1988**
n	1 ♀	3 ♀	1 ♂	1 ♂	1 ♀
Anterior genital tract length	–	489 ± 75.9 (420–570)	–	–	–
Posterior genital tract length	–	433 ± 36.2 (393–464)	–	–	–
Spicule length	–	–	63	66	–
Number of supplements	–	–	1+4	1+4	–
Tail length	44	43 ± 1.9 (40–44)	42	42	181
Peg length (if present)	13	10 ± 1.5 (8–11)	9	9	
Hyaline length	18	16 ± 0.6 (15–16)	14	15	15
Tail hyaline length as percentage of tail length	41	37 ± 1.6 (35–38)	34	36	8
Z differentiation	Pseudo–Z–organ consisting of a variable number of granular structures and spines	Pseudo–Z–organ containing a large number of granular structures, arranged in longitudinal rows, sometimes spiralled.	–		No
Uterine inclusions	Spines sporadically distributed over the uterus	No spines or other inclusions	–		No

– Indicates structure not present or unable to measure accurately.

***Xiphinema vanderlindei* Heyns, 1962**

Figure 2.23 G–H

Measurements: Table 2.16

Xiphinema vanderlindei Heyns, 1962 was described from soil around the root of *Z. mays* and *Vigna unguiculata* (L.) Walp. near Bothaville (Free State Province) and Ventersdorp (North West Province), as well as from *Arachis hypogaea* L. near Lichtenburg, North West Province (Heyns, 1962a). This species is one of the most commonly found species of *Xiphinema* in South Africa and has been recorded from all the provinces in all seven biomes, mostly in the Savanna (42 %) and Grassland (26 %) biomes (SAPPNS). The current specimens were identified as *X. vanderlindei* based on a set off lip region, no uterine differentiation, more posterior position of the vulva and a combination of other morphometrics (Table 2.16). The current specimens correspond well with descriptions in Heyns (1962) and Heyns (1976b) with the only difference from the type description being a longer odontostyle (84–93 µm *vs* 66–84 µm). However, this measurement corresponds to the amended description of the species by Heyns (1976b).

***Xiphinema xenovariabile* Kruger and Heyns, 1984**

Figure 2.24 E–G

Measurements: Table 2.16

Xiphinema xenovariabile Kruger and Heyns, 1984 was described from the soil around roots of unidentified grasses and *Z. mays* near Rustenburg, North West Province (Kruger and Heyns, 1984). This species has since been recorded from the Gauteng, Limpopo, Mpumalanga and Northern Cape

provinces mostly in the Savanna Biome (86 %) (SAPPNS). There are only three records of this species from the Grassland Biome (14 % of the total records) from Gauteng Province. The current specimen from Telperion was identified as *X. xenovariabile* based on the more posteriorly situated vulva, a concentration of spines in the pseudo–Z organ, conoid tail, dorsally convex, ventrally concave with a depression on the dorsal side near the base of the hyaline tip. The specimen from Telperion compares well with the description of the species in Kruger and Heyns (1984) with the only difference observed being a slightly shorter tail (51 µm *vs* 61–74 µm). However, this was regarded as a minor difference and the specimen from Telperion is positively identified as belonging to *X. xenovariabile*.

***Xiphinema zyzy* Heyns and Swart, 2002**

Figure 2.24 A–D

Measurements: Table 2.16

Xiphinema zyzy Heyns and Swart, 2002 was described from natural vegetation near Modimolle, Limpopo Province in the Savanna Biome (Heyns and Swart, 2002). According to the SAPPNS, this species was also recorded from natural vegetation in Namibia. The current specimens represent the second report for this species in South Africa and the first report from the Grassland Biome, as well as the Mpumalanga Province. The current specimens were identified as *X. zyzy* based on slightly set off lip region, uterine differentiation with irregularly shaped, small inclusions in the pseudo–Z organ and a variable number of spines in the uterus, presence of males, and sexual dimorphism of the tail region (elongate, ventrally curved in female and short, digitate in male). These specimens correspond well to the type and only description of this species with no major differences between these populations. The Telperion specimens are therefore regarded as conspecific to *X. zyzy*.

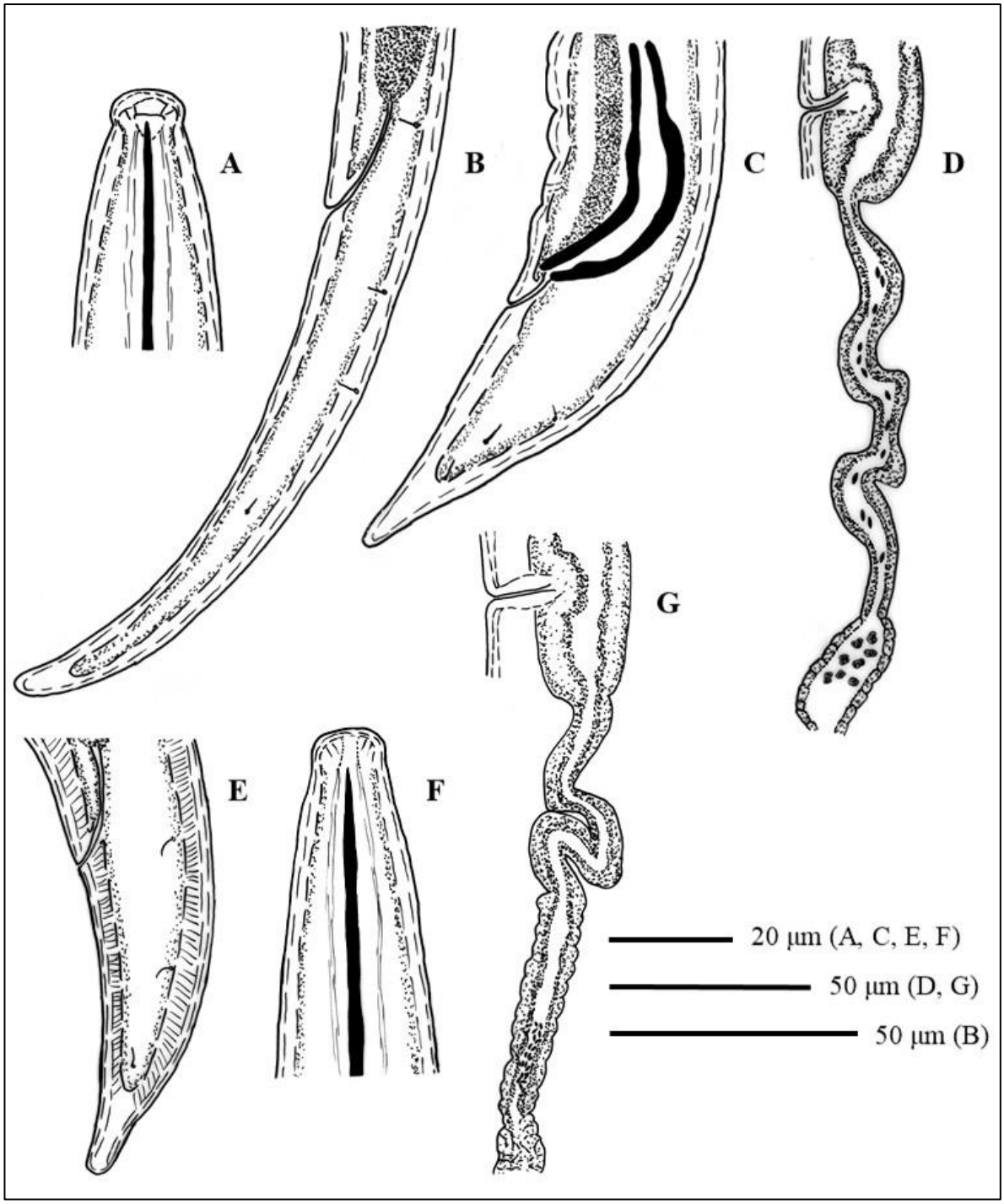


Figure 2.24: E–G: *Xiphinema xenovariabile* Kruger and Heyns, 1984; and A–D: *Xiphinema zyzy* Heyns and Swart, 2002 from the Telperion Nature Reserve. A, F: female anterior region; B, E: female tail; C: male tail; and D, G: part of the reproductive system indicating uterine differentiation.

Table 2.16: Morphometrics of *Xiphinema vanderlindei* Heyns, 1962; *Xiphinema xenovariabile* Kruger and Heyns, 1984; *Xiphinema zyzy* Heyns and Swart, 2002 populations in the Telperion Nature Reserve. All measurements in µm and in the form: mean ± standard deviation (range).

Species	***Xiphinema vanderlindei*** **Heyns, 1962**	***Xiphinema xenovariabile*** **Kruger and Heyns, 1984**	***Xiphinema zyzy*** **Heyns and Swart, 2009**		
n	4 ♀	1 ♀	3 ♀	1 ♂	1 ♂
L	3525 ± 345.9 (3133–3924)	2203	2778 ± 172.1 (2588–2925)	2875	3110
a	104.4 ± 10.0 (94.7–117.8)	56.5	78.4 ± 9.7 (68.4–87.8)	79.9	89.8
b	9.4 ± 1.8 (82–12.1)	6.5	7.4 ± 0.9 (6.5–8.3)	6.1	8.0
c	27.6 ± 2.3 (25.0–29.7)	42.8	23.1 ± 0.4 (22.9–23.5)	55.1	65.2
c'	5.9 ± 0.1 (5.8–6.0)	2.3	5.9 ± 0.5 (5.4–6.5)	2.1	1.9
V	47 ± 0.7 (46–48)	52	49 ± 1.9 (47–51)	–	–
OV1	8 ± 1.9 (6–10)	17	14[a]	–	–
OV2	7 ± 1.0 (6–8)	16	10[a]	–	–
Body width at mid body	34 ± 0.8 (33–35)	39	36 ± 2.3 (33–38)	36	35
Body width at vulva	33 ± 1.8 (31–35)	39	35 ± 1.7 (33–37)	–	–
Body width at anus	20 ± 0.8 (20–21)	22	20 ± 0.6 (20–21)	24	25
Lip region width	12 ± 0.8 (11–13)	11	12[a]	11	12
Anterior to guide ring	80 ± 7.6 (91–89)	74	73 ± 2.5 (71–75)	78	77
Total spear length	150 ± 6.5 (144–159)	131	138 ± 4.6 (133–141)	146	137
Odontostyle length	87 ± 4.1 (84–93)	72	78 ± 2.5 (75–79)	86	78
Odontophore length	63 ± 3.4 (58–66)	58	61 ± 2.1 (59–62)	60	59
Flanges width	10 ± 1.6 (8–12)	12	9 ± 0.4 (9–10)	10	12
Oesophagus length	380 ± 55.9 (324–446)	341	378 ± 23.8 (351–396)	471	387
Basal bulb length	123 ± 7.5 (115–128)	90	113 ± 6.5 (106–119)	120	116
Basal bulb width	15 ± 1.9 (12–17)	21	15 ± 0.9 (14–16)	20	17

[a] Only measured in one specimen.

– Indicates structure not present or unable to measure accurately.

Table 2.16 (continued): Morphometrics of *Xiphinema vanderlindei* Heyns, 1962; *Xiphinema xenovariabile* Kruger and Heyns, 1984; *Xiphinema zyzy* Heyns and Swart, 2002 populations in the Telperion Nature Reserve. All measurements in µm and in the form: mean ± standard deviation (range).

Species	***Xiphinema vanderlindei*** **Heyns, 1962**	***Xiphinema xenovariabile*** **Kruger and Heyns, 1984**	***Xiphinema zyzy*** **Heyns and Swart, 2009**		
n	4 ♀	1 ♀	3 ♀	1 ♂	1 ♂
Anterior to vulva	1654 ± 165.3 (1489–1850)	1150	1355 ± 34.4 (1316–1377)	0	0
Anterior genital tract length	272 ± 50.3 (234–329)	377	409[a]	0	0
Posterior genital tract length	264 ± 53.8 (231–326)	353	301[a]	0	0
Spicule length	–	–	–	50	47
Number of supplements	–	–	–	–	–
Tail length	123 ± 2.8 (120–125)	51	120 ± 7.3 (113–128)	52	48
Peg length (if present)	–	–	–	11	16
Hyaline length	16 ± 4.6 (13–21)	15	18 ± 9.6 (12–29)	18	19
Tail hyaline length as percentage of tail length	13 ± 3.5 (10–17)	29	15 ± 8.0 (11–25)	35	41
Z differentiation	No	No	Pseudo–Z–organ weakly developed, containing inconspicuous, irregularly shaped, small inclusions.	–	
Uterine inclusions	No	Specialized, slightly enlarged area containing closely associated elongated spines	Scattered spines in uterus directing away from vulva	–	

[a] Only measured in one specimen.
– Indicates structure not present or unable to measure accurately.

2.5 Discussion and Conclusion

The number of species found during the survey of the Telperion Nature Reserve conforms well to the number of species previously reported from surveys in South Africa (Botha and Heyns, 1990b, 1993b; Van den Berg, 1993, 1996; Van den Berg et al., 2003, Marais et al., 2004; Van den Berg et al., 2007; Marais and Swart, 2013, 2014). Thirty genera were found during the survey with forty seven species identified. High diversity was expected from the undisturbed natural habitat, especially for the larger dorylaims such as *Xiphinema* (with 13 known species identified) as these nematodes are particularly sensitive to disturbance. Compared to similar surveys, the Telperion Nature Reserve hosts the highest number of *Xiphinema* species in South Africa. *Pratylenchus pseudopratensis* is reported for the first time in South Africa while several species are reported for the first time in the Mpumalanga Province, namely *C. sirgeli, C. zea, H. demani, L. moniloides, L. rotundicaudatus, R. gracilidens, X. pongolense, X. ripogranum,* and *X. zyzy*. Three species are reported here from the Grassland Biome for the first time in South Africa i.e. *L. moniloides, L. rotunidcaudatus* and *X. zyzy*. The current paper also reports *X. zyzy* for the second time in South Africa, the third time since its description (also recorded from Namibia).

The present paper presents more insight into the distribution and diversity of plant–parasitic nematodes in South Africa. The results in the present paper also reveal the importance of nematode surveys in natural, protected areas of endangered biomes such as the Grassland Biome, not only for conservation purposes but also to have a baseline knowledge of potential pests if the land had to be used for agricultural purposes. Future work on the plant–parasitic nematodes of Telperion will be to describe the undescribed species in the present paper.

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CHAPTER 3: ARTICLE 2

Compendium of the Pratylenchidae Thorne, 1949 of South Africa with keys to the species

Prepared to be published as a manual as part of the **ARC-PHP handbook and manual series**

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3.1 Abstract

Several members of Pratylenchidae are well-known in South Africa due to their economic importance in agriculture. However, the identification of species is difficult because of the lack of taxonomic expertise in South Africa. In the present study species of the genus *Pratylenchus* were collected, and included is a first report for the species *P. pseudopratensis* as well as the first time *P. dellatrei* is morphologically characterised in South Africa. Based on the first report from Telperion, the realisation that some species reported from South Africa were never morphologically characterised and from literature it was clear that the members of Pratylenchidae in South Africa need attention and therefore a species compendium was constructed for the family Pratylenchidae including all reported species in South Africa. Diagnostic keys for their identification were also constructed.

Keywords: Apratylenchoides, diagnostic keys, distribution, *Hirschmanniella,* host range, morphology, morphometrics, *Pratylenchus, Radopholoides, Radopholus,* taxonomy, *Zygotylenchus.*

3.2 Introduction

Several members of the family Pratylenchidae Thorne, 1949 are well-known in South Africa due to their economic importance in agriculture. These nematodes are known to parasitize various major crops and some species are listed as quarantine organisms for example *Radopholus similis* (Cobb, 1893) Thorne, 1949, a quarantine pest of *Musa* L. (Knoetze *et al.* 2017). Pratylenchidae are widespread in South Africa (Figures 3.34–3.43) and is associated with various economically important crops (Table 3.25). *Pratylenchus* Filipjev, 1936 is the most commonly found genus of the family in South Africa and in many cases the dominant nematode pest in various crops. Cereal crops such as *Zeae mays* L., *Sorghum bicolor* subsp. *bicolor* (L.) Moench and *Triticum* L. are some of the most susceptible hosts to *Pratylenchus* of which *P. brachyurus* (Godfrey, 1929) Filipjev & Schuurmans Stekhoven, 1941 and *P. zeae* Graham, 1951 are the most abundant (Mc Donald *et al*. 2017). *Pratylenchus brachyurus* is also reported as one of the most common nematode species in South Africa and is associated with *Gossypium* L. (Van Biljon 2017), *Arachis hypogaea* L. (Fourie *et al.* 2017) and *Solanum tuberosum* L. (Jones *et al.* 2017). *Pratylenchus zeae* is the most abundant species in *Saccharum officinarum* L. in South Africa (Berry *et al.* 2017) and has an extremely wide host range (according to SAPPNS, 104 reported host plants in South Africa, see also Table 3.25).

Although members of the family Pratylenchidae are widespread in South Africa and a major agricultural pest in the country, a compendium of the morphology and geographical distribution of members of this family in South Africa has not yet been published. The first work of the Pratylenchidae in South Africa dates back to 1971 where Van den Berg (1971) described ten Pratylenchus species from South Africa and included the distribution of the species. The most recent summary of the Pratylenchidae in South Africa was published by Kleynhans *et al.* (1996) with a key to the genera, host range and geographical distribution of the species found in South Africa. Since then several new species and new reports for South Africa have been published (Van den Berg *et al.* 2017). In the present study a compendium of the members of the family Pratylenchidae are constructed, which include host range,

geographical distribution and morphometrics of all reported species in South Africa. Keys to their identification are also included, using selected diagnostic characters.

The family Pratylenchidae consists of 11 genera (Table 3.1) of which *Pratylenchus* is the most species rich (101 species) (Geraert 2013). Members of the family Pratylenchidae are polyphagous migratory, sedentary in the case of *Nacobbus* Thorne & Allen, 1944, endoparasitic nematodes, which according to Castillo *et al.* (2012) include some of the most economically important species of nematodes, i.e. *Pratylenchus*, *Radopholus* Thorne, 1949, *Hirschmanniella* Luc & Goodey, 1964 and *Nacobbus*. In fact, according to Castillo & Vovlas (2007) members of the family Pratylenchidae are the second most economically important nematodes after *Meloidogyne* Göldi, 1892. Of the eleven known genera of Pratylenchidae, six have been reported in South Africa i.e. *Apratylenchoides* Sher, 1973, *Hirschmanniella*, *Pratylenchus*, *Radopholus, Radopholoides* De Guiran, 1967 and *Zygotylenchus* Siddiqi, 1963, of which the species of *Apratylenchoides* are unidentified.

3.2.1 Taxonomy of Pratylenchidae Thorne, 1949:

The family Pratylenchidae belongs to the class Secernentia Von Linstow, 1905 and the order Tylenchida Thorne, 1949. Thorne (1949) established the subfamily Pratylenchinae Thorne, 1949 in the family Tylenchidae Örley, 1880 which included the genera *Pratylenchus, Chitinotylenchus* Micoletzky, 1922, *Nacobbus, Rotylenchulus* Linford & Oliveira, 1940 and *Radopholus*. Chitwood (1950) placed the genera *Pratylenchus*, *Radopholus*, *Hoplolaimus* Daday, 1905, *Helicotylenchus* Steiner, 1945, *Rotylenchus* Filipjev, 1936 and *Tylenchorhynchus* Cobb, 1913 in the subfamily Hoplolaiminae Filipjev, 1934 based on the females being vermiform. Genera with swollen females were place under two subfamilies: Heteroderinae Filipjev & Schuurmans Stekhoven, 1941 (*Heterodera* Schmidt, 1871 and *Meloidogyne*) and Nacobbinae Chitwood, 1950 (*Nacobbus* and *Rotylenchulus*). Goodey (1963) recognised two subfamilies in Hoplolaimidae Filipjev, 1934: Pratylenchinae (*Pratylenchus, Pratylenchoides* Winslow, 1958, *Hoplotylus* s'Jacob, 1960, *Radopholoides* and *Hirschmannia* Luc &

Goodey, 1962) and Nacobbinae (*Nacobbus* and *Rotylenchulus*). Siddiqi (1963) elevated Pratylenchinae to family rank with two subfamilies: Pratylenchinae (*Pratylenchus, Radopholus, Pratylenchoides, Hirschmannia* and *Zygotylenchus*) and Nacobbinae (*Nacobbus*). *Chitinotylenchus* was excluded as a doubtful genus and *Hoplotylus* and *Rotylenchulus* (following Allen [1960]) was removed from Pratylenchidae sharing more characteristics with Hoplolaimidae (Siddiqi 1963). Allen & Sher (1967) did not recognise Pratylenchidae and placed the pratylenchids into three subfamilies within Tylenchidae: Pratylenchinae, genera with vermiform females and only one genital branch (*Pratylenchus* and *Hoplotylus*); Radopholinae Allen & Sher, 1967, genera with vermiform females and two genital branches (*Radopholus, Pratylenchoides, Hirschmanniella* and *Zygotylenchus*); and Nacobbinae, a single genus with swollen females (*Nacobbus*) excluding *Rotylenchulus*.

Golden (1971) accepted Pratylenchinae and Radopholinae in Pratylenchidae as defined by Allen & Sher (1967), however *Nacobbus* was placed in Heteroderoidea Filipjev & Schuurmans Stekhoven, 1941 based on the swollen females and Nacobbinae was elevated to family rank with two subfamilies Nacobbinae (*Nacobbus*) and Rotylenchulinae Husain & Khan, 1967 (*Rotylenchulus*). Siddiqi (1971) recognised the subfamilies as in Allen & Sher (1967) but in the family Pratylenchidae and, based on the position of the oesophageal overlap, changed the generic content of these subfamilies: Pratylenchinae, genera with a mainly ventral overlap (*Pratylenchus*, *Zygotylenchus* and *Hirschmanniella*); Radopholinae, genera with a mainly dorsal overlap (*Radopholus, Radopholoides* and *Pratylenchoides*); Nacobbinae (*Nacobbus*). The genera *Hoplotylus* and *Acontylus* Meagher, 1968 was placed in Hoplotylinae Fotedar & Handoo, 1978 by Siddiqi (1971). Later, Andrássy (1976) based the classification of the pratylenchids on the morphology of the female reproductive system and recognised two subfamilies in Pratylenchidae, taxa with one genital branch in Pratylenchinae (*Hoplotylus, Pratylenchus, Radopholoides* and *Acontylus*) and Nacobbinae (*Nacobbus* and *Nacobbodera* Golden & Jensen, 1974). Andrássy (1976) then placed taxa with two genital branches in Radopholinae (*Apratylenchoides, Hirschmanniella, Pratylenchoides, Radopholus, Rotylenchoides* and *Rotylenchulus*) in the family Hoplolaimidae. A different classification of Tylenchida was produced by Fotedar &

Handoo (1978) following Khan & Nanjappa (1972) who elevated Radopholinae to family rank containing the genera *Radopholus* and *Radopholoides* and in Pratylenchidae they recognised three subfamilies: Pratylenchinae (*Pratylenchus*); Hirschmanniellinae (*Hirschmanniella, Pratylenchoides, Zygotylenchus* and *Apratylenchoides*); and Hoplotylinae (*Hoplotylus*). *Nacobbus* was moved to Heteroderoidea in Nacobboderidae Golden & Jensen, 1974 (subfamily Nacobbinae).

Siddiqi (1986) used the presence or absence of sexual dimorphism to classify subfamilies: Radopholinae, with sexual dimorphism (*Radopholus, Pratylenchoides, Hoplotylus, Apratylenchoides* and *Radopholoides*); and two subfamilies without sexual dimorphism Pratylenchinae (*Pratylenchus* and *Zygotylenchus*) and Hirschmanniellinae (*Hirschmanniella*) separated by tail shape and the position of the phasmids. *Nacobbus* was placed in a separate family, Nacobbidae. Luc (1987) recognised two subfamilies in Pratylenchidae: Pratylenchinae (*Pratylenchus, Radopholus, Pratylenchoides, Hoplotylus, Zygotylenchus, Hirschmanniella* and *Apratylenchoides*) and Nacobbinae (*Nacobbus*). The classification of Luc (1987) was supported by Maggenti *et al*. (1988) in the reappraisal of Tylenchina. More recently, Geraert (2013) presented the classification of Pratylenchidae based on Thorne (1949) and Siddiqi (2000) and will be used in the present work along with the higher classification of Maggenti *et al*. (1988) (Table 3.1). However, Geraert (2013) placed the genus *Pratylenchoides* in the family Pratylenchidae. Ryss (1993) suggested that this genus be placed in the family Merliniidae Siddiqi, 1971. Siddiqi (2000) placed the genus in Radopholinae (Pratylenchidae). Sturhan (2012) placed *Pratylenchoides* in a new subfamily, Pratylenchoidinae Sturhan, 2012, within the family Merliniidae which is now supported by recent morphological (Sturhan 2011; 2012) and molecular (Bert *et al.* 2008; Holterman *et al.* 2009; Van Megen *et al.* 2009; Ghaderi & Karegar 2014; Ghaderi *et al*. 2014; Panahandeh *et al.* 2014) studies of *Pratylenchoides* and is accepted in this paper. Therefore, as *Pratylenchoides* belongs to another family, it will not be discussed further in this study.

Due to the agricultural importance, several papers have been published on members of the family Pratylenchidae. Apart from the papers published on the classification of Pratylenchidae

mentioned above, important work on the family was done by Loof (1991), Castillo & Vovlas (2007) and Geraert (2013). Keys and compendia to members of the family were also published in several papers of *Hirschmanniella* (Sher 1968a; Ebsary & Anderson 1982; Sivakumar & Khan 1982), *Pratylenchus* (Sher & Allen 1953; Loof 1960; Van den Berg 1971; Loof 1978; Ryss 1988; Tarjan & Frederick 1988; Café-Filho & Huang 1989; Frederick & Tarjan 1989; Handoo & Golden 1989; Ryss 2002a; Ryss 2002b; Castillo & Vovlas 2007); *Radopholus* (Sher, 1968b; Ryss, 1997; Ryss, 2003), *Radopholoides* (Sturhan, 2008) and *Zygotylenchus* (Geraert, 2013).

Table 3.1: Classification of the family Pratylenchidae Thorne, 1949 and the genera in this family, number of species indicated in square brackets.

Class Secernentia Von Linstow, 1905
Order Tylenchida Thorne, 1949
Suborder Tylenchina Chitwood, 1950
Superfamily Tylenchoidea Örley, 1880
Family Pratylenchidae Thorne, 1949
= Nacobbidae Chitwood, 1950
= Radopholidae Allen & Sher, 1967
Subfamily Pratylenchinae Thorne, 1949
Genus *Pratylenchus* Filipjev, 1936 [98]
Genus *Zygotylenchus* Siddiqi, 1963 [4]
Subfamily Apratylenchidae Trinh, Waeyenberge, Nguyen, Baldwin, Karssen & Moens, 2009
Genus *Apratylenchus* Trinh, Waeyenberge, Nguyen, Baldwin, Karssen & Moens, 2009 [2]
Subfamily Hirschmanniellinae Fotedar & Handoo, 1978
Genus *Hirschmanniella* Luc & Goodey, 1964 [37]
= *Hirschmannia* Luc & Goodey, 1962 (name preoccupied)
Subfamily Nacobbinae Chitwood, 1950
Genus *Nacobbus* Thorne & Allen, 1944 [2]
Subfamily Radopholinae Allen & Sher, 1967
= Hoplotylinae Khan, 1969
Genus *Radopholus* Thorne, 1949 [23]
= *Neoradopholus* Khan & Shakil, 1973
Genus *Achlysiella* Hunt, Bridge & Machon, 1989 [6]
Genus *Apratylenchoide*s Sher, 1973 [3]
Genus *Hoplotylus* s'Jacob, 1960 [4]
Genus *Radopholoides* De Guiran, 1967 [5]
Genus *Zygradus* Siddiqi, 1991 [2]

3.2.2 Morphology of Pratylenchidae.

According to Castillo *et al.* (2012), the following seven main characteristics, mainly based on the morphology of females, can be used to identify the genera within Pratylenchidae (also see Figure 3.1 for illustrations of the characteristics):

1. Oesophageal gland expansion overlaps the intestine either ventrally or dorsally;
2. Reproductive systems with either one or two functional ovaries and the position of the vulva;
3. Mature females vermiform or swollen (*Achlysiella* Hunt, Bridge & Machon, 1989, *Apratylenchus* Trinh, Waeyenberge, Nguyen, Baldwin, Karssen & Moens, 2009 and *Nacobbus* swollen);
4. Body annuli with ornamentations by single or double rows of rounded tubercles;
5. Tail similarity between sexes;
6. Prominence of sexual dimorphism in anterior region;
7. Position of bursa in males.

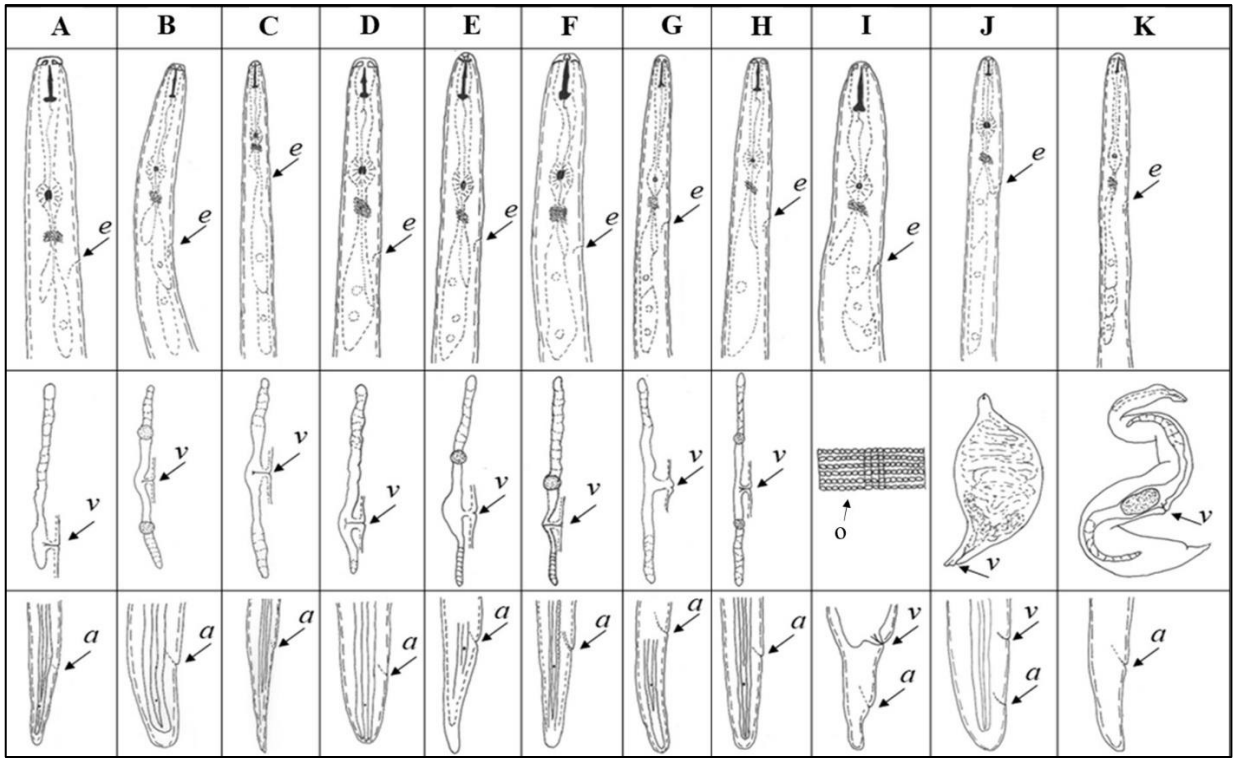


Figure 3.1: Basic morphology of the members of the family Pratylenchidae Thorne, 1949; A: *Pratylenchus* Filipjev, 1936; B: *Zygotylenchus* Siddiqi, 1963; C: *Hirschmanniella* Luc & Goodey, 1964; D: *Apratylenchoides* Sher, 1973; E: *Hoplotylus* s'Jacob, 1960; F: *Radopholoides De* Guiran, 1967; G: *Zygradus* Siddiqi, 1991; H: *Radopholus* Thorne, 1949; I: *Apratylenchus* Trinh, Waeyenberge, Nguyen, Baldwin, Karssen & Moens, 2009; J: *Nacobbus* Thorne & Allen, 1944; K: *Achlysiella* Hunt, Bridge & Machon, 1989; *e: excretory pore; v: vulva; d: deirid; a: anus, o: ornamentation on cuticle.* Redrawn and adapted from Castillo *et al.* (2012).

3.3 Materials and methods

The following compendium was constructed by collecting all relevant literature on members of the family Pratylenchidae in South Africa. The species illustrations were redrawn from original (where possible) and subsequent species descriptions. The morphometrics include that of the South African populations (if available), as well as a morphometric range, which includes the type descriptions and all relevant descriptions of the species. The description of taxa were combined from the original descriptions, as well as the information provided by various descriptions in literature. The diagnosis of taxa were combined from Castillo & Vovlas (2007) and Geraert (2013), as this is the most recent work on the family. Dichotomous keys to the species for all genera were compiled except in the case of *Radopholus, Radopholoides* and *Apratylenchoides*, where only one species of the genus has been found in South Africa. As the species of the genus *Pratylenchus* are morphologically very near to each other, with the range of variation of characteristics sometimes overlapping, it was decided to construct a polytomous key for accurate identification. Polytomous keys have the advantage over dichotomous keys because several species can share the same code for one or more characteristics. Distribution maps for all species of the family Pratylenchidae were compiled based on the distribution in each of the nine provinces of South Africa (Figure 3.2) with information provided by the South African Plant-Parasitic Nematode Survey (SAPPNS) (Marais *et al*., 2017; SAPPNS). The classification of the family as suggested by Castillo & Vovlas (2007) and Geraert (2013), excluding *Pratylenchoides*, was followed except for higher classification, where Maggenti *et al.* (1988) was followed (Table 3.1).

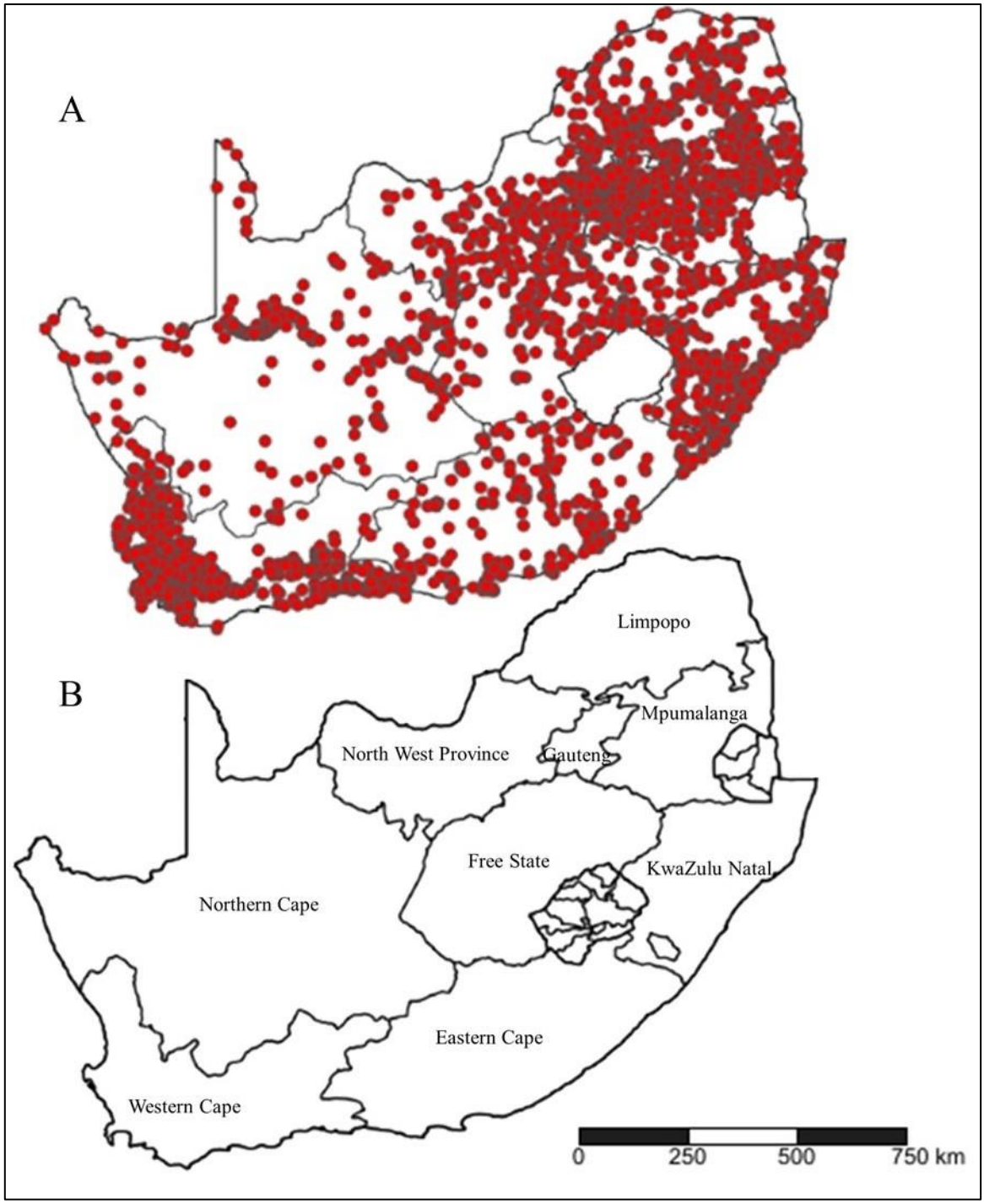


Figure 3.2: A: Total locality records listed in the South African Plant–Parasitic Nematode Survey where nematodes were recorded from South Africa. Each red dot represent a locality captured in the database; B: The nine provinces of South Africa for reference (Shorthouse, 2010).

3.4 Family Pratylenchidae Thorne, 1949

= Nacobbidae Chitwood, 1950

= Radopholidae Allen & Sher, 1967

According to Loof (1991), Castillo *et al.* (2012) and Geraert (2013), the diagnosis of the family Pratylenchidae is as follows:

Tylenchoidea. Mature females vermiform to obese (*Achlysiella*, *Apratylenchoides* and *Nacobbus*). Cuticle distinctly annulated. Lateral field with four to six incisures, rarely aerolated. Amphid apertures small. Labial region annulated, low, anteriorly flattened to broadly round. Labial framework strongly sclerotized, labial disc indistinct, *en face* labial pattern variable, often characterizing genera. Stylet strong, length not exceeding three labial region widths (except in *Hirschmanniella*), conus as long as shaft, stylet knobs large, rounded. Dorsal gland opening close behind stylet knobs. Procorpus slender, median bulb muscular with prominent refractive thickenings, isthmus short. Oesophageal-intestinal junction with refringent valve. Oesophageal glands extending over intestine ventrally or dorsally. Deirids absent. Vulva a transverse slit, positioned submedian to more posterior but not subterminal, lips not modified, lateral membranes absent. Reproductive system didelphic, amphidelphic or pseudo-monodelphic. Ovaries outstretched, posterior ovary degenerate in pseudo-monodelphic forms. Spermatheca large, rounded, with small round or rod-like sperm when impregnated. Tail conoid, sub-cylindrical to elongate-conoid, approximately two or more times anal width, with round to pointed terminus that may bear a mucro (*Hirschmanniella*), generally with inconspicuous hyaline portion. Phasmids pore-like, on tail, located far posterior to anus.

Male vermiform with head, stylet and oesophagus similar to that of female but can be reduced (*Apratylenchus*, *Radopholus* and *Radopholoides*). Tail elongate-conoid, bursa terminal or sub-terminal,

phasmids extending caudal alae as pseudoribs (except in *Hirschmanniella*). Testis single, outstretched, spermatocytes in one or two rows. Spicules similar, cephalated, arcuate, pointed with sub-terminal opening on dorsal or ventral side. Gubernaculum simple, fixed, or complex with titillate, protrusible. Hypotygma present or absent.

Juveniles similar to females in anterior region and tail.

3.4.1 Key to the Genera of Pratylenchidae in South Africa

The following key to the genera of Pratylenchidae reported from South Africa was constructed based on the characteristics listed in section 3.2.2.

Table 3.2: Key to the genera of Pratylenchidae Thorne, 1949 in South Africa

1	Oesophageal gland overlapping intestine ventrally and ventro-laterally……..	**2**
	Oesophageal gland overlapping intestine dorsally and dorso-laterally……	**3**
2	One functional ovary……	***Pratylenchus***
	Two functional ovaries……..	**4**
3	Two functional ovaries……	***Radopholus***
	Posterior ovary reduced, non-functional……	**5**
4	Tail similar between sexes, phasmid near tail terminus……..	***Hirschmanniella***
	Tail dissimilar between sexes, phasmid not near tail terminus……	***Zygotylenchus***
5	Vulva 65-73, sexual dimorphism in anterior region prominent……..	***Radopholoides***
	Vulva 77-82, sexual dimorphism in anterior region not prominent…….	***Apratylenchoides***

3.5 Subfamily Pratylenchinae Thorne, 1949

According to Thorne (1949) and Geraert (2013), the diagnosis of the subfamily Pratylenchinae is as follows:

Pratylenchidae: Small sized (under 1 000 μm). No marked sexual dimorphism in anterior region. Lateral field with four to six incisures. Labial region low, flattened, with two to four annuli. Stylet generally less than 20 μm. Oesophageal gland lobe-like, mostly overlapping intestine ventrally. Ovaries paired or single. Female tail three anal body widths or less, subcylindrical to conoid, lacking a mucro. Male tail conoid, ventrally curved, bursa peloderan. Phasmids near middle of tail. Spicules arcuate, cephalated, with subterminal pore on ventral or dorsal side. Gubernaculum fixed. Hypotygma generally present.

3.5.1 Pratylenchus Filipjev, 1936

(*Pratylenchus* Filipjev, 1934 = *nomen nudum*)

The genus *Pratylenchus* consists of 101 valid species (Geraert 2013) and is regarded as the most economically important genus in the family Pratylenchidae. This is due to their wide host range and worldwide distribution in a wide range of environments. *Pratylenchus* species are found on every continent including Antarctica with Asia hosting the most species (40 species) and in all agricultural regions (Castillo & Vovlas 2007). Twenty species have been reported from South Africa as listed in the National Collection of Nematodes (NCN) and SAPPNS. Although Waeyenberge *et al*. (2000) stated that a population of *P. subranjani* Mizukubo, Toida, Keereewan & Yoshida, 1990 was received from South Africa in their paper, no record of this population is available in the SAPPNS or NCN databases. It is therefore uncertain if this species is present in South Africa. This species will therefore not be included in the present paper.

Diagnosis (Castillo & Vovlas 2007; Geraert 2013)

Pratylenchinae: No marked sexual dimorphism in anterior region. Lateral field with four to six lines, occasionally with oblique median markings. Deirids absent. Labial region low, flattened anteriorly, rarely rounded, continuous with body contour or slightly offset, sclerotization massive, labial disc inconspicuous, amphidial apertures pore-like or slit-like, near indistinct labial disc. *En face* oral disc and first head annulus may form one of three patterns (Figure 3.3 for illustration of characters): 1) plain undivided face with no division between sub-median and lateral segments; 2) sub-median segments fused to oral disc, narrowing towards stoma, widening towards outer edge of face (like a bow tie), separated from the larger, lateral segments that complete the circular face; 3) dumb-bell shaped pattern of the sub-median segments with slightly smaller lateral segments to complete the circular face. Stylet 10-30 μm long with round, anteriorly flattened or anteriorly indented stylet knobs. Median bulb oval to round, muscular. Oesophageal gland generally less than two body widths long, overlapping intestine ventrally. Vulva posterior, at 70-90 % of body length. Genital tract pseudo-monodelphic, only anterior ovary functional. Post vulval uterine branch present, with or without rudimentary ovary. Spermatheca oval to rounded, usually axial, functional (filled with sperm) or indistinct (empty). Female tail sub-cylindrical to conoid, two to three anal body widths long, terminus smooth or annulated. Phasmids near mid tail. Bursa peloderan. Spicules with subterminal pore on dorsal side. Gubernaculum simple, trough-like, fixed.

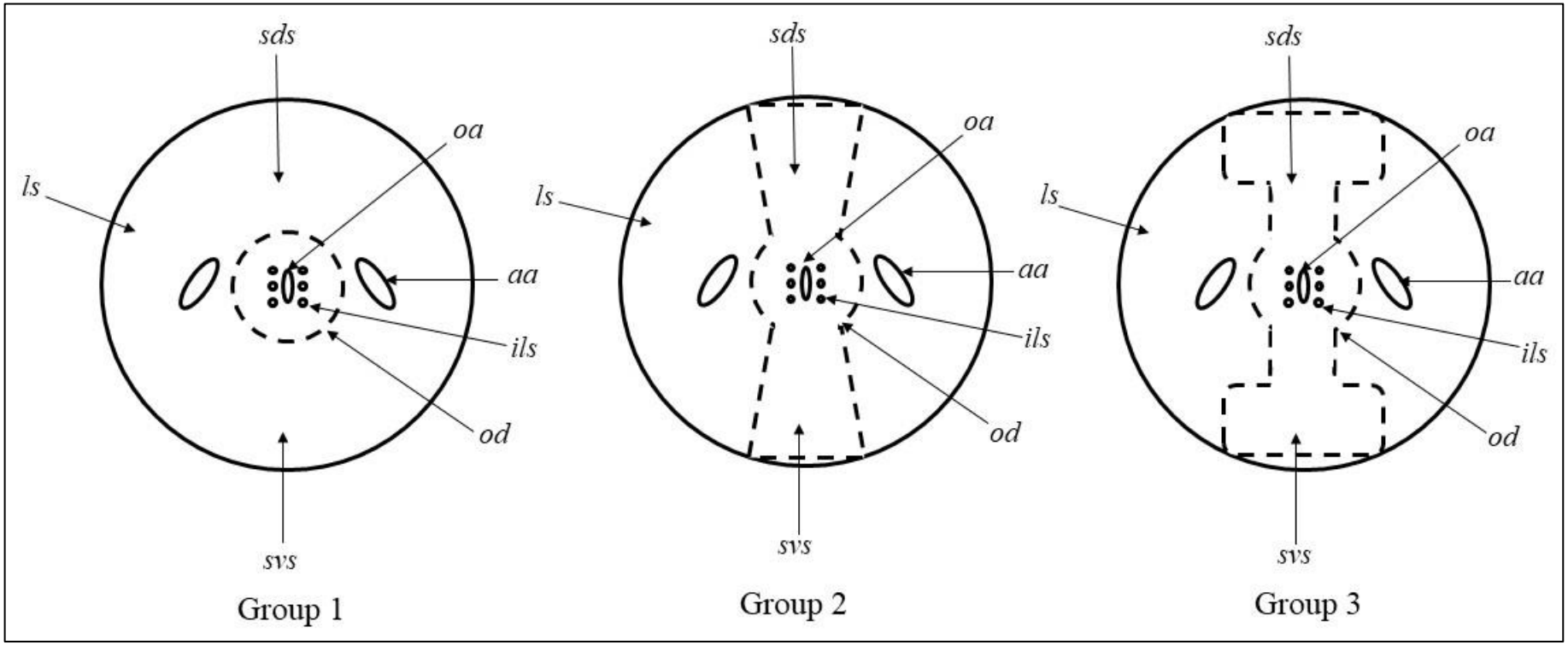


Figure 3.3: Variations in the pattern of the oral disc and the first labial annule; Group: 1 no division between the submedian and lateral segments; Group 2: submedian segments fused to the oral disc, narrower at the inner extremity and widening towards the outer extremities, submedian segments separated from lateral segments; Group 3: distinctive dumb-bell shape, submedian segments slightly larger than lateral segments. where *aa* = amphid aperture; *ils* = inner labial sensilla; *ls* = lateral segment; *oa* = oral aperture; *od* = oral disc; *sds* = subdorsal segment; *svs* = subventral segment. Adapted from Castillo & Vovlas (2007).

3.5.2 Species of P*ratylenchus* Filipjev, 1936 reported in South Africa:

3.5.2.1 *Pratylenchus pratensis* (De Man, 1880) Filipjev, 1936

= *Tylenchus pratensis* De Man, 1880

= *Anguillulina pratensis* (De Man, 1880) Goffart, 1929

= *Pratylenchus helophilus* Seinhorst, 1959

= *Pratylenchus irregularis* Loof, 1960

Figure 3.4

Measurements: Table 3.3

Pratylenchus pratensis (De Man, 1880) Filipjev, 1936 is the type species of the genus and apart from the type locality in the Netherlands; this species has been reported from the Afrotropical, Nearctic, Palearctic, and Oriental biogeographic regions and has been associated with various host plants (Castillo & Vovlas 2007). The morphometrics of *P. pratensis* (Table 3.3) is a combination of data from Goodey (1933); Seinhorst (1959); Das (1960); Loof (1960); Seinhorst (1968); Brzeski (1969); Van den Berg (1971); Loof (1974); Inserra *et al*. (1979); Ryss (1988); Brzeski (1998); Castillo & Vovlas (2007) and Geraert (2013). The morphometrics of the South African population are those given in Van den Berg (1971). See section 3.8-3.9 for distribution and host range in South Africa (Figure 3.37, Table 3.25).

Description:

Lectotype (Loof 1960): Female: L = 460 µm; a = 28.0; b = 3.6; c = 20.0; V = 77 %; stylet length = 15 µm.

Female: Habitus almost straight. Cuticular annulation fine, 0.9 µm mid body. Lateral field with four lines. Labial region not set off, bearing three annuli with rounded outer margins, labial framework extending into body for one annulus. *En face* view with distinctive dumb-bell shape, submedian segments slightly larger than lateral segments (Group 3, Figure 3.3). Stylet stout, stylet knobs well separated, flattened or anteriorly indented. Dorsal gland opening 2.5 µm posterior to base of stylet knobs. Oesophageal gland overlaps intestine ventrally to ventrolaterally, overlap 22-53 µm long. Excretory pore situated just anterior to junction of oesophagus and intestine, 71-101 µm from anterior end. Hemizonid immediately anterior to excretory pore. Vulva transverse slit, spermatheca large, oval to rectangular in shape, filled with sperm, oocytes in single row except in a short multiplication zone at anterior tip. Post-vulval uterine branch slightly longer than corresponding body diameter, 11-39 µm long, undifferentiated, sometimes with one separate cell. Phasmids situated mid tail. Tail with 20-33 annuli, excluding those around terminus, tail tip annulated, variable in shape, usually oblique, sometimes more symmetrically conoid or slightly mucronate.

Male: Similar to female. Testis single, outstretched. Spicules 16-19 µm long, curved, gubernaculum 4-7 µm long. Bursa enveloping tail tip with faintly crenate margins. Phasmids extending into bursa, posterior to mid tail.

Diagnosis (Castillo & Vovlas 2007):

Pratylenchus pratensis is characterised by labial region bearing three annuli, oval to rectangular spermatheca, post-vulval uterine branch one body diameter long, tail with annulated tip, 20-28 (33 in some reports) ventral tail annuli.

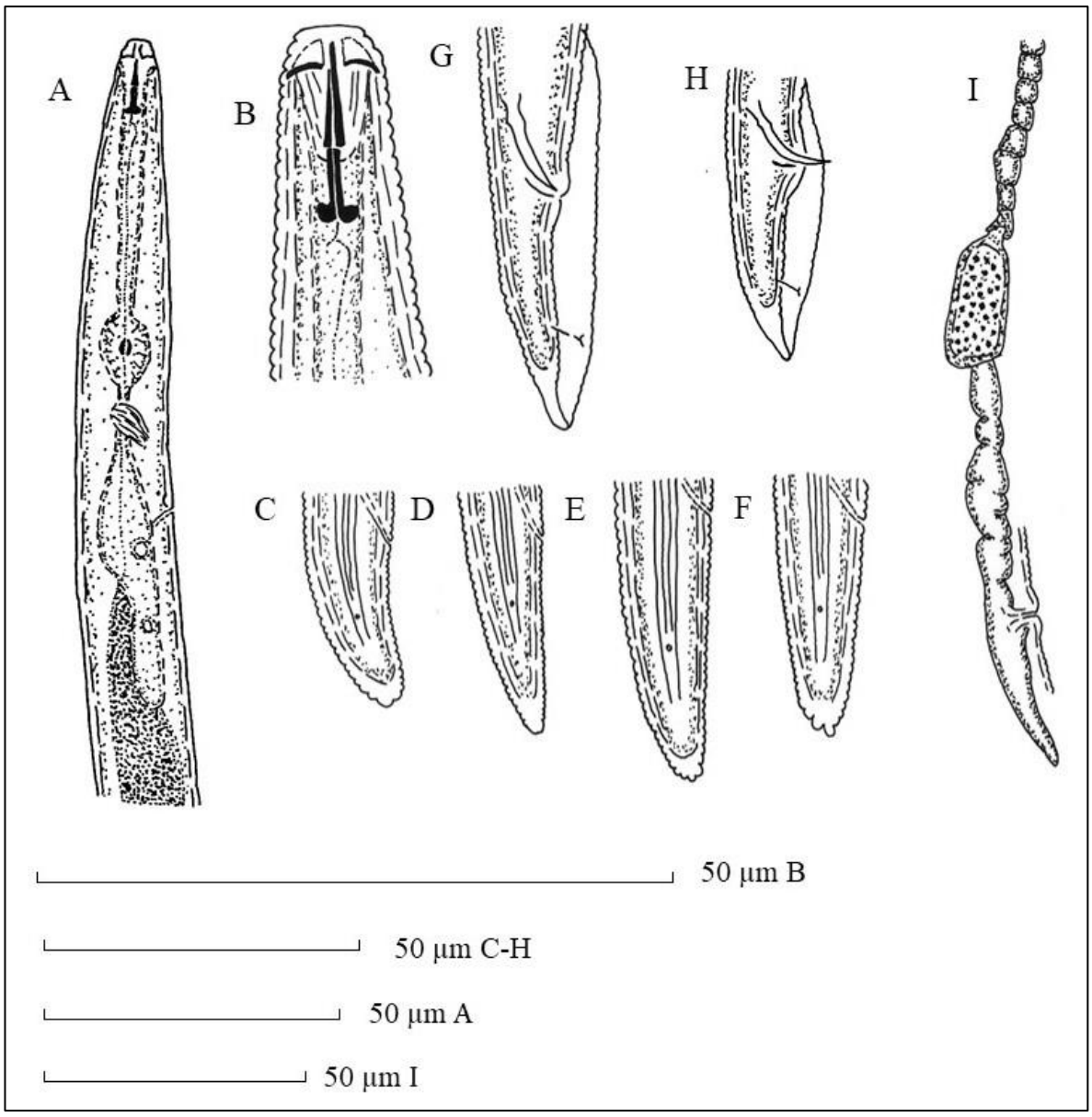


Figure 3.4: *Pratylenchus pratensis* (De Man, 1880) Filipjev, 1936; A: Female oesophageal region; B: Female labial region; C-D: Variation in female tails; G-H: Male tails; I: Vulval region showing spermatheca and post-vulval uterine branch (Redrawn from Loof [1974]).

Table 3.3: Morphometrics of females and males of *Pratylenchus pratensis* (De Man, 1880) Filipjev, 1936. All measurements are in µm unless stated otherwise.

Species	***P. pratensis* (De Man, 1880) Filipjev, 1936**		
Population	**South African**	**Range**	
	♀	♀	♂
L	400–610	380–700	450–640
a	19.4–35.0	16.0–38.0	18.0–43.0
b	–	3.6–9.0	4.3–9.0
b'	3.6–5.3	3.4–5.3	3.4–4.3
c	13.1–19.6	12.0–23.0	16.0–25.0
c'	–	1.8–3.1	2.2–2.7
o	–	–	–
m	–	–	–
V / T	72–78	71–83	34–61
OV1	28–37	28–51	–
Number of annuli in labial region	3	3	3
Stylet length	13–17	12–17	13–16
Dorsal gland opening from base of stylet knobs	–	2.5[a]	–
Oesophageal gland overlap length	–	22–53	–
Excretory pore from anterior end	71–101	71–101	–
Oesophagus length	–	100–120	–
Post–vulval uterine branch length	11–39	11–39	–
Spicule length	–	–	16–19
Gubernaculum length	–	–	4–7
Number of annuli from phasmid to anus	–	–	–
Number of annuli posterior to phasmid	6–13	6–13	–
Ventral tail annuli	22–28	20–33	–
Tail length	24–39	18–39	–

– Indicates structure not present or value not available.

[a] Indicates range not available.

3.5.2.2 *Pratylenchus bolivianus* Corbett, 1983

=*Pratylenchus australis* Valenzuela & Raski, 1985

Figure 3.5

Measurements: Table 3.4

Pratylenchus bolivianus was described from *Avena sativa* L. from Bolivia (Corbett 1983). The species has been reported from the Afrotropical, Nearctic, Neotropical, and Palearctic biogeographical regions (Castillo & Vovlas 2007; Daramola *et al.* 2018). The morphometrics of the species (Table 3.4) is based on those of the type population and the morphometrics by Valenzuela & Raski (1985); Castillo & Vovlas (2007) and Geraert (2013). The South African populations are yet to be characterised. See section 3.8-3.9 for distribution and host range in South Africa (Figure 3.34, Table 3.25).

Description:

Holotype (Corbett 1983): Female: L = 610 µm; a = 25.0; b = 5.4; b' = 4.0; c = 22.0; V = 82 %; stylet length = 19 µm.

Female: Habitus slightly curved ventrally, often twisted about 180 °, tapering from posterior to vulva to ventrally bent tail. Four lines in lateral field, outer bands narrower than inner band, aerolated, occasionally with faint slanted striae in middle band at mid body. Labial region with three, rarely four, annuli, labial framework extending into body for at least two annuli. In *en face* view by SEM, subdorsal and subventral segments of first annulus fused to form rectangular shape with convex dorsal and ventral margins and longer, concave lateral sides separated from lateral sectors, each bearing an amphidial opening on inner edge (Group 2, Figure 3.3). Oral aperture oval, surrounded by six small pores. Stylet robust, stylet knobs rounded, dorsal gland opening 2.7-4.0 µm behind base of stylet knobs. Median bulb

oval, well developed. Nerve ring at level of isthmus just anterior to oesophageal gland which overlaps intestine ventrally and laterally, overlap 18-49 µm long. Oesophageal-intestinal junction more than one body diameter posterior to excretory pore located 89-105 µm from anterior end. Anterior genital tract with inconspicuous spermatheca. Post-vulval uterine branch 22-31 µm or 1.25 times vulval body width, undifferentiated. Lateral field in tail with four lines extending posterior to inconspicuous phasmid located 10-15 annuli from terminus, on anterior half of tail. Tail convex dorsally, straight to concave ventrally, conoid. Ventral side with 15-19, rarely 24, irregular annuli, tail tip smooth, rounded.

Male: Unknown.

Diagnosis (Castillo & Vovlas 207):

Pratylenchus bolivianus is characterised by habitus irregular (twisted about 180 °), tapering posterior to vulva, labial region with three annuli, stylet generally 19 µm long, tail tip rounded and smooth.

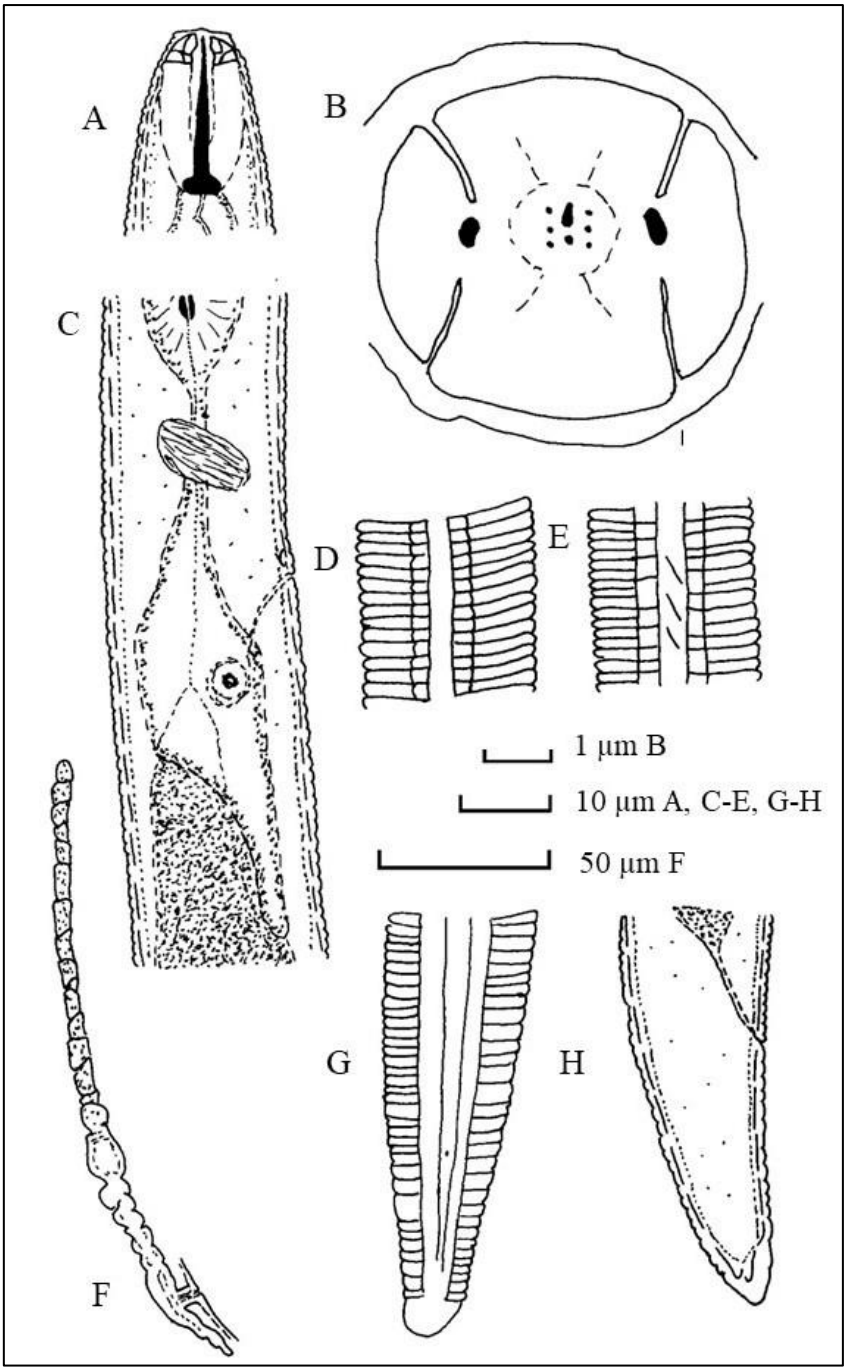


Figure 3.5: *Pratylenchus bolivianus* Corbett, 1983; A: Labial region; B: *En face* view of labial region; C: Oesophageal region; D-E: Lateral field; F: Genital tract; G-H: Tail region (Redrawn from Corbett [1983]).

3.5.2.3 *Pratylenchus brachyurus* (Godfrey, 1929) Filipjev & Schuurmans Stekhoven, 1941

= *Tylenchus brachyurus* Godfrey, 1929

= *Anguillulina brachyurus* (Godfrey, 1929) Goodey, 1932

= *Pratylenchus leiocephalus* Steiner, 1949

= *Pratylenchus pratensis* apud Thorne, 1940

= *Pratylenchus steineri* Lordello, Zamith & Boock, 1954

Figure 3.6

Measurements: Table 3.4

Pratylenchus brachyurus was described from *Ananas comosus* var. *comosus* (L.) Merr. in Hawaii (Godfrey 1929). This species of *Pratylenchus* is cosmopolitan and polyphagous (Castillo & Vovlas 2007) and has been reported from the Afrotropical, Australasian, Nearctic, Neotropical, Oceanian, Oriental, and Palearctic, biogeographical bioregions. The morphometrical range of this species (Table 3.4) is a combination of the type population and several other populations (Sher & Allen 1953; Loof 1960; Williams 1960; Thorne 1961; Román & Hirschmann 1969; Van den Berg 1971; Corbett 1976; Boonduang & Pliansinchai 1980; Rashid *et al.* 1987; Ryss 1988; Loof 1991; Sakwe & Geraert 1994; Crozzoli *et al.* 1998; Castillo & Vovlas 2007; Geraert 2013). The South African population of *P. brachyurus* (Table 3.4) was described by Van den Berg (1971). See section 3.8-3.9 for distribution and host range in South Africa (Figure 3.34, Table 3.25).

Description:

Topotype (Sher & Allen 1953): Female: L = 560 µm; a = 27; b = 5; c = 21; V = 86 %; stylet length = 20 µm.

Female: Habitus slightly curved ventrally to almost straight. Lateral fields usually with four lines in mid body, occasionally five or six in vulval region. Labial region set off from body contour, with angular anterior margin and two distinct annuli. In *en face* view with SEM median and lateral lips fused with oral disc, separated from first annulus by an incisure, apical region undivided with six papillae around oval oral aperture and two large amphidial openings (Group 1, Figure 3.3). Labial framework strong. Stylet with stout, rounded stylet knobs. Excretory pore just posterior to hemizonid in region of often indistinct oesophageal-intestinal junction, situated 49-121 µm from anerior end. Oesophageal gland overlaps intestine ventrally and laterally, overlap 31-80 µm long. Anterior genital tract outstretched, occasionally reflexed, rarely extending past oesophageal lobe. Oocytes in single row, occasionally forming two rows, spermatheca inconspicuous, not functional. Vulva at 82-89 % of body length (in rare cases 71 %), post-vulval uterine branch short, less than one body diameter long, 5-30 µm. Lateral field terminating as two lines near tail. Phasmid located mid-tail, 8-12 annuli posterior to anus. Tail broadly conoid, tip smooth, broadly rounded, truncate or spatulate with 12-24 ventral annuli, usually less than 20.

Male: Extremely rare. Similar to female except in reproductive organs. Single outstretched testis. Spicules arcuate. Gubernaculum simple. Bursa delicate. Phasmid slightly posterior to mid-tail, not extending into bursa.

Diagnosis (Castillo & Vovlas 2007):

Pratylenchus brachyurus is separated from all other species by labial region with two annuli, angular contour of first lip annulus, stylet stout, stylet knobs rounded, vulva at 82-89 % of body length, short post-vulval uterine branch (less than one body dimeter), inconspicuous spermatheca not functional, tail broadly conoid with smooth, broadly rounded, truncate of spatulate tip, and the rarity of males.

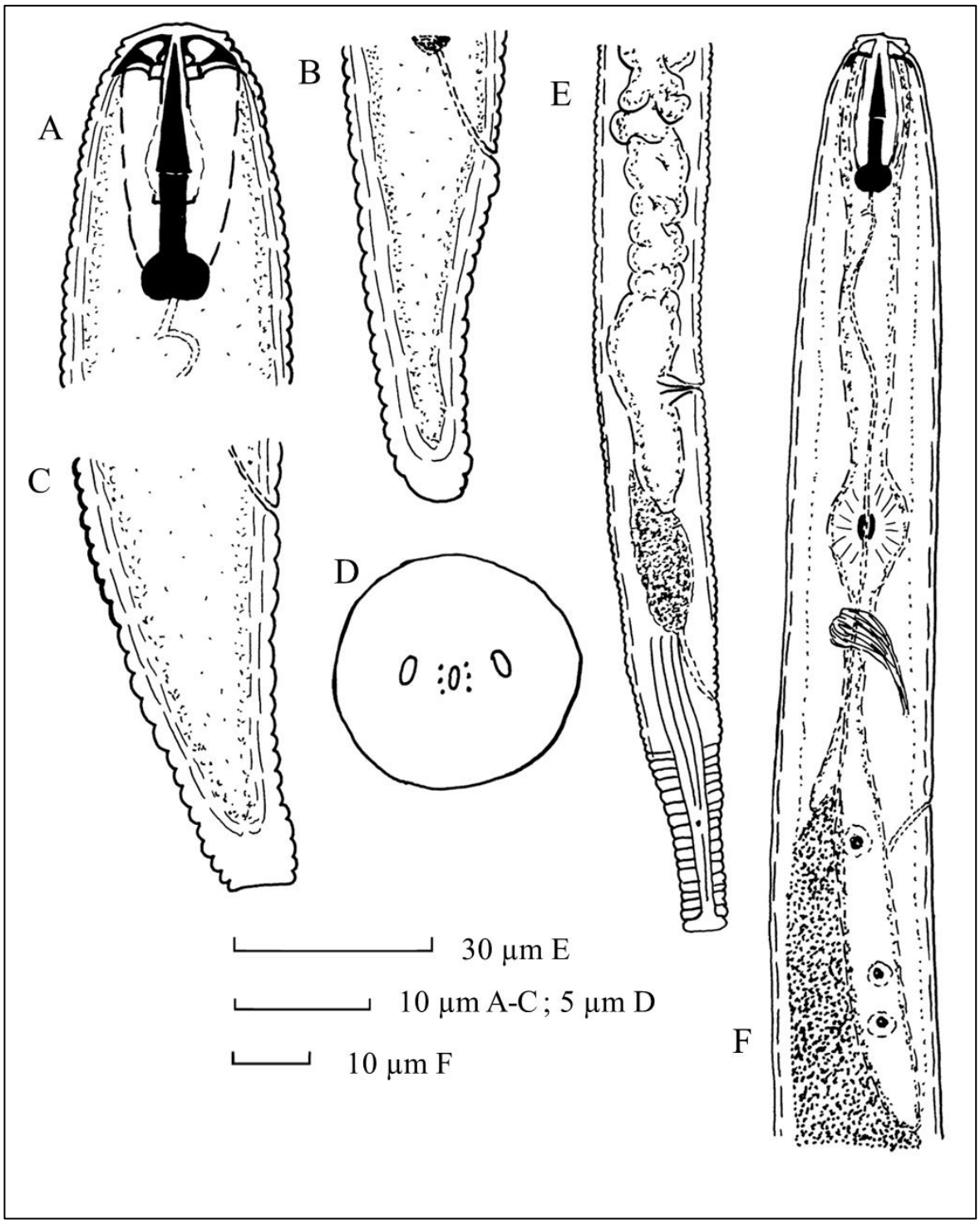


Figure 3.6: *Pratylenchus brachyurus* (Godfrey, 1929) Filipjev & Schuurmans Stekhoven, 1941 A: Labial region; B-C: Tail region; D: *En face* view of labial region; E: Posterior region showing post-vulval uterine branch and lateral field in tail region; F: Oesophageal region (A-E redrawn from Corbett [1976]; F redrawn from Román & Hirschmann [1969]).

Table 3.4: Morphometrics of females and males of *Pratylenchus bolivianus* Corbett, 1983 and *Pratylenchus brachyurus* (Godfrey, 1929) Filipjev & Schuurmans Stekhoven, 1941. All measurements are in µm unless stated otherwise.

Species	***P. bolivianus* Corbett, 1983**	***P. brachyurus* (Godfrey, 1929) Filipjev & Schuurmans Stekhoven, 1941**		
Population	**Range**	**South African**	**Range**	
	♀	♀	♀	♂
L	530–720	420–570	390–750	460–560
a	25.0–33.0	15.3–22.7	15.0–34.0	27.0–29.0
b	3.9–8.2	–	3.3–10.0	6.0
b'	3.4–4.9	3.4–5.2	2.9–5.2	–
c	16.0–22.0	14.7–21.9	11.8–22.0	21.0
c'	–	–	1.6–2.8	–
V / T	77–83	84–89	[71]82–89	51–53
OV1	–	30–57	30–57	–
Number of annuli in labial region	3–4	2	2–[4]	2–[4]
Stylet length	17–20	17–21	17–22	19
Dorsal gland opening from base of stylet knobs	2.7–4.0	–	2.0–2.5	–
Oesophageal gland overlap length	18–49	–	31–80	–
Excretory pore from anterior end	89–105	49–121	49–121	–
Oesophagus length	104–153	–	111–167	–
Post–vulval uterine branch length	22–31	9–28	5–30	–
Spicule length	–	–	–	–
Gubernaculum length	–	–	–	–
Number of annuli posterior to phasmid	10–15	–	–	–
Number of annuli from anus to phasmid	–	8–12	8–12	–
Ventral tail annuli	15–19 [24]	15–21	12–24	–
Tail length	28[a]	22–34	20–35	–

– Indicates structure not present or value not available.

[a] Indicates range not available.

Values in [] indicated values found in extreme cases.

3.5.2.4 *Pratylenchus coffeae* (Zimmermann, 1898) Filipjev & Schuurmans Stekhoven, 1941

= *Tylenchus coffeae* Zimmermann, 1898

= *Anguillulina coffeae* (Zimmermann, 1898) Goodey, 1932

= *Tylenchus musicola* Cobb, 1919

= *Anguillulina musicola* (Cobb, 1919) Goodey, 1932

= *Pratylenchus musicola* (Cobb, 1919) Filipjev, 1936

= *Tylenchus mahogani* Cobb, 1920

= *Anguillulina mahogani* (Cobb, 1920) Goodey, 1932

= *Pratylenchus mahogani* (Cobb, 1920) Filipjev, 1936

= *Radopholus brassicae* Shahina & Maqbool, 1996

= *Pratylenchus brassicae* (Shahina & Maqbool, 1996) Siddiqi, 2000

Figure 3.7

Measurements: Table 3.5

Pratylenchus coffeae (Zimmermann, 1898) Filipjev & Schuurmans Stekhoven, 1941 was described from the roots of *Coffeae* L. in Indonesia (Zimmermann 1898) and has since been reported from all the biogeographic regions (except Antarctic) associated with several hosts including crops, cultivated ornamentals and weeds (Castillo & Vovlas 2007). The morphometrics of *P. coffeae* (Table 3.5) is a combination of populations reported globally (Goodey 1932; Sher & Allen 1953; Loof 1960; Thorne 1961; Román & Hirschmann 1969; Van den Berg 1971; Knobloch & Laughlin 1973; Tarjan & Frederick 1974; Boonduang & Pliansinchai 1980; Bajaj & Bhatti 1984; Ryss 1988; Loof 1991; Mizukubo 1992; Pourjam *et al.* 1997; Inserra *et al.* 2001; Castillo & Vovlas 2007; Tuyet *et al.* 2012;

Geraert 2013). A population of *P. coffeae* from South Africa was described by Van den Berg (1971). See section 3.8-3.9 for distribution and host range in South Africa (Figure 3.34, Table 3.25).

Description:

Neotype (Sher & Allen 1953): Female: L = 590 µm; a = 34.0; b = 6.3; c = 21.0; V = 82 %; stylet length = 18 µm.

Female: Cuticle distinctly annulated. Lateral field with four, occasionally five or six lines. Labial region slightly off set from body contour, two distinct lip annuli, sometimes three on one side. The *en face* view of labial region with fusion of subdorsal, subventral and lateral lips with oral disc (Group 1, Figure 3.3). Stylet knobs round to oblong. Excretory pore situated at 68-114 µm from anterior end. Oesophageal gland overlap 24-85 µm long. Spermatheca large, broadly oval to nearly rounded with sperm. Intra-uterine eggs may contain embryos. Post-vulval uterine sac up to 100 µm long, 1.0-1.5 times the corresponding body diameter, with terminal rudimentary ovary, sometimes with distinct oocytes. Phasmid slightly posterior to mid tail. Tail 1.5-2.5 times anal body diameter, tail tip indented, sometimes appearing smoothly rounded, truncate or irregularly crenate.

Male: Abundant. Similar to female. Spicules slender, 15-21 µm long, manubrium well demarcated and ventrally arcuate shaft. Gubernaculum 4-7 µm long, hypoptygma prominent. Margins of bursa faintly crenate.

Diagnosis (Castillo & Vovlas 2007):

Pratylenchus coffeae is characterised by slightly off set labial region with two distinct annuli, stylet shorter than 15 µm, post-vulval uterine sac with rudimentary ovary, 1.0-1.5 times body diameter, female tail tip truncate or hemispherical and abundance of males.

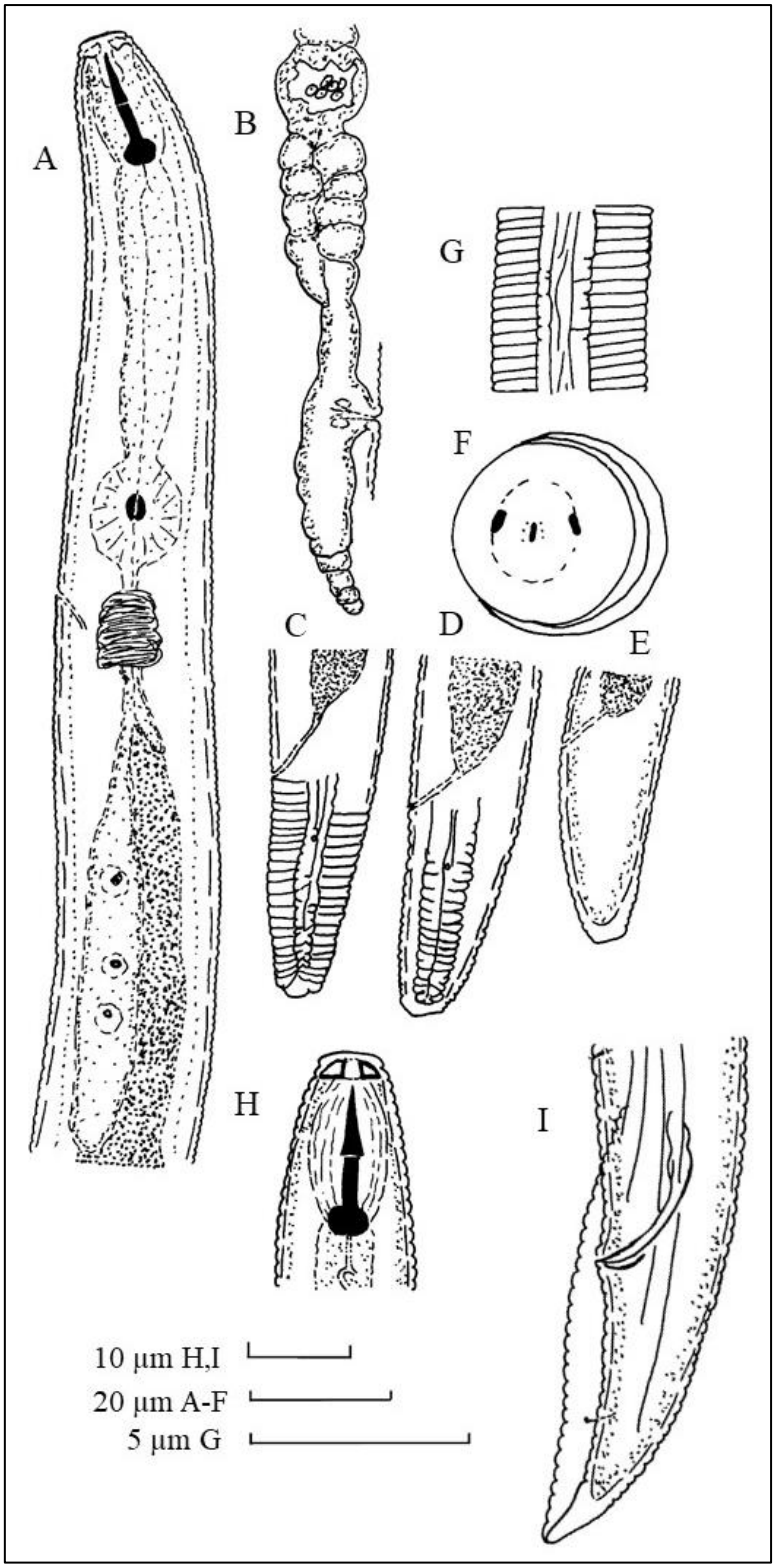


Figure 3.7: *Pratylenchus coffeae* (Zimmermann, 1898) Filipjev & Schuurmans Stekhoven, 1941; A: Female oesophageal region; B: Female genital tract showing post-vulval uterine branch and spermatheca; C-E: female tails; F: *En face* view of labial region; G: Lateral field; H: Male labial region; I: Male tail (A-G redrawn from Inserra *et al.* [2001]; H-I redrawn from Román & Hirschmann [1969]).

Table 3.5: Morphometrics of females and males of *Pratylenchus coffeae* (Zimmermann, 1898) Filipjev & Schuurmans Stekhoven, 1941. All measurements are in µm unless stated otherwise.

Species	***P. coffeae* (Zimmermann, 1898) Filipjev & Schuurmans Stekhoven, 1941**			
Population	**South African**		**Range**	
	♀	♂	♀	♂
L	590–620	490–551	330–839	310–700
a	14.1–20.3	18.4–20.3	14.1–38.0	23.8–40.0
b	–	–	4.1–8.7	4.5–7.7
b'	4.1–4.6	3.9–5.1	2.3–7.6	3.1–5.1
c	25.9–33.4	22.1–22.3	13.7–33.4	15.0–25.4
c'	–	–	1.6–2.8	2.3–2.8
o	–	–	–	–
m	–	–	46.2–54.6	–
V / T	83–84	–	[69]76–86	32–58
OV1	–	–	25–63	–
Number of annuli in labial region	2	2	2	2
Stylet length	17–18	15[a]	13–18	12–17
Dorsal gland opening from base of stylet knobs	–	–	0.7–4.0	–
Oesophageal gland overlap length	–	–	24–85	35–60
Excretory pore from anterior end	85–114	85–87	68–114	78–87
Oesophagus length	–	–	90–140	80–120
Post–vulval uterine branch length	24–56	–	17–50 [100]	–
Spicule length	–	17–18	–	15–21
Gubernaculum length	–	5[a]	–	4–7
Ventral tail annuli	15–21	–	15–32	–
Tail length	19–23	–	19–27[40]	25–35

– Indicates structure not present or value not available.
[a] Indicates range not available.
Values in [] indicated values found in extreme cases.

3.5.2.5 *Pratylenchus crenatus* Loof, 1960

= *Pratylenchus clavicaudatus* Baranovskaya & Haque, 1968

= *Pratylenchus pratensis* apud Thorne, 1949

Figure 3.8

Measurements: Table 3.6

Pratylenchus crenatus Loof, 1960 is a polyphagous species, with cosmopolitan distribution (reported from the Afrotropical, Australasian, Nearctic, Neotropical, Oriental, and Palearctic biogeographic regions). This species has been reported from various hosts, although cereals and grasses seems to be favoured (Castillo & Vovlas 2007). The morphometrics of *P. crenatus* (Table 3.6) is a combination of the type population as well as morphometrics of other populations (Baranovskaya & Haque 1968; Seinhorst 1968; Loof 1978; Inserra *et al*. 1979; Ryss 1988; Loof 1991; Brzeski 1998; Torres & Chaves 1999; Karssen & Brinkman 2003; Castillo & Vovlas 2007; Geraert 2013) including the South African populations by Van den Berg (1971; 1986). See section 3.8-3.9 for distribution and host range in South Africa (Figure 3.34, Table 3.25).

Description:

Holotype (Loof 1960): Female: L = 450 µm; a = 25.0; b = 6.0; c = 21.0; V = 81 %; stylet length = 17 µm.

Female: Habitus usually coiled. Body annuli prominent. Lateral field with six lines, outer lines strongly crenate, inner lines interrupted. Labial region with three annuli, *en face* with no division between the submedian and lateral segments (Groups 1, Figure 3.3). Oesophageal gland overlap 18-42 µm long. Duct of excretory pore swollen, situated 62-93 µm from anterior end. Vulva at 78-86 % of

body length. Oocytes in anterior tract in a single row, except at anterior tip with more than one row, spermatheca absent or small, empty, not functional. Post-vulval uterine branch undifferentiated, two body diameters long, 40-50% distance form vulva to anus or 22-32 µm long. Phasmid situated 3-11 annuli posterior to anus. Tail broadly rounded, 18-30 annuli on ventral side, tail tip distinctly annulated, often spatulate.

Male: Extremely rare, only known from one specimen (Karsen & Brinkman 2003). Morphology similar to females, except in reproductive organs. Spicules (17 µm) and gubernaculum (4 µm) ventrally curved. Tail 20 µm long, tail tip conical and pointed. Bursa arising slightly anterior to spicule head, enveloping tail.

Diagnosis (Castillo & Vovlas 2007):

Pratylenchus crenatus is characterised by lip region with three annuli, long post-vulval uterine branch, crenate tail tip and males usually absent.

3.5.2.6 *Pratylenchus delattrei* Luc, 1958

= *Pratylenchus singhi* Das & Sultana, 1979

= *Pratylenchus portulacus* Zarina & Maqbool, 1998

= *Pratylenchus graminis* Subramaniyan & Sivakumar, 1991

Figure 3.9

Measurements: Table 3.6

Pratylenchus delattrei Luc, 1958 was described from the roots of *Gossypium* sp. in southern Madagascar (Luc 1958). The species has been reported from the Afrotropical, Oriental and Palearctic biogeographical regions and has been associated with several hosts (Castillo & Vovlas 2007). The morphometrics of *P. delattrei* is a combination of the type population by Luc (1958) as well as information in several other descriptions (Das & Sultana 1979; Subramaniyan & Sivakumar 1991; Zarina & Maqbool 1998; Castillo & Vovlas 2007; Geraert 2013). The first record of *P. delattrei* from South Africa was in 1978 from the Gauteng Province, however a South African population of *P. delattrei* is described for the first time in the current paper (Table 3.5, and Chapter 2: Article 1, Plant–Parasitic Nematodes of the Telperion Nature Reserve). See section 3.8-3.9 for distribution and host range in South Africa (Figure 3.35, Table 3.25).

Description:

Type population (Luc 1958): Female: L = 386-470 µm; a = 20.4-25.8; b = 3.7-4.8; c = 18.0-22.3; V = 73-81 %; stylet length = 17-18 µm.

Female: Habitus slightly ventrally arcuate. Annuli 1.0-1.5 µm wide at mid body. Lateral field with four lines, one third of corresponding body diameter. Labial region not off set from body contour, with three distinct annuli, labial framework well developed. Stylet robust, stylet knobs flattened. Dorsal gland opening 1.5-2.4 µm posterior to base of stylet knobs. Oesophageal gland overlaps intestine ventrally, overlap 11-24 µm long, median bulb rounded to oval with distinct valve. Nerve ring encircles isthmus in posterior half, anterior to excretory pore located 54-77 µm from anterior end. Hemizonid three annuli long, situated just anterior to excretory pore. Spermatheca rounded, empty. Post-vulval uterine branch longer than vulval body diameter, 11-21 µm long. Phasmid situated mid tail. Tail with 14-20 ventral annuli, subcylindrical, tail tip smooth, conical to rounded.

Male: Unknown.

Diagnosis (Castillo & Vovlas 2007):

Pratylenchus delattrei is characterised by labial region with three annuli, lateral field with four incisures, tail subcylindrical with smooth, conical to rounded tip, and absence of males.

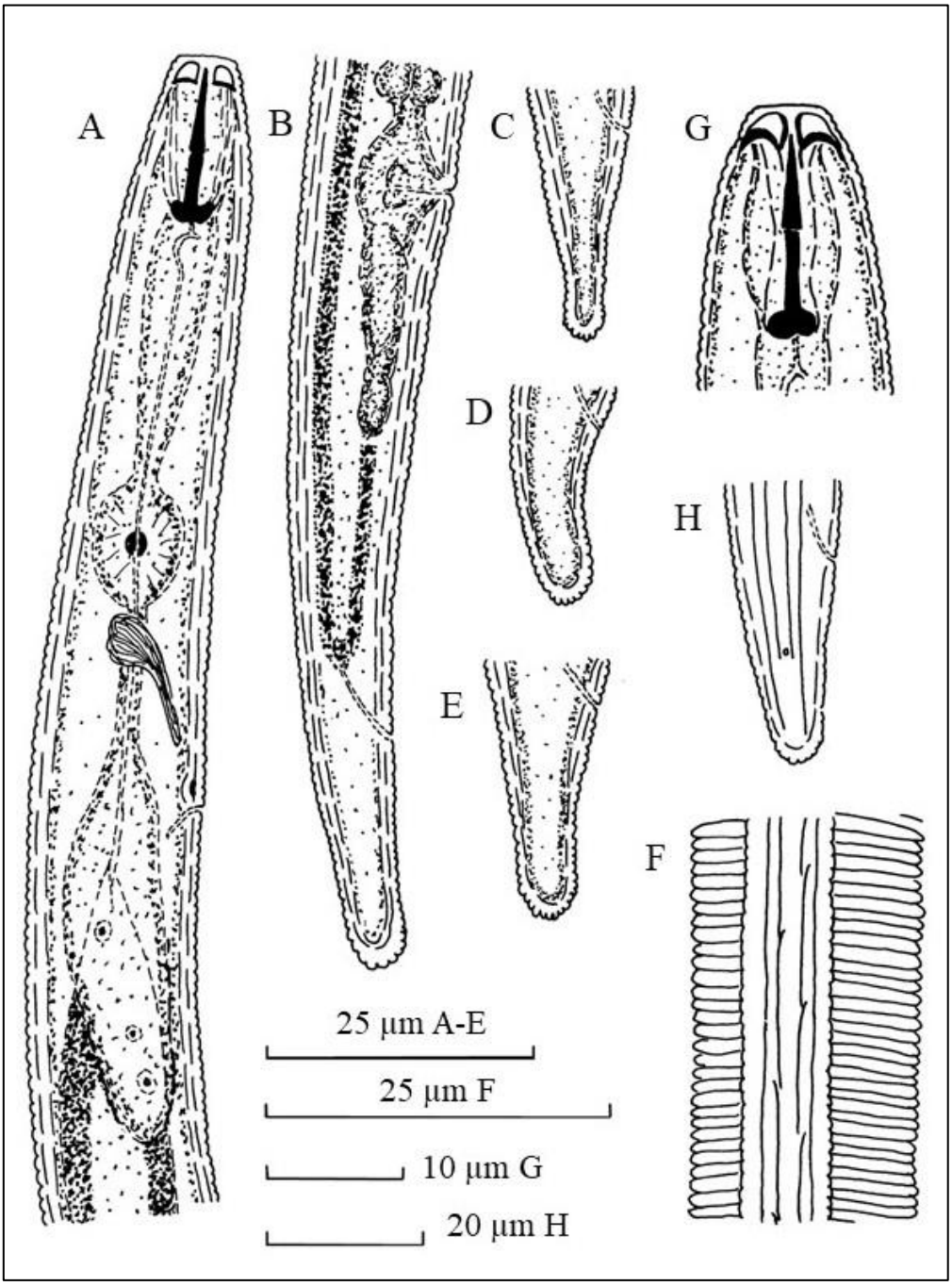


Figure 3.8: *Pratylenchus crenatus* Loof, 1960 A: Oesophageal region; B: Posterior region showing post-vulval uterine branch and tail; C-E: Variation in tail shape; F: Lateral field; G: Labial region; H: Tail region showing end of lateral field (A-E redrawn from Loof [1991]; F redrawn from Castillo & Vovlas [2007]; G-H redrawn from Brzeski [1998]).

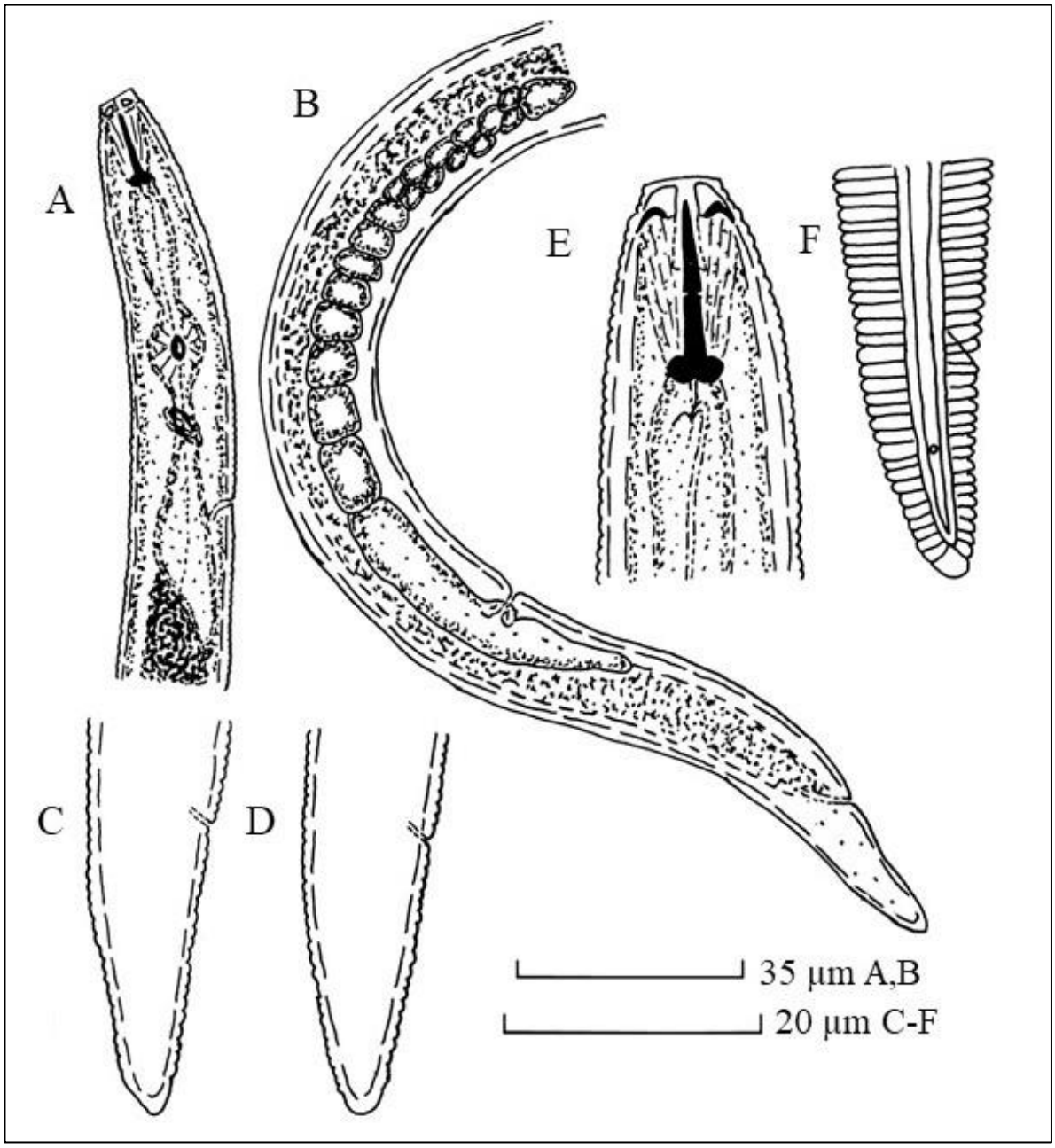


Figure 3.9: *Pratylenchus delattrei* Luc, 1958 A: Oesophageal region; B: Posterior region showing genital tract and tail; C-D: Tail region; E: Labial region; F: Tail region showing end of lateral field (Redrawn from Luc [1958]).

Table 3.6: Morphometrics of females and males of *Pratylenchus crenatus* Loof, 1960 and *Pratylenchus delattrei* Luc, 1958. All measurements are in µm unless stated otherwise.

Species	***P. crenatus* Loof, 1960**			***P. delattrei* Luc, 1958**	
Population	**South African**	**Range**		**South African**	**Range**
	♀	♀	♂	♀	♀
L	500–608	320–700	432 [a]	297–389	297–570
a	24.2–33.1	18.0–33.1	28.4 [a]	17.6–28.0	17.6–30.7
b	3.8–5.1	3.8–7.9	6[a]	3.0–3.5	3.0–8.8
b'	4.2–6.1	4.2–6.1		–	3.0–4.1
c	16.6–21.4	16.0–27.0	21.4 [a]	15.9–22.0	14.4–26.3
c'	2.2–2.9	2.0–3.0	–	1.6–2.3	1.2–3.1
o	9.9–15.4	–	–	11.4–15.8	11.4–15.8
m	47.0–49.0	–	–	33.0–45.0	33.0–45.0
V / T	81–84	78–86	57 [a]	74–77	72–86
OV1	20–29	20–48	–	26–35	26–35
Number of annuli in labial region	3	3	3 [a]	3	3
Stylet length	17–19	14–19	16 [a]	12–15	12–18
Dorsal gland opening from base of stylet knobs	1.8–2.9	1.8–2.9	2.5[a]	1.5–2.2	1.5–2.4
Oesophageal gland overlap length	18–34	18–42	25[a]	14–36	14–36
Excretory pore from anterior end	81–93	62–93	76[a]	54–74	54–77
Oesophagus length	106–143	106–143	–	85–121	85–121
Post–vulval uterine branch length	22–32	22–32	–	11–21	11–21
Spicule length	–	–	17[a]	–	–
Gubernaculum length	–	–	4[a]	–	–
Number of annuli from anus to phasmid	3–11	3–11	–	–	–
Ventral tail annuli	21–25	18–30	–	14–20	14–20
Tail length	28–37	18–37	20[a]	16–20	16–20

– Indicates structure not present or value not available.
[a] Indicates range not available.

3.5.2.7 *Pratylenchus fallax* Seinhorst, 1968

Figure 3.10

Measurements: Table 3.7

Pratylenchus fallax Seinhorst, 1968 was described from the Netherlands in loam soils in a *Malus* Mill. orchard with grass cover and have been reported from the Afrotropical, Nearctic, Oriental, and Palearctic biogeographical regions associated with several crops (Seinhorst 1968; Castillo & Vovlas 2007). The morphometrical range of *P. fallax* (Table 3.7) is a combination of the morphometrics in the type description by Seinhorst (1968) as well as other described populations (Brzeski & Szczygiel 1977; Seinhorst 1977a; Loof 1978; Ryss 1988; Loof 1991; Brzeski 1998; Castillo & Vovlas 2007; Geraert 2013). No morphometrical data is available on the populations of *P. fallax* reported from South Africa. See section 3.8-3.9 for distribution and host range in South Africa (Figure 3.35, Table 3.25).

Description:

Holotype (Seinhorst 1968): Female: L = 520 µm; a = 29.0; b = 6.2; c = 18.0; V = 80 %; stylet length = 17 µm.

Female: Habitus straight to slightly curved. Lateral field with four lines, occasionally some additional oblique lines in inner band, outer two lines extending past phasmids, sometimes aerolated past phasmid. Labial region with three, often flat, obscure annuli, labial framework extending two annuli into body, *en face* view with characteristics of Group 3 (Figure 3.3); distinctive dumb-bell shape, submedian segments slightly larger than lateral segments. Stylet knobs anteriorly flattened or pointing forward. Excretory pore at level of or just posterior to nerve ring, 69-90 µm. Oesophageal gland overlap 20-71 µm long. Ovary outstretched, oocytes filed in a single row, except at anterior tip, spermatheca

round, filled with sperm or empty. Post-vulval uterine branch slightly longer than body diameter, often with two to three rudimentary cells at end. Phasmids located 9-13 annuli from terminus. Tail conical, 16-26 annuli on ventral side, tail tip rounded, distinctly crenate, occasionally with irregular contour, rarely smooth.

Male: Similar to female. Testis with double row spermatocytes. Spicules slender, well marked manubrium and ventrally arcuate shaft, gubernaculum 4 µm long. Bursa with distinctly crenate margins.

Diagnosis (Castillo & Vovlas 2007):

Pratylenchus fallax is characterised by labial region with three, often flat and obscure annuli, tail conical with rounded distinctly crenate tip, spermatheca round, often filled with sperm, abundance of males, 1:5 females.

3.5.2.8 *Pratylenchus flakkensis* Seinhorst, 1968

Figure 3.11

Measurements: Table 3.7.

Pratylenchus flakkensis Seinhorst, 1968 was described from a pasture in The Netherlands (Seinhorst 1968). Since then, this species was reported from various hosts in the Afrotropical, Nearctic, Neotropical, and Palearctic biogeographical regions (Castillo & Vovlas 2007). Apart from the type population of *P. flakkensis*, by Seinhorst (1968), morphometric data on this species were obtained from Van den Berg (1976); Brzeski & Szczygiel (1977); Loof (1978); Ryss (1988); Brzeski (1998); Zarina & Maqbool (1998); Van den Berg & Quénéhervé (2000); Castillo & Vovlas (2007) and Geraert (2013) and was combined to create a morphometrical range for the species (Table 3.7). The South African

population of the species was described in Van den Berg (1976). See section 3.8-3.9 for distribution and host range in South Africa (Figure 3.35, Table 3.25).

Description:

Holotype (Seinhorst 1968): Female: L =480 µm; a = 25.0; b = 5.5; c = 19.0; V = 76 %; stylet length = 17 µm.

Female: Habitus slightly curved to almost straight. Lateral field with four lines, inner band plain, outer bands aerolated on posterior third of tail. Labial region with two annuli, labial framework strongly sclerotised, extending two annuli into body. Stylet knobs with forward pointed anterior margins. Oesophageal lobe 1.3-3.6 body widths long, 24-62 µm. Excretory pore at level of or slightly posterior to nerve ring, 63-91 µm from anterior end. Ovary outstretched, oocytes filed in a single row, spermatheca round to angular, filled with sperm. Post-vulval uterine branch 11-27 µm long, 0.9-1.9 corresponding body widths. Phasmid located 12-18 annuli from terminus. Tail conical, 14-27 annuli on ventral side, tip faintly annulated.

Male: Similar to female except in the following. First labial annulus distinctly wider than second. Testis with double row spermatocytes. Spicules 14-18 µm long, gubernaculum 4-7 µm long. Bursa with coarsely crenate margins. Phasmids located slightly over one third tail length from terminus.

Diagnosis (Castillo & Vovlas 2007):

Pratylenchus flakkensis is distinguished from other species by labial region with two annuli, stylet knobs with forward pointing anterior margins, annulated tail tip and presence of males.

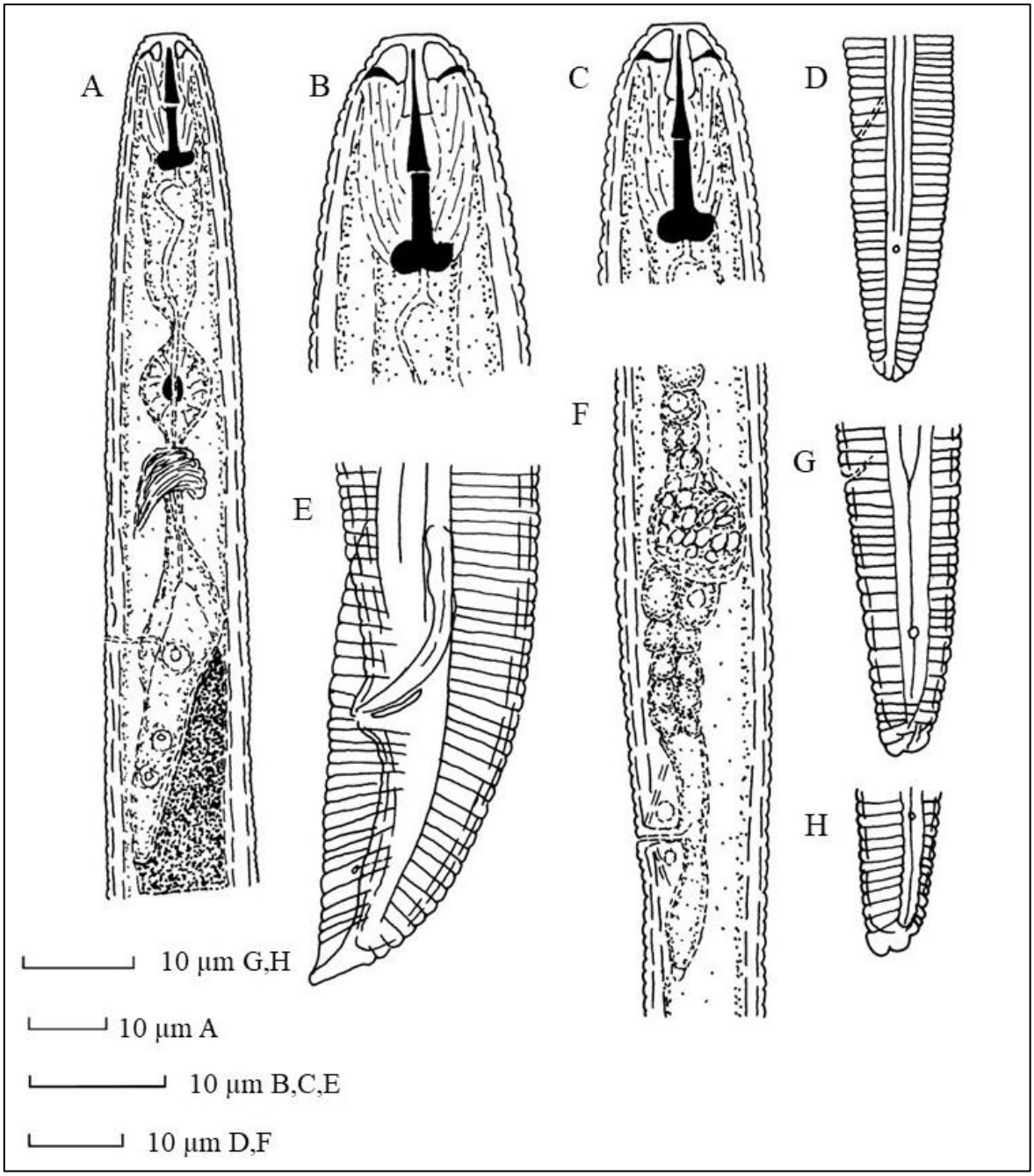


Figure 3.10: *Pratylenchus fallax* Seinhorst, 1968 A: Female oesophageal region; B: Female labial region; C: Male labial region; D, G-H: Female tails; E: Male tail; F: Vulval region showing spermatheca and post-vulval uterine branch (B-F redrawn from Seinhorst [1968]; A, G-H redrawn from Seinhorst [1977a]).

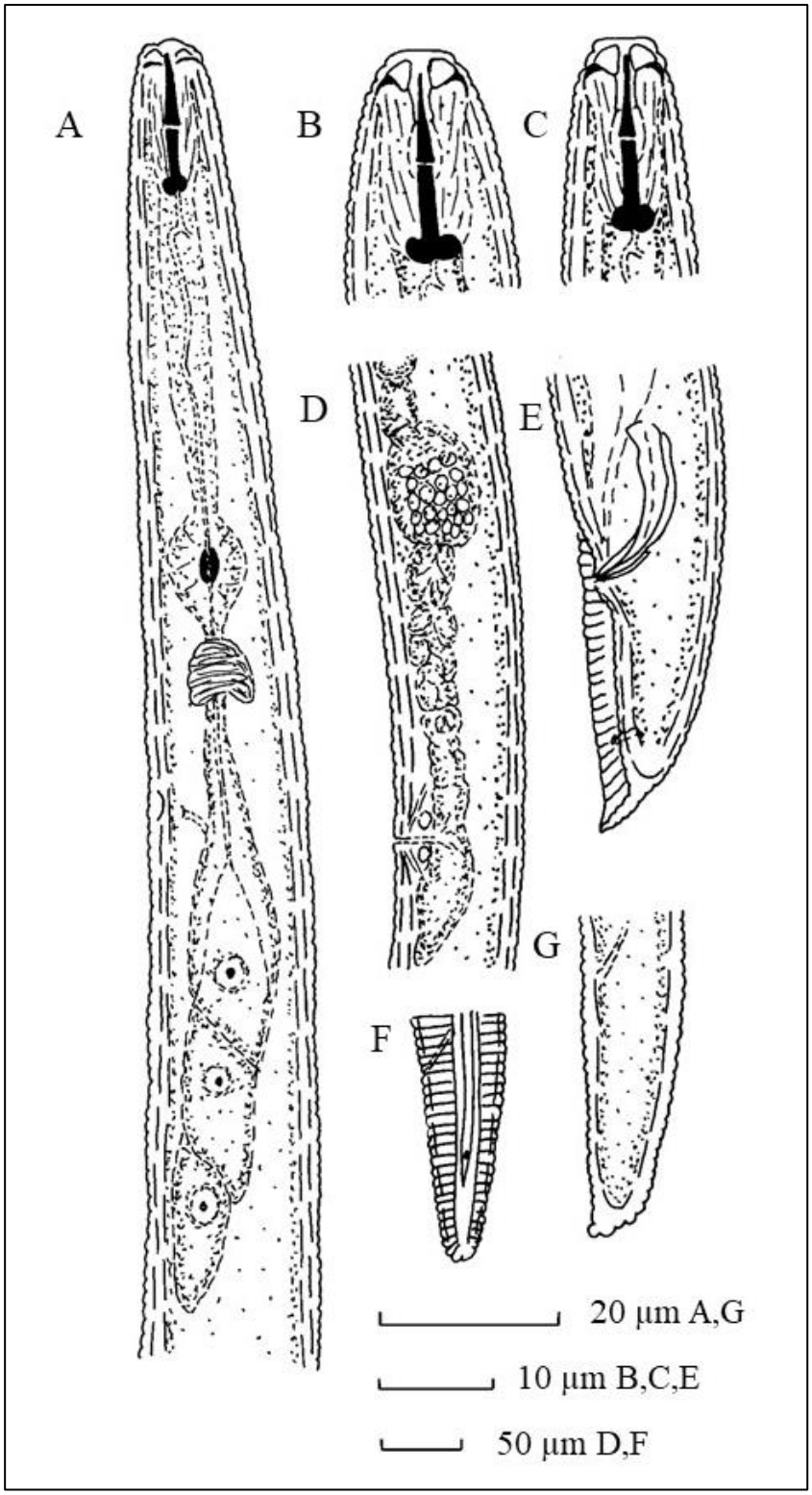


Figure 3.11: *Pratylenchus flakkensis* Seinhorst, 1968 A: Female oesophageal region; B: Female labial region ; C: Male labial region; D: Vulval region showing spermatheca and post-vulval uterine branch; E: Male tail; F-G: Female tails (B-F redrawn from Seinhorst [1968]; A, G redrawn from Van den Berg & Queneherve [2000]).

Table 3.7: Morphometrics of females and males of *Pratylenchus fallax* Seinhorst, 1968 and *Pratylenchus flakkensis* Seinhorst, 1968. All measurements are in µm unless stated otherwise.

Species	***P. fallax* Seinhorst, 1968**		***P. flakkensis* Seinhorst, 1968**			
Population	**Range**		**South African**		**Range**	
	♀	♂	♀	♂	♀	♂
L	410–580	380–530	388–497	410	380–600	340–530
a	19.0–33.0	24.0–32.0	21.3–33.3	30.9	18.0–33.3	25.0–37.0
b	4.8–7.4	5.0–6.3	4.3–6.1	–	3.5–7.1	3.5–7.5
b'	3.2–5.3	3.7–4.6	–	–	3.6–4.8	3.8–4.9
c	13.0–26.0	15.0–25.0	13.9–18.2	15.5	12.0–34.7	14.6–24.0
c'	1.6–3.0	1.9–3.1	1.7–3.0	3.0	1.4–3.0	1.4–3.0
o	–	–	8.9–24.4	15.8	8.9–30	15.8[a]
M	–	–	–	–	7.5–8.5	–
V / T	76–83	–	70–79	–	70–83	30–47
OV1	–	–	26–51	–	21–51	–
Number of annuli in labial region	3	3	2	2	2	2
Stylet length	14–17	15–16	14–17	14	13–18	13–16
Dorsal gland opening from base of stylet knobs	–	–	–	–	4.0[a]	–
Oesophageal gland overlap length	20–71	–	–	–	24–62	–
Excretory pore from anterior end	69–90	–	63–84	–	63–91	–
Post–vulval uterine branch length	–	–	14–27	–	11–27	–
Spicule length	–	14–16	–	16	–	14–18
Gubernaculum length	–	4[a]	–	5	–	4–7
Number of annuli from anus to phasmid	–	–	7–14	–	7–14	–
Number of annuli posterior to phasmid	9–13	–	–	–	12–18	–
Ventral tail annuli	16–26	–	20–24	–	14–27	–
Tail length	19–38	–	21–29	–	20–38	–

– Indicates structure not present or value not available.
[a] Indicates range not available.

3.5.2.9 *Pratylenchus goodeyi* Sher & Allen, 1953

= *Tylenchus musicola* apud Goodey, 1928

= *Anguillulina musicola* apud Goodey, 1932

Figure 3.12

Measurements: Table 3.8

Described from the roots of *Musa* sp. in the United Kingdom, *Pratylenchus goodeyi* Sher & Allen, 1953, has been reported from the Afrotropical, Australasian, and Palearctic biogeographical regions associated with several hosts (Sher & Allen 1953; Castillo & Vovlas 2007). Its main host seems to be species of *Musa*, with several reports from Afromontane areas (Castillo & Vovlas 2007). The morphometrical data of the original description by Sher & Allen (1953) in combination with several other descriptions of the species was used to establish a morphometric range (Table 3.8) for the species (Loof 1960; Thorne 1961; Loof 1978; Machon & Hunt, 1985; Loof 1991; Sakwe & Geraert 1994; Nico *et al.* 1999; Castillo & Vovlas 2007; Geraert 2013). No morphometrical data on South African populations is available. See section 3.8-3.9 for distribution and host range in South Africa (Figure 3.35, Table 3.25).

Description:

Holotype (Sher & Allen 1953): Female: L = 590 µm; a = 27.0; b = 5.8; c = 18.0; V = 74 %; stylet length = 17 µm.

Female: Habitus almost straight, slightly ventrally arcuate posteriorly. Lateral field with four inconspicuous lines, outer bands partially aerolated. Labial region with four annuli, rarely three, slightly set off from body contour. *En face* view characterised by fusion of subdorsal, subventral and lateral lips

with oral disc (Group 1, Figure 3.3). Stylet with distinct anteriorly flattened stylet knobs. Hemizonid just anterior to excretory pore. Oesophageal gland overlap 27-63 µm long. Excretory pore 65-99 µm from anterior end. Anterior ovary with single row of oocytes except for two rows near anterior end, spermatheca large, sub-rectangular, filled with sperm. Post-vulval uterine branch about one body diameter long, 14-35 µm, undifferentiated. Phasmid conspicuous, 0-14 annuli from terminus. Tail tapering to a narrow, almost pointed, unstriated terminus, 18-35 (usually 22-24) annuli on ventral side.

Male: Common. Similar to female. Single outstretched testis with spermatocytes arranged in double row. Spicules slender, arcuate, 14-21 µm long. Gubernaculum simple, 5-8 µm long. Bursa peloderan.

Diagnosis (Castillo & Vovlas 2007):

Pratylenchus goodeyi is characterised by labial region with four annuli, four inconspicuous lines in lateral field, two outer bands partially aerolated, large, sub-rectangular spermatheca, and conoid, ventrally concave tail with smooth tip.

3.5.2.10 *Pratylenchus hexincisus* Taylor & Jenkins, 1957

Figure 3.13

Measurements: Table 3.8

Pratylenchus hexincisus Taylor & Jenkins, 1957 was described from *Z. mays* in the United States of America and has since been reported from various crops in the Afrotropical, Nearctic, Neotropical and Palearctic biogeographical regions (Castillo & Vovlas 2007). The morphometrical range (Table 3.8) of this species includes data of the type population as well as other populations (Taylor

& Jenkins 1957; Loof 1960; Thorne 1961; Loof 1964; Thorne & Malek 1968; Loof 1978; Doucet 1988; Castillo & Vovlas 2007; Geraert 2013). Although this species has been reported from South Africa, no population have been described. See section 3.8-3.9 for distribution and host range in South Africa (Figure 3.36, Table 3.25).

Description:

Type population (Taylor & Jenkins 1957): Female: L = 340-540 µm; a = 19.0-31.0; b = 4.2-5.8; c = 17.0-24.0; V = 75-82 %; stylet length = 15 µm.

Female: Body short and stout, tapering towards both extremities. Cuticle with annuli, 1 µm wide. Lateral field with six lines except in extremities, at mid body lines irregular or broken, outer bands crenate, only four lines in tail region. Labial region low, flattened, bearing two annuli, second annulus larger than first, labial framework extending 1 µm into body. *En face* view divided into dumbbell-shaped submedian lip sectors fused with oval oral disc (Group 3, Figure 3.3). Stylet with somewhat rounded knobs. Dorsal gland opening 1.7-3.0 µm posterior to base of stylet knobs. Oesophageal gland overlaps intestine ventrally, which contains granules that are larger and denser towards ventral surface, overlap as long as corresponding body width, overlap 21-48 µm long. Hemizonid just anterior to excretory pore, situated 79-86 µm long. Vagina extending transversely one half of the corresponding body width, vulva prominent. Anterior ovary outstretched, single row oocytes except short region in anterior end, no spermatheca observed. Post-vulval uterine branch short, 12-31 µm long, as long as corresponding body diameter, undifferentiated. Phasmid in anterior one quarter to two fifths of tail. Tail slightly convex-conoid to broad, 15-22 obscure annuli on ventral side, tail tip rounded and smooth.

Male: Unknown.

Diagnosis (Castillo & Vovlas 2007):

Pratylenchus hexincisus is characterised by low, flat labial region with two annulilateral field with six lines, reduced to four lines towards extremities, rounded stylet knobs and convex-conoid tail with broad, smooth, rounded terminus.

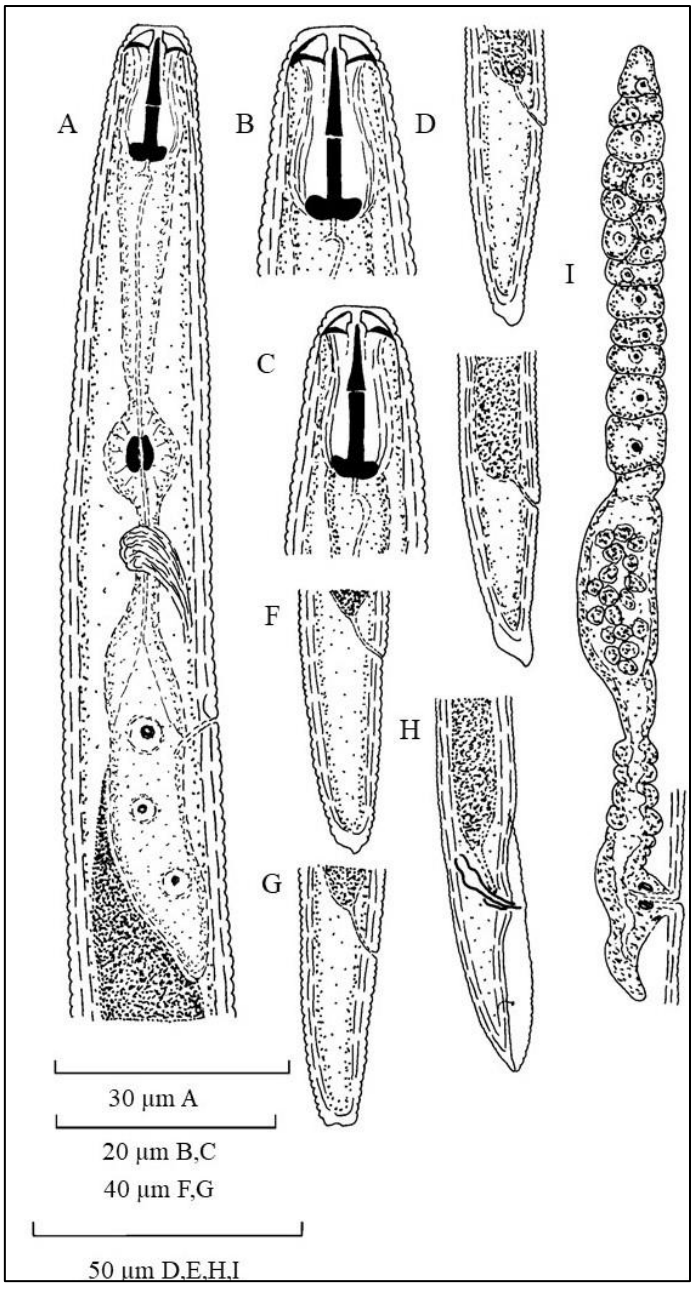


Figure 3.12: *Pratylenchus goodeyi* Sher & Allen, 1953 A: Female oesophageal region; B: Female labial region; C: Male labial region; D-G: Female tails; H: Male tail; I: Female genital tract showing spermatheca and post-vulval uterine branch (A-C, F-G redrawn from Sakwe & Geraert [1994]; D-E, H-I redrawn from Machon & Hunt [1985]).

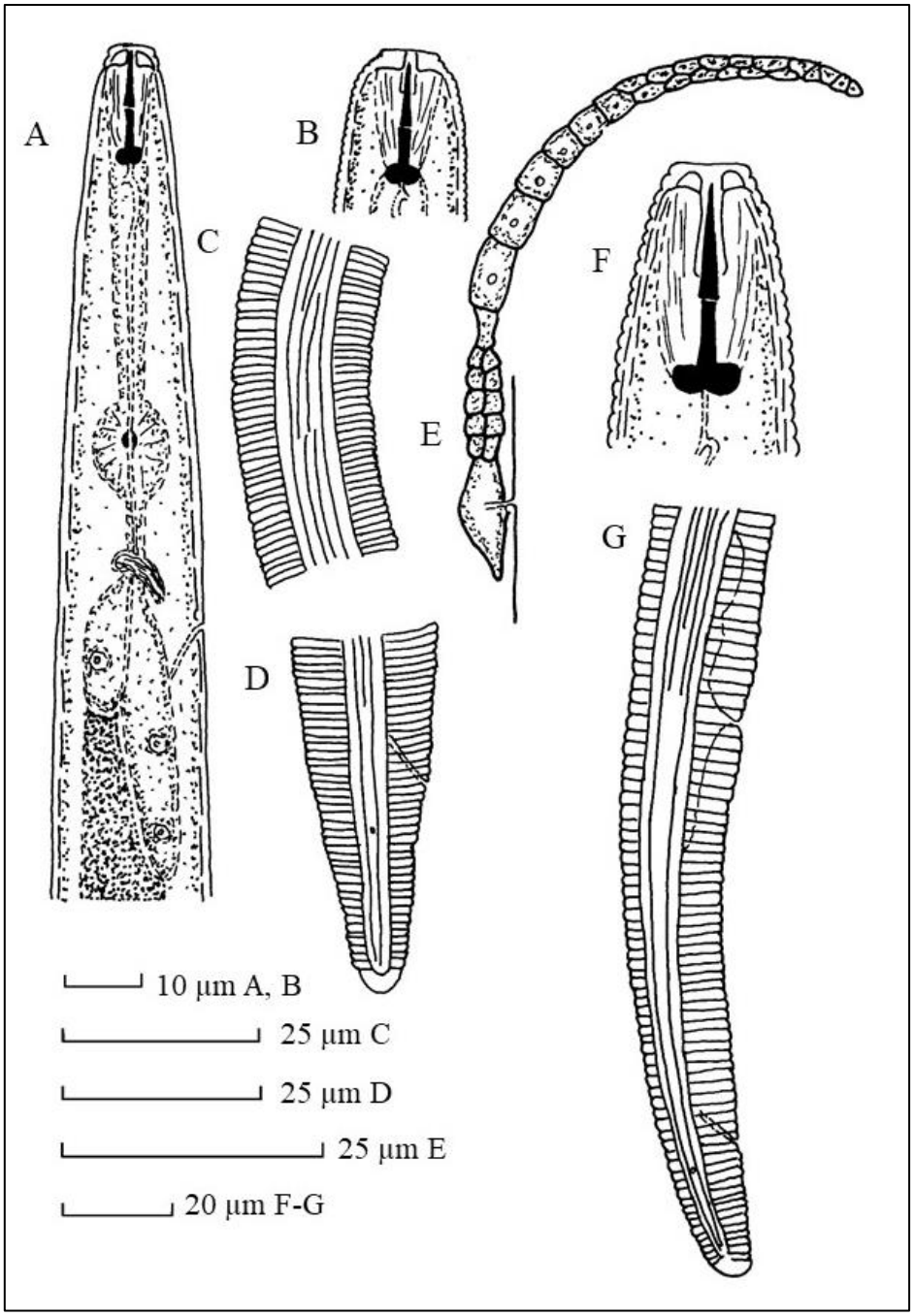


Figure 3.13: *Pratylenchus hexincisus* Taylor & Jenkins, 1957 A: Oeosphageal region; B, F: Labial region; C: Lateral field; D: Tail region; E: Genital tract showing post-vulval uterine branch; G: Posterior region showing lateral field in vulval and tail region (A redrawn from Thorne & Malek [1968]; B-D redrawn from Taylor & Jenkins [1957]; F-G redrawn from Doucet [1988]).

3.5.2.11 *Pratylenchus loosi* Loof, 1960

= *Pratylenchus coffeae* apud Loos, 1953

Figure 3.14

Measurements: Table 3.8

Pratylenchus loosi Loof, 1960 was described in Sri Lanka from *Camellia sinensis* (L.) Kuntze by Loof (1960). This species has since been reported from several crops in the Afrotropical, Nearctic, Oriental, and Palearctic biogeographical regions and is an important pathogen of *C. sinensis* in various parts of the world (Castillo & Vovlas 2007). Morphometrical data of *P. loosi* from a number of publications was combined with the type description by Loof (1960) to give a morphometrical range (Table 3.8) of the species (Seinhorst 1977b; Loof 1978; Ryss 1988; Loof 1991; Pourjam *et al.* 1997; Inserra *et al.* 2001; Castillo & Vovlas 2007; Geraert 2013). Even though *P. loosi* has been reported from South Africa before, no morphometric information is available on the South African populations. See section 3.8-3.9 for distribution and host range in South Africa (Figure 3.36, Table 3.25).

Description:

Holotype (Loof 1960): Female: L = 604 µm; a = 27.7; b = 6.8; c = 17.8; V = 82 %; stylet length = 16 µm.

Female: Body slender, habitus almost straight, narrowing abruptly posterior to vulva, then cylindrical to anus. Lateral field broad, four lines (occasionally five to six), three lines posterior to anus. Labial region rounded with two annuli (rarely two on one side three on other, some with three one side and four on the other side), labial framework extending one or two annuli into body. *En face* view a distinct labial disc with lateral labial sectors fused, no separation labial disc and submedian lips (Group

1, Figure 3.3). Stylet knobs rounded, broadly fused to shaft. Oesophageal gland overlap 35-60 µm long. Excretory pore situated 77-100 µm from anterior end. Spermatheca oval, rarely round, up to 20 µm long, filled with sperm. Post-vulval uterine branch 15-40 µm long, 0.9-2.0 body diameters. Phasmids situated mid tail. Tail 2.5 times anal body diameter long, 22-35 ventral annuli, tapering gradually to smooth conical tip, with narrowly rounded terminus.

Male: Abundant. Similar to female. Spicules slender 15-20 µm long, with well demarcated manubrium and ventrally arcuate shaft. Gubernaculum 4-7 µm long. Bursa with faintly crenate margins.

Diagnosis (Castillo & Vovlas 2007):

Pratylenchus loosi is separated by other species based on rounded labial region bearing two annuli, oval spermatheca, rarely rounded, tail tapering gradually to smooth conical tip with narrowly rounded terminus and the abundance of males.

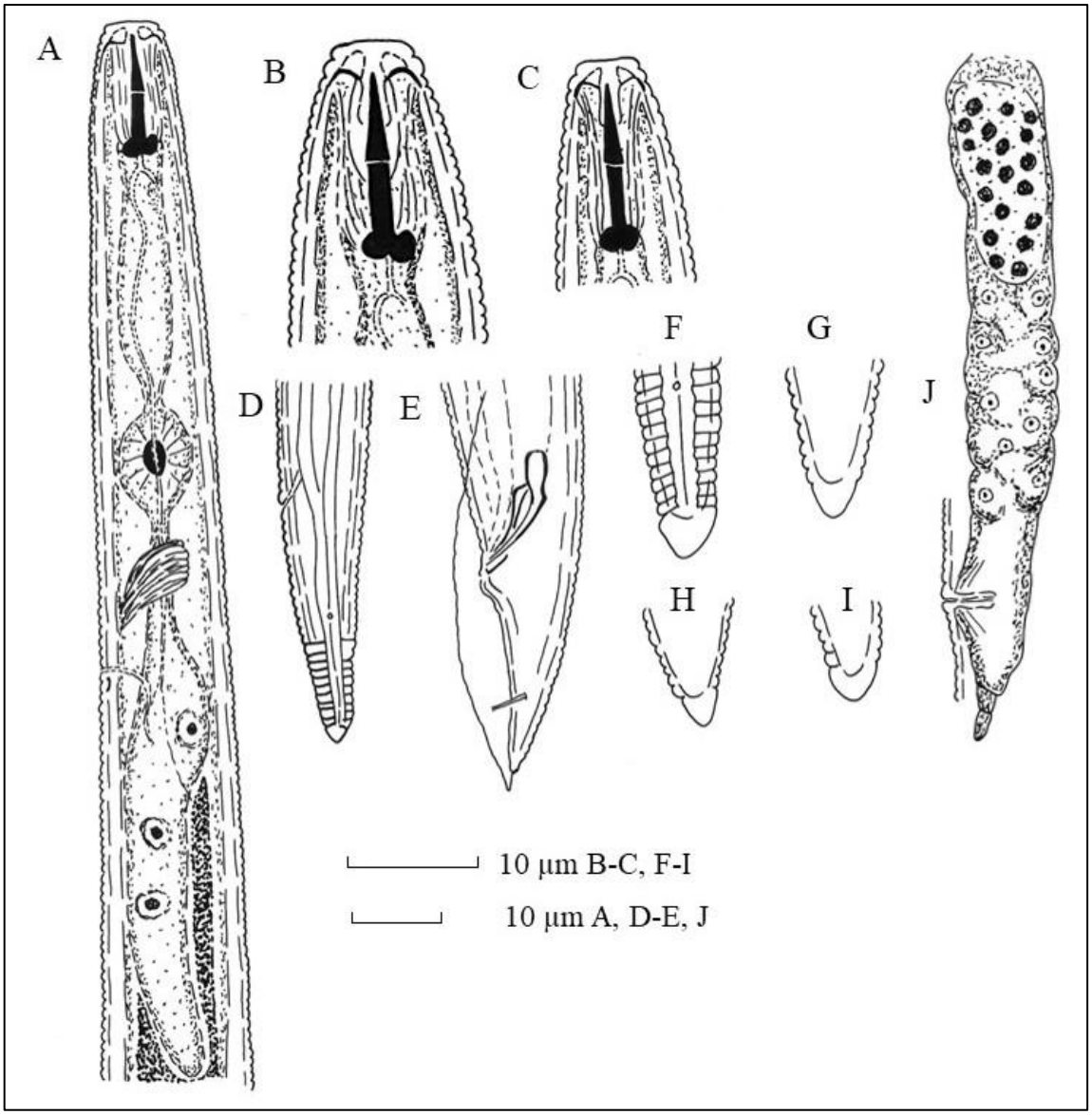


Figure 3.14: *Pratylenchus loosi* Loof, 1960 A: Female oesophageal region; B: Female labial region; C: Male labial region; D, F-I: Variation in shape of female tail; E: Male tail; J: Vulval region showing spermatheca and post-vulval uterine branch (Redrawn from Seinhorst [1977b]).

Table 3.8: Morphometrics of females and males of *Pratylenchus goodeyi* Sher & Allen, 1953; *Pratylenchus hexincisus* Taylor & Jenkins, 1961 and *Pratylenchus loosi* Loof, 1960. All measurements are in µm unless stated otherwise.

Species	***P. goodeyi* Sher & Allen, 1953**		***P. hexincisus* Taylor & Jenkins, 1961**	***P. loosi* Loof, 1960**	
Population	**Range**		**Range**	**Range**	
	♀	♂	♀	♀	♂
L	400–710	380–650	340–540	420–700	380–610
a	19.7–37	22.8–37	18.2–31.0	19.5–38.0	25.7–41.4
b	4.1–8.1	5.4–7.3	4.2–8.4	4.7–7.1	5.2–6.7
b'	2.9–5.3	3.8–5.1	3.9–4.6	3.5–5.0	3.4–4.5
c	13.8–20.0	12.5–25.0	16.1–28.9	16.2–25.0	17–24
c'	1.7–3.2	1.9–3.3	1.7–2.5	2.3–3.6	2.4–3.0
o	–	–	14.7–22.1	–	–
m	–	–	–	–	–
V / T	72–79	35–64	74–82	79–86	32–59
OV1	27–50	–	47[a]	–	–
Number of annuli in labial region	[3]–4	4	2	2	2
Stylet length	14–18	13–16	14–18	14–18	12–16
Dorsal gland opening from base of stylet knobs	2.0–4.3	2.0–3.7	1.7–3.0	2–3	34–38
Oesophageal gland overlap length	27–63	27–53	21–48	35–60	65–74
Excretory pore from anterior end	65–99	57–91	79–86	77–100	–
Oesophagus length	115–150	93–139	107–130	81–105	75–89
Post–vulval uterine branch length	14–35	–	12–31	15–40	–
Spicule length	–	14–21	–	–	15–20
Gubernaculum length	–	5–8	–	–	4–7
Number of annuli posterior to phasmid	0–14	–	–	–	–
Ventral tail annuli	[18] 22–24 [35]	–	15–22	22–35	–
Tail length	25–43	27–36	25–30	24–35	22–25

– Indicates structure not present or value not available.

[a] Indicates range not available.

Values in [] indicated values found in extreme cases.

3.5.2.12 *Pratylenchus neglectus* (Rensch, 1924) Filipjev & Schuurmans Stekhoven, 1941

= *Aphelenchus neglectus* Rensch, 1924

= *Tylenchus neglectus* (Rensch, 1924) Steiner, 1928

= *Anguillulina (Pratylenchus) neglecta* (Rensch, 1924) Schneider, 1939

=*Pratylenchus minyus* Sher & Allen, 1953

= *Pratylenchus capitatus* Ivanova, 1968

= *Pratylenchus neocapitatus* Khan & Singh, 1975

= *Pratylenchus similis* Khan & Singh, 1975

= *Pratylenchus gotohi* (Mizukubo & Minagawa, 1991) Pourjam, Kheiri, Geraert & Alizadeh, 1999

Figure 3.15

Measurements: Table 3.9.

Pratylenchus neglectus (Rensch, 1924) Filipjev & Schuurmans Stekhoven, 1941 was described from the roots of *Lolium* L. in Germany. It has since been reported from various crops in the Afrotropical, Australasian, Nearctic, Neotropical, Oriental and Palearctic biogeographical regions (Castillo & Vovlas 2007). This species has been described in numerous papers and these morphometrics, combined with the type description, was used to compile a morphometric range (Table 3.9) of the species (Rensch 1924; Sher & Allen 1953; Loof 1960; Thorne 1961; Ivanova 1968; Seinhorst 1968; Van den Berg 1971; Geraert *et al.* 1975; Khan & Singh 1975; Townshend & Anderson 1976; Koliopanos & Vovlas 1977; Loof 1978; Inserra *et al.* 1979; Ryss 1988; Lal & Khan 1990; Loof 1991; Mizukubo & Minagawa 1991; Zeidan & Geraert 1991; Pourjam *et al.* 1997; Doucet & Cagnolo 1998; Torres & Chaves 1999; Castillo & Vovlas 2007; Geraert 2013). *Pratylenchus neglectus* was initially reported as *P. minyus* Sher & Allen, 1953 by Van den Berg (1971). See section 3.8-3.9 for distribution and host range in South Africa (Figure 3.36, Table 3.25).

Description:

Neotype (Loof, 1960): Female: L = 510 µm; a = 21.2; b = 6.3; c = 22.3; V = 79 %; stylet length = 16 µm.

Female: Habitus of young females coiled and older females almost straight. Lateral field marked by four lines, middle band often containing one or two longitudinal or oblique striations. Labial region bluntly rounded bearing two annuli, second annulus wider than first. *En face* view with dorsal and ventral submedian lips are fused, forming a large cap while lateral lips are delineated, amphidial apertures at an oblique angle (Group 2, Figure 3.3). Stylet knobs indented at anterior margins. Oesophageal gland overlaps intestine ventrally or ventrolaterally, overlap 20-42 µm long, subventral gland nuclei at end of lobes not in tandem. Hemizonid immediately anterior to excretory pore, which is situated 60-87 µm from anterior end. Anterior ovary outstretched, oocytes arranged in tandem, occasionally extending to base of oesophagus, intra-uterine eggs in older females may be segmented or contain early first-stage juveniles. Post-vulval uterine branch 6-21 µm long, less than or equal to one body diameter, undifferentiated. Phasmids in posterior half of tail, 5-13 annuli from anus. Tail variable in shape, usually conoid curved on ventral surface bearing 15-27 annuli, tail tip smooth, usually rounded, occasionally obliquely truncate or slightly digitate.

Male: Rare. Similar to female. Single outstretched testis. Spicules rather slender, shaft not set off. Phasmids slightly posterior to mid tail, extending into delicate bursa.

Diagnosis (Castillo & Vovlas 2007):

Pratylenchus neglectus is characterised by labial region with two annuli, second wider than first, stylet knobs indented on anterior margins, post vulval uterine branch less than or equal to one body diameter, tail variable in shape, usually conoid curved on ventral surface, tail tip smooth, usually rounded, occasionally obliquely truncate or slightly digitate.

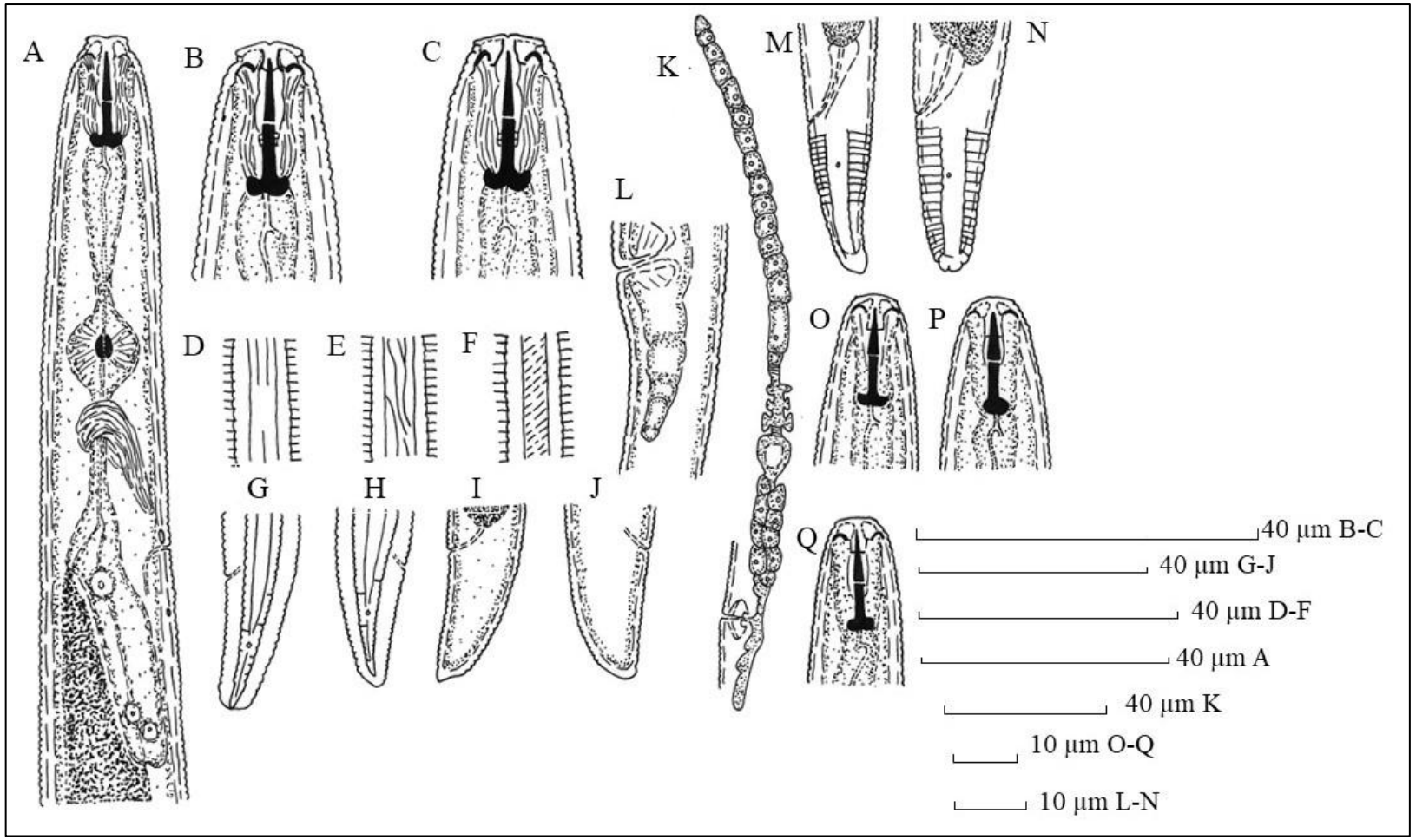


Figure 3.15: *Pratylenchus neglectus* (Rensch, 1924) Filipjev & Schuurmans Stekhoven, 1941 A: Oesophageal region; B-C, O-Q: Labial region; D-F: Variation of lateral field; G-J, M-N: Tail region showing variation in tail shape; K: Genital tract; L: Vulval region showing post-vulval uterine branch (A-K redrawn from Townshed & Anderson [1976]; L-Q redrawn from Mizukubo & Minagawa [1991]).

3.5.2.13 *Pratylenchus penetrans* (Cobb, 1917) Filipjev & Schuurmans Stekhoven, 1941

= *Tylenchus penetrans* Cobb, 1917 male, *nec* female

= *Anguillulina* (*Pratylenchus*) *penetrans* (Cobb, 1917) Goodey, 1932

= *Tylenchus gulosus* Kühn, 1890 (=*nome oblitum*)

= *Pratylenchus gulosus* (Kühn, 1890) Filipjev & Schuurmans Stekhoven, 1941

= *Pratylenchus globulicola* Romániko, 1960

Figure 3.16

Measurements: Table 3.9

Pratylenchus penetrans (Cobb, 1917) Filipjev & Schuurmans Stekhoven, 1941 was described from glasshouse soil in the United States of America. This species of *Pratylenchus* is cosmopolitan and polyphagous, reported from over 350 host types in the Afrotropical, Australasian, Nearctic, Neotropical, Oriental and Palearctic biogeographical regions (Castillo & Vovlas 2007). The morphometrics of *P. penetrans* (Table 3.9) are a combination of information given in Sher & Allen (1953); Taylor & Jenkins (1957); Loof (1960); Ryss (1988); Vovlas & Troccoli (1990); Castillo & Vovlas (2007) and Geraert (2013). Van den Berg (1971) described the South African population. See section 3.8-3.9 for distribution and host range in South Africa (Figure 3.36, Table 3.25).

Description:

Neotype (Loof 1960): Female: L = 530 µm; a = 26.0; b = 5.8; c = 16.0; V = 81 %.

Female: Habitus almost straight. Body slender, cuticular annulation fine. Lateral field with four lines, outer bands partly aerolated, central band occasionally with oblique striae in vulval region,

becoming aerolated posterior to vulva, not extending to tail tip. Labial region slightly set off from body contour, low, flat anteriorly with rounded outer margins, bearing three annuli, labial framework strong. *En face* view with rectangular subdorsal and subventral lips clearly wider than oral disc and separated from lateral lips by two incisures perpendicular to each other (Group 3, Figure 3.3). Stylet knobs broadly rounded occasionally cupped anteriorly. Oesophagus overlaps intestine ventrally, overlap 1.5 times corresponding body diameter, 30-77 µm long. Excretory pore opposite junctions of oesophagus and intestine, 77-84 µm from anterior end. Hemizonid two body annuli anterior to excretory pore. Spermatheca in anterior genital branch spherical, filled with sperm. Post-vulval uterine branch 0.6-1.5 corresponding body width long, undifferentiated. Phasmid situated 9-12 annuli posterior anus. Tail with 15-28 annuli on ventral side, rounded, tip smooth.

Male: Common. Similar to females only slightly smaller. Lateral field with four lines ending on bursa, sometimes with oblique lines in central band near mid body. Spicules slender, 14-19 µm long, with well demarcated manubrium and ventrally arcuate shafts. Gubernaculum simple, 4-5 µm long. Tail twice as long as cloacal body diameter, bursa irregularly crenate along outer margins, enveloping tail tip.

Diagnosis (Castillo & Vovlas 2007):

Pratylenchus penetrans is characterised by a labial region with three annuli, slightly off set, low and flat in anterior surface with rounded outer margins, oesophagus overlapping intestine ventrally, overlap 1.5 body diameters long, post vulval uterine branch short, undifferentiated, tail generally rounded with smooth tip.

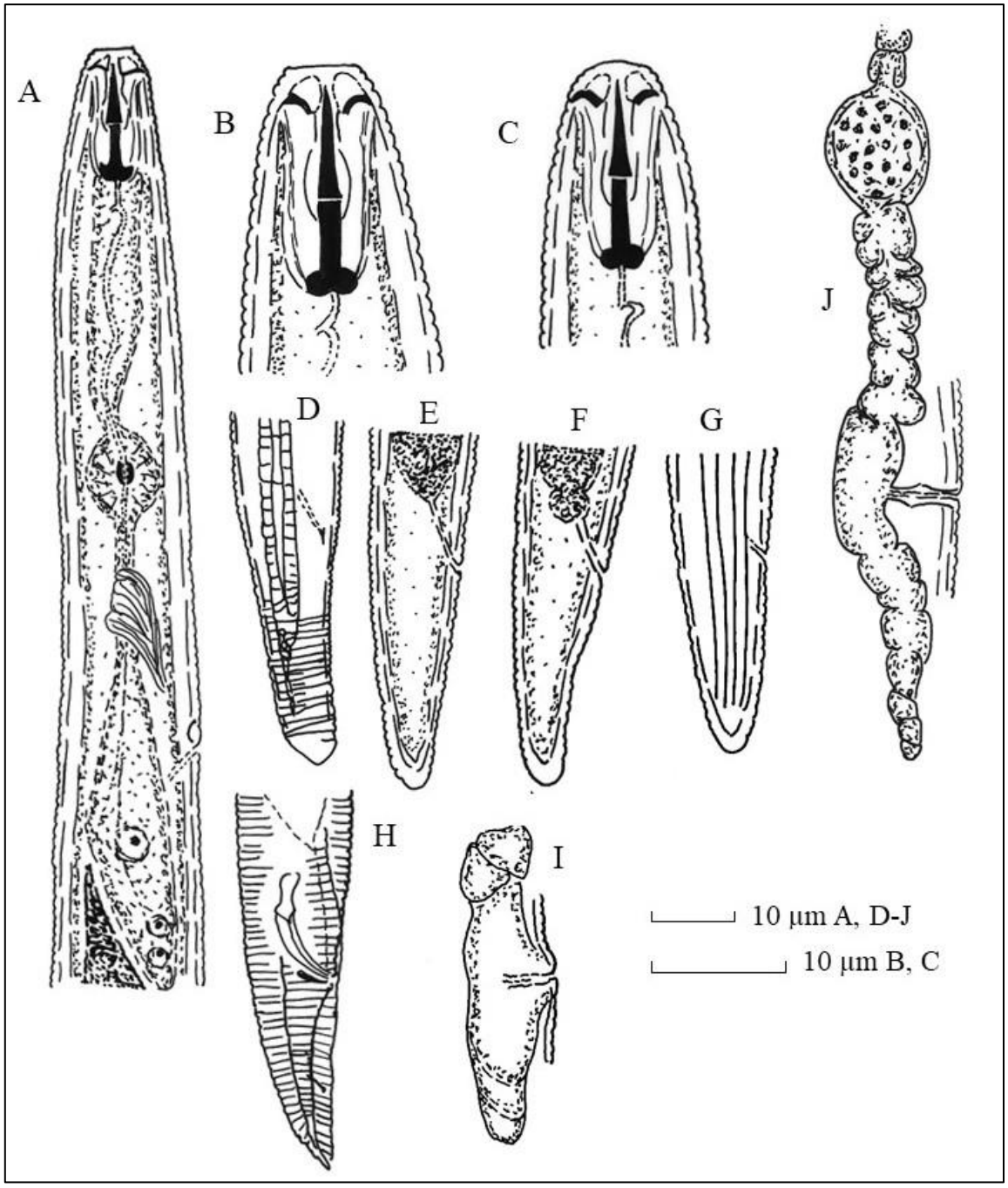


Figure 3.16: *Pratylenchus penetrans* (Cobb, 1917) Filipjev & Schuurmans Stekhoven, 1941 A: Female oesophageal region; B: Female labial region; C: Male labial region; D-G: Female tail; H: Male tail; I: Vulval region showing post-vulval uterine branch; J: Vulval region showing spermatheca and post-vulval uterine branch (A-H, J redrawn from Corbett [1973]; G, I redrawn from Ryss [1988]).

Table 3.9: Morphometrics of females and males of *Pratylenchus neglectus* (Rensch, 1924) Filipjev & Schuurmans Stekhoven, 1941 and *Pratylenchus penetrans* (Cobb, 1917) Filipjev & Schuurmans Stekhoven, 1941. All measurements are in µm unless stated otherwise.

Species	***P. neglectus* (Rensch, 1924) Filipjev & Schuurmans Stekhoven,1941**			***P. penetrans* (Cobb, 1917) Filipjev & Schuurmans Stekhoven,1941**			
Population	**South African**	**Range**		**South African**		**Range**	
	♀	♀	♂	♀	♂	♀	♂
L	340–480	310–700	340–524	430–520	470	350–810	310–570
a	13.3–30.2	13.3–32.2	22–28.9	21.2–30.9	28.6	17.0–33.0	23.0–43.0
b	–	3.6–7.8	4.8–6.3	–	–	4.6–8.1	5.4–7.3
b'	3.0–5.7	3.0–5.7	–	3.6–4.2	3.7	3.6–4.9	3.7–5.2
c	12.8–28.7	12.8–31.0	17.3–21.7	14.0–18.9	18.4	14.0–27.7	15.0–23.0
c'	–	1.5–3.0	–	–	–	1.5–2.6	2.0–2.4
o	–	–	–	–	–	–	–
m	–	45–49	–	–	–	–	–
V / T	81–85	76–88	42–56	76–81	–	73–85	36–58
OV1	30–48	25–60	–	35	–	–	–
Number of annuli in labial region	2	2	2	3	3	3	3
Stylet length	15–20	15–20	14–17	15–16	15	15–19	13–18
Dorsal gland opening from base of stylet knobs	–	2.0–4.0	–	–	–	2.0[a]	–
Oesophageal gland overlap length	–	20–42	–	–	–	30–77	–
Excretory pore from anterior end	60–82	60–87	–	77–84	80	77–84	80[a]
Oesophagus length	–	–	–	–	–	–	–
Post–vulval uterine branch length	6–21	6–21	–	18–27	–	18–27	–
Spicule length	–	–	–	–	15	–	14–19
Gubernaculum length	–	–	–	–	5	–	4–5
Number of annuli from anus to phasmid	5–13	5–13	–	9–12	–	9–12	–
Ventral tail annuli	16–24	15–27	–	26–28	–	15–28	–
Tail length	13–32	13–33	–	24–37	26	21–38	26[a]

– Indicates structure not present or value not available.

[a] Indicates range not available.

3.5.2.14 *Pratylenchus pseudopratensis* Seinhorst, 1968

= *Pratylenchus sefaensis* Fortuner, 1973

Figure 3.17

Measurements: Table 3.10

Pratylenchus pseudopratensis Seinhorst, 1968 was described from a *Malus* Mill. orchard in the Netherlands and has since been reported from several hosts in the Afrotropical, Nearctic, Neotropical, and Palearctic biogeographic regions (Castillo & Vovlas 2007). Morphometrics of *P. pseudopratensis* (Table 3.10) are a combination of the type and other publications (Seinhorst 1968; Fortuner 1973; Loof 1978; Ryss 1988; Sakwe & Geraert 1994; Pourjam *et al.* 1997; Castillo & Vovlas 2007; Geraert 2013). The report from the Telperion Nature Reserve is the first of this species from South Africa (Chapter 2: Article 1, Plant–Parasitic Nematodes of the Telperion Nature Reserve).

Description:

Holotype (Seinhorst, 1968): Female: L = 520 µm; a = 29.0; b = 6.2; c = 18.0; V = 80 %; stylet length = 17 µm.

Female: Habitus straight to slightly curved ventrally. Lateral field with four lines, usually additional oblique lines in middle band, only outer lines exceeds phasmids. Labial region bearing three, rather flat and often obscure annuli, labial framework extending two annuli into body. *En face* view with first annulus with prominent labial disc and perioral pad (Group 1, Figure 3.3). Stylet knobs flattened anteriorly. Opening of dorsal gland 2-3 µm posterior to stylet knobs. Excretory pore located at or just posterior to nerve ring, 64-96 µm from anterior end. Oesophageal gland overlap 19-57 µm long. Anterior ovary outstretched, oocytes in single row except for anterior tip, spermatheca rectangular,

filled with sperm, sometimes empty. Post-vulval uterine branch 0.4-1.3 body widths long, 15-42 µm long, often with two or three rudimentary elements. Phasmids between 9-13 annuli from terminus. Tail conical bearing 16-29 annuli, tip rounded or slightly irregular in shape, distinctly crenate to almost smooth.

Male: Similar to female. Spicule 14-16 µm long. Gubernaculum 3-5 µm long. Testis with spermatocytes in double row. Bursal margins coarsely crenate. Phasmid at two fifths of tail length from terminus.

Diagnosis (Castillo & Vovlas 2007):

Pratylenchus pseudopratensis is characterised by labial region with three annuli, lateral field with four lines, usually some additional lines in middle band, spermatheca round, tail conical with rounded tip and males present.

3.5.2.15 *Pratylenchus scribneri* Steiner in Sherbakoff & Stanley, 1943

= *Tylenchus penetrans* Cobb, 1917; female only

= *Pratylenchus agilis* Thorne & Malek, 1968

= *Pratylenchus crossandrae* Subramaniyan & Sivakumar, 1991

Figure 3.18

Measurements: Table 3.10

Pratylenchus scribneri Steiner in Sherbakoff & Stanley, 1943 was described from *S. tuberosum* in the United States of America (Sherbakoff & Stanley 1943). The species has since been reported from

the Afrotropical, Australasian, Nearctic, Neotropical, Oriental and Palearctic biogeographic regions and is a polyphagous species (Castillo & Vovlas 2007). The morphometrics of *P. scribneri* (Table 3.10) are based on the type description with additional population descriptions by Sher & Allen (1953); Loof (1960); Thorne (1961); Thorne & Malek (1968); Román & Hirschmann (1969); Van den Berg (1971); Knobloch & Laughlin (1973); Geraert et al. (1975); Loof (1978); Hashim (1984); Loof (1985); Doucet (1988); Loof (1991); Subramaniyan & Sivakumar (1991); Sakwe & Geraert (1994); Doucet & Cagnolo (1998); Van den Berg & Quénéhervé (2000); Castillo & Vovlas (2007) and Geraert (2013). A South African population of *P. scribneri* was described by Van den Berg (1971). See section 3.8-3.9 for distribution and host range in South Africa (Figure 3.37, Table 3.25).

Description:

Lectotype (Loof, 1985): Female: L = 530 µm; a = 22.0; b = 6.2; b' = 4.7; c = 21.0; c' = 2.1; V = 80 %; stylet length = 14 µm.

Female: Habitus almost straight. Body stout, more slender in younger females. Cuticular annulation fine, shallow, 1 µm apart, becoming more pronounced on tail. Lateral field with four lines, occasionally a fifth in pre-vulval region. Labial region slightly set off from body, bearing two annuli, first annuli distinctly narrower than second, angles rounded, anterior margin flat, labial framework extending into body for one annulus. *En face* view shows subdorsal and subventral lips wider than oral disc and separated from lateral lips by two convex incisures, inner part of lateral lips narrower than outer part (Group 3, Figure 3.3). Stylet stout with knobs variable in shape. Opening of dorsal gland 2-3 µm posterior to base of stylet knobs. Hemizonid opposite to posterior half of isthmus, anterior to excretory pore, situated 67-79 µm from anterior end. Oesophageal gland overlap of medium length, 26-60 µm long. Anterior ovary with oocytes in single row except short zone in anterior end, spermatheca empty, uterus tricolumella. Post-vulval uterine branch equal to or slightly longer than vulval body width, or 8-43 µm in length. Phasmids inconspicuous, situated mid tail. Tail tapering slightly, 14-28

annuli on ventral side, tail tip smooth, mostly broadly rounded, varying from narrower to almost truncate. Protoplasmic core ending in some finger-like processes.

Male: Similar to female. Spicules typical for genus, 17 µm long. Gubernaculum 7 µm long, slightly curved. Bursa typical, phasmids 14 µm posterior to cloaca.

Diagnosis (Castillo & Vovlas 2007):

Pratylenchus scribneri is characterised by labial region slightly set off from body, bearing two annuli, the first distinctly narrower than second, stout stylet, stylet knobs rounded, spermatheca oval, tail tapering slightly with smooth terminus.

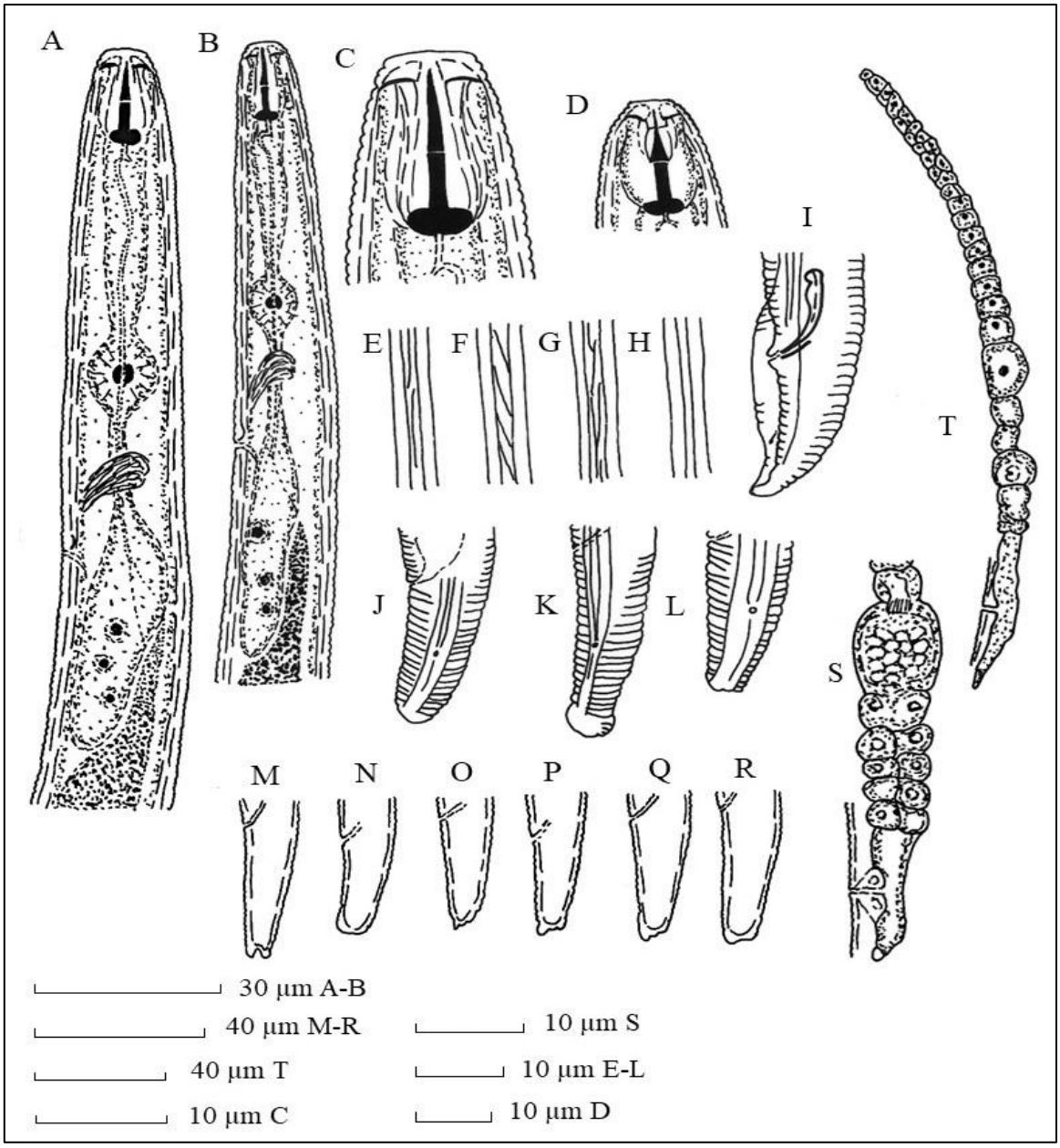


Figure 3.17: *Pratylenchus pseudopratensis* Seinhorst, 1968 A: Female oesophageal region; B: Male oesophageal region; C-D: Female labial region; E-H: Variation in the lateral field; I: Male tail; J-R: Variation in female tail shape; S: Vulval region showing spermatheca and post-vulval uterine branch; T: Female genital tract (A-C, M-R, T redrawn from Sakwe & Geraert [1994]; D, E-L, S redrawn from Seinhorst [1968]).

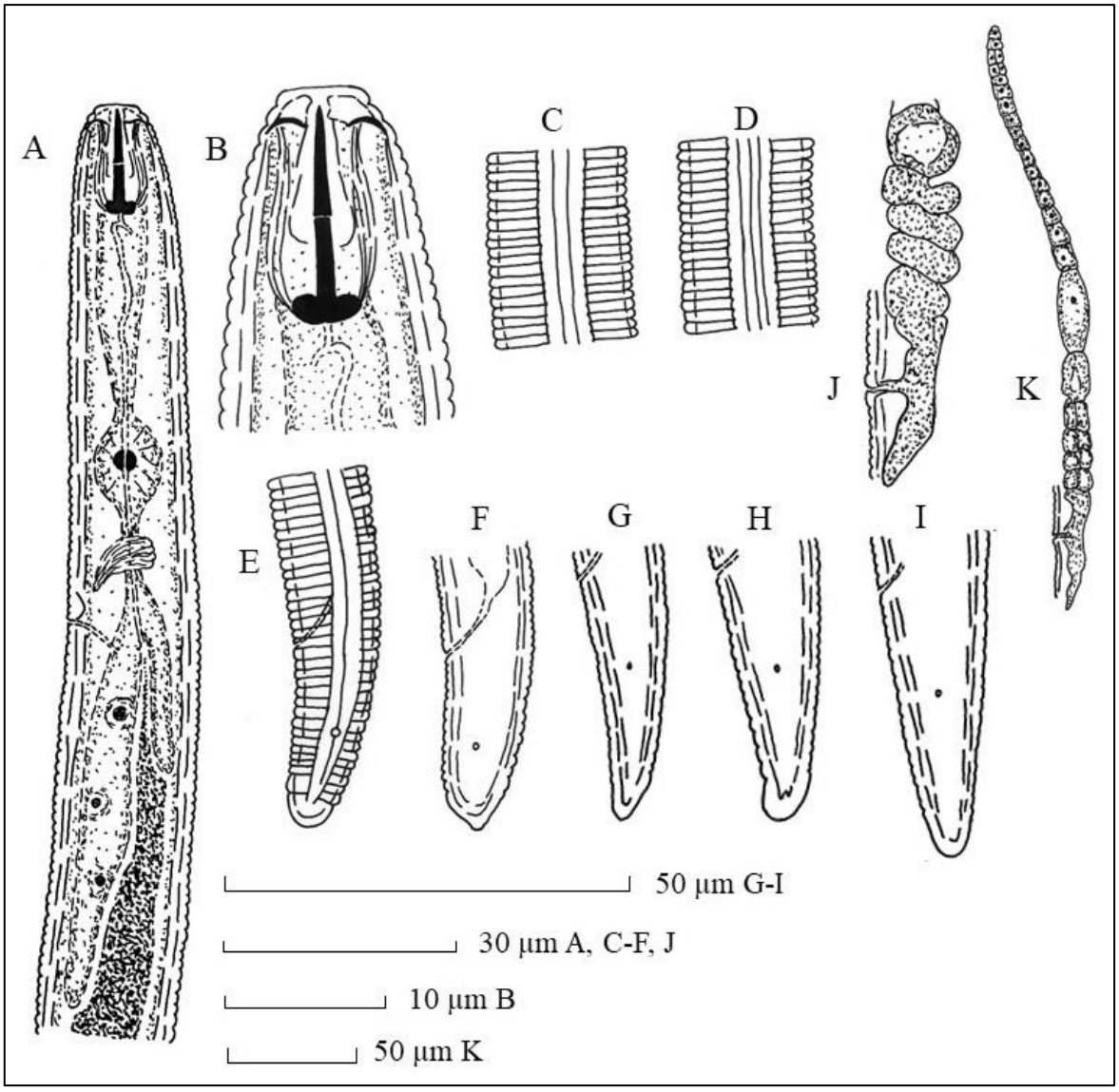


Figure 3.18: *Pratylenchus scribneri* Steiner in Sherbakoff & Stanley, 1943 A: Oesophageal region; B: Labial region; C-D: Variation of lateral field; E-I: Variation in shape of the tail; J: Vulval region showing post-vulval uterine branch; K: Genital tract (A-F, J redrawn from Sakwe & Geraert [1994]; K, G-I redrawn from Loof [1985]).

Table 3.10: Morphometrics of females and males of *Pratylenchus pseudopratensis* Seinhorst, 1968 and *Pratylenchus scribneri* Steiner in Sherbakoff & Stanley, 1943. All measurements are in µm unless stated otherwise.

Species	***P. pseudopratensis* Seinhorst, 1968**			***P. scribneri* Steiner in Sherbakoff & Stanley, 1943**		
Population	**South African**	**Range**		**South African**	**Range**	
	♀	♀	♂	♀	♀	♂
L	516	340–705	310–500	340–470	278–731	400–470
a	28.9	20.0–36.7	22.0–33.0	18.5–26.2	17.0–33.0	27.0–28.0
b	5.9	5.0–7.6	4.7–6.5	–	4.5–8.0	5.5–6.6
b'	–	3.2–5.6	3.2–4.2	3.3–4.6	3.0–6.0	–
c	13.6	15.0–25.0	16.0–29.0	13.6–17.9	13.0–32.4	17.0–18.0
c'	3.0	1.7–3.3	1.7–2.8	–	1.5–4.0	–
o	29.6	–	–	–	10.0–17.1	–
m	38.6	–	–	–	–	–
V / T	77	74–84	–	72–78	69–82	42–53
OV1	29	–	–	26–32	22–66	–
Number of annuli in labial region	3	3	3	2	2	2
Stylet length	12	12–17	13–15	14–16	12–18	12–17
Dorsal gland opening from base of stylet knobs	3	2–3	–	–	2–3	–
Oesophageal gland overlap length	30	19–57	–	–	26–60	–
Excretory pore from anterior end	73	64–96	–	53–73	67–79	–
Oesophagus length	87	87–149	–	–	100–145	–
Post–vulval uterine branch length	15	15–42	–	17–35	8–43	–
Spicule length	–	–	14–16	–	–	17
Gubernaculum length	–	–	3–5	–	–	7
Number of annuli posterior to phasmid	–	9–13	–	–	–	–
Number of annuli from anus to phasmid	–	–	–	10–13	10–13	–
Ventral tail annuli	–	16–29	–	18–24	14–28	–
Tail length	38	18–39	20–23	24–30	20–30	–

– Indicates structure not present or value not available. [a] Indicates range not available.

3.5.2.16 *Pratylenchus tenuis* Thorne & Malek, 1968

Figure 3.19

Measurements: Table 3.11

Pratylenchus tenuis Thorne & Malek, 1968 was described from cultivated soils in United States of America and has since only been reported a few times in other localities in the Afrotropical (South Africa) and Nearctic (United States of America) biogeographical regions (Castillo & Vovlas 2007; SAPPNS). This species was considered species inquirenda by Loof (1978), however according to Handoo & Golden (1989) the type specimens of the species presented unique characteristics such as tulip-shaped stylet knobs and an unusually long oesophageal lobe and the species was therefore considered valid by Castillo & Vovlas (2007) and is accepted in this paper. The morphometrics of the species are based on Thorne & Malek (1968); Handoo & Golden (1989); Castillo & Vovlas (2007) and Geraert (2013). No morphometric data for the South African populations reported are available. See section 3.8-3.9 for distribution and host range in South Africa (Figure 3.37, Table 3.25).

Description:

Type population (Thorne & Malek 1968): Female: L = 400 µm; a = 25.0; b = 8.0; c = 22.0; V = 79 %; stylet length = 17 µm (15 µm types measured by Handoo & Golden [1989]).

Female: Body cylindroid, tapering slightly anteriorly. Cuticular annulation fine, 1.7 µm near labial region and 1.0 µm at mid body. Lateral field with four obscure lines, middle two lines end near anal region. Labial region low, flattened, four times as wide as high, bearing two annuli. Stylet knobs peculiar, tulip-shaped. Excretory pore and hemizonid opposite nerve ring. Oesophageal lobe overlaps intestine ventrally for more than two body widths. Anterior ovary outstretched, oocytes in single row

except in anterior zone for multiplication. Post-vulval uterine branch shorter than corresponding body diameter. Phasmids near mid tail. Tail conoid-rounded, slightly bent, not clear whether smooth or annulated tail tip.

Male: Unknown.

Diagnosis (Castillo & Vovlas 2007):

Pratylenchus tenuis is characterised by a low, flattened labial region bearing two annuli, lateral field with four obscure lines, middle two lines disappearing near anus, stylet knobs tulip-shaped, tail conoid-rounded, slightly bent.

3.5.2.17 *Pratylenchus teres* Khan & Singh, 1975

Figure 3.20

Measurements: Table 3.11

Pratylenchus teres Khan & Singh, 1975 was described from soil around the roots of *S. tuberosum* in India (Khan & Singh 1975). The species has since been reported from various hosts in the Afrotropical, Australasian, Palearctic and Oriental biogeographical regions (Castillo & Vovlas 2007). Carta *et al.* (2002) split this species into two subspecies i.e. *P. teres teres* and *P. teres vandenbergae* and although there were slight morphological differences between these subspecies, molecular sequences were identical and any morphological differences are herein regarded as variations within the species. The morphometrical range of the species is a combination of the type description as well as additional populations described from various hosts and localities (Khan & Singh 1975; Van den Berg & Quénéhervé 2000; Carta *et al.* 2002; Castillo & Vovlas 2007; Geraert 2013). A South African

population was described from a cultured population in Carta *et al*. (2002). See section 3.8-3.9 for distribution and host range in South Africa (Figure 3.37, Table 3.25).

Description:

Holotype (Khan & Singh 1975): Female: L = 400 µm; a = 21.1; b = 4.2; c = 14.2; c' = 1.9; V = 70 %; stylet length = 18 µm.

Female: Habitus straight or slightly arcuate, tapering symmetrically and gradually towards vulva. Cuticular annulation fine. Lateral field with six strongly crenate lines, occasionally with perpendicular striations, first lateral line beginning at level of stylet, increasing to two, then four near posterior at level of median bulb, then six in vulval region, reducing to three between anus and phasmid then finally reducing to one or two just before tail terminus. Labial region conoid, low, bearing three annuli, labial sclerotization strong, extending two body annuli behind labial region. *En face* view broad hour-glass shape with fusion of lip sectors (Group 2, Figure 3.3). Stylet with slightly anchor shaped knobs. Oesophageal gland overlaps intestine ventrally, 35-47 µm long, one dorsal and two subventral gland nuclei in overlap. Excretory pore situated opposite the junction of the oesophagus and intestine, 77-90 µm from anterior end, hemizonid slightly anterior to excretory pore. Spermatheca inconspicuous, empty. Post-vulval uterine branch one to one and a half body diameters long, 20-40 µm. Phasmid located 18 annuli from tail terminus. Tail conoid, over two anal body diameters long, with 23-35 annuli on ventral side, tail tip crenate.

Male: Unknown.

Diagnosis (Castillo & Vovlas 2007):

Pratylenchus teres can be differentiated by conoid labial region bearing three annuli, lateral field with six lines and conoid tail with crenate tip.

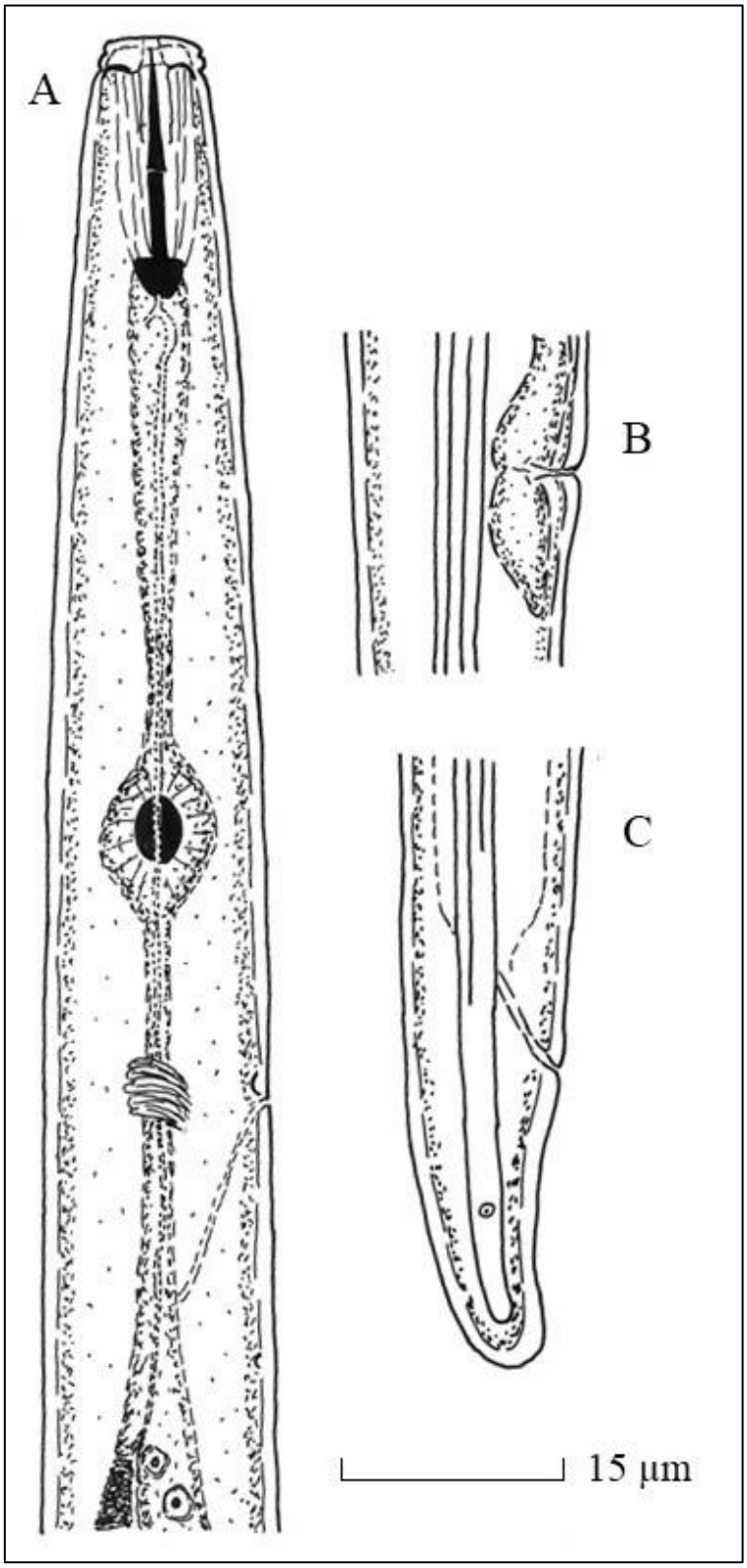


Figure 3.19: *Pratylenchus tenuis* Thorne & Malek, 1968 A: Oesophageal region; B: Vulval region showing post-vulval uterine branch; C: Tail region (Redrawn from Thorne & Malek [1968]).

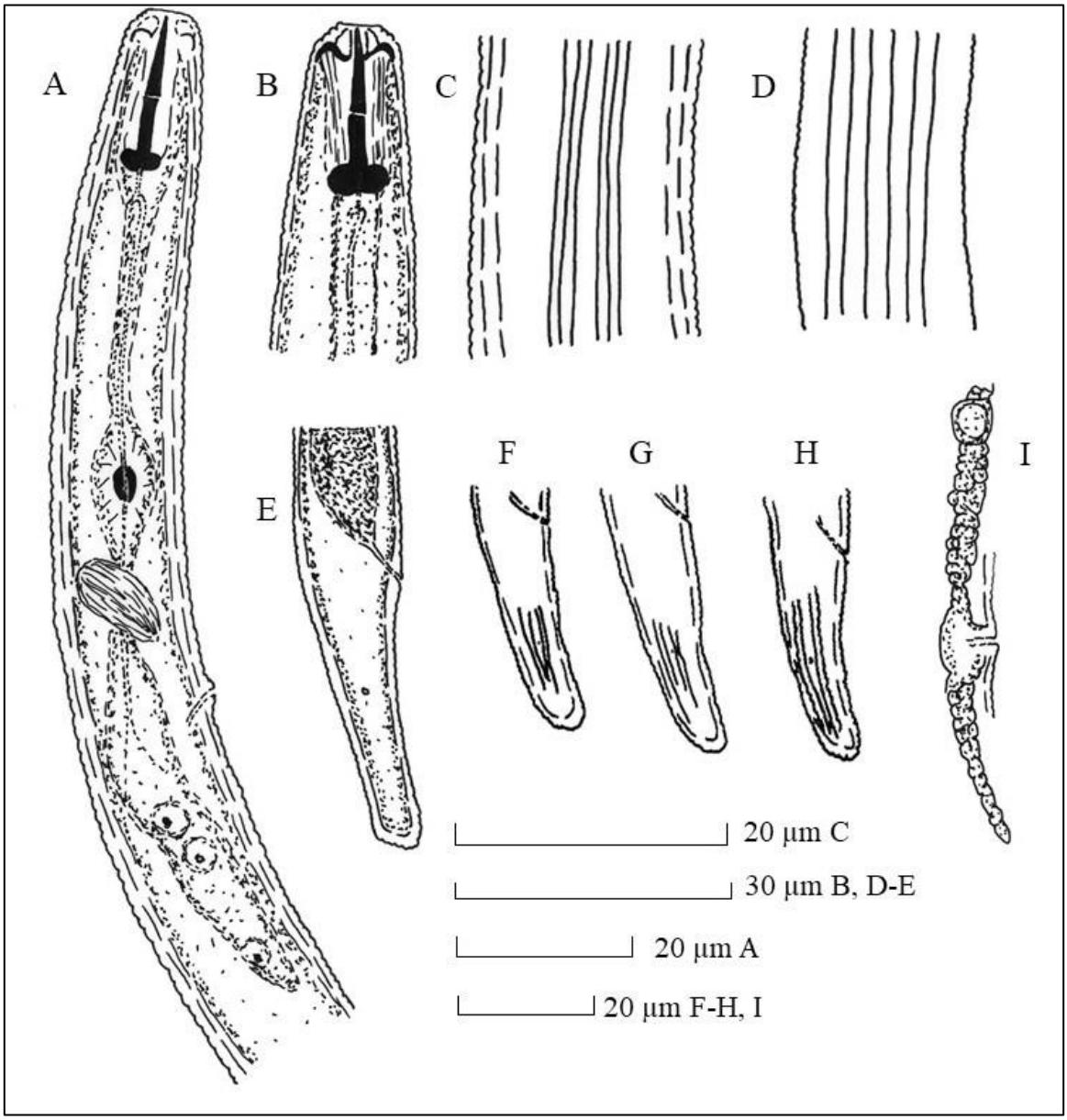


Figure 3.20: *Pratylenchus teres* Khan & Singh, 1975 A: Oesophageal region; B: Labial region; C-D: Lateral field; E-H: Variation in tail shape; I: Vulval region showing spermatheca and post-vulval uterine branch (B, E-D redrawn from Khan & Singh [1975]; A, C, F-I redrawn from Van den Berg & Queneherve [2000]).

Table 3.11: Morphometrics of females and males of *Pratylenchus tenuis* Thorne & Malek, 1968 and *P. teres* Khan & Singh, 1975. All measurements are in µm unless stated otherwise.

Species	***P. tenuis*** **Thorne & Malek, 1968**	***P. teres*** **Khan & Singh, 1975**	
Population	**Range**	**South African**	**Range**
	♀	♀	♀
L	400	472–640	420–640
a	25.0	22.0–39.0	20.0–40.0
b	3.1–3.6	3.2–5.6	3.2–5.6
b'	–	5.0–6.0	5.0–6.0
c	22.0	11.5–27.0	11.5–27.0
c'	–	1.5–3.2	1.5–3.2
o	–	14–21	14–21
m	–	9[a]	9[a]
V / T	79	69–78	69–78
OV1	–	20–34	20–34
Number of annuli in labial region	2	3	3 [4]
Stylet length	15[17]	15–18	15–18
Dorsal gland opening from base of stylet knobs	–	2.0–3.0	2.0–3.0
Oesophageal gland overlap length	–	35–47	35–47
Excretory pore from anterior end	–	77–90	77–90
Oesophagus length	–	115–155	115–155
Post–vulval uterine branch length	–	20–40	20–40
Spicule length	–	–	–
Gubernaculum length	–	–	–
Ventral tail annuli	–	23–35	23–35
Tail length	18	30–39	28–39

– Indicates structure not present or value not available.
[a] Indicates range not available.
Values in [] indicated values found in extreme cases.

3.5.2.18 *Pratylenchus thornei* Sher & Allen, 1953

=*Pratylenchus ranjani* (Khan & Singh, 1975) Pourjam, Kheiri, Geraert & Alizadeh, 1999

= *Pratylenchus peelari* in Chawla & Prasad, 1973 (= *nomen nudum*)

= *Pratylenchus allius* (Shahina & Maqbool, 1996) Siddiqi, 2000

= *Radopholus allius* Shahina & Maqbool, 1996

Figure 3.21

Measurements: Table 3.12

Pratylenchus thornei Sher & Allen, 1953 is a polyphagous, cosmopolitan species that was originally described from soil around the roots of grass in the Unites States of America now reported from the Afrotropical, Australasian, Nearctic, Neotropical, Oriental and Palearctic biogeographical regions (Sher & Allen 1953). Apart from the type population by Sher & Allen (1953), morphometrics for *P. thornei* are available in several papers and was combined (Table 3.12) to present a species range (Loof 1960; Thorne 1961; Brzeski 1969; Van den Berg 1971; Khan & Singh 1975; Fortuner 1977; Inserra *et al.* 1979; Doucet 1988; Ryss 1988; Loof 1991; Dobrin & Geraert 1994; Pourjam *et al.* 1997; Yu 1997; Elbadri *et al.* 2001; Castillo & Vovlas 2007; Geraert 2013). A South African population of the species (Table 3.12) was described by Van den Berg (1971). See section 3.8-3.9 for distribution and host range in South Africa (Figure 3.38, Table 3.25).

Description:

Holotype (Sher & Allen 1953): Female: L = 570 µm; a = 31.0; b = 5.8; c = 20.0; V = 77 %; stylet length = 18 µm.

Female: Habitus an open C-shape. Cuticular annulation inconspicuous, 1 µm wide. Lateral field with four lines, outer bands often weakly crenate, rarely oblique striae in central band. Labial region not off set, bearing three annuli, labial framework sclerotised extending two body annuli posterior to labial region. *En face* view shows lateral lips clearly separated from subdorsal and subventral lips by respective incisures, concave with respect to lateral lips (Group 2, Figure 3.3). Stylet with broadly rounded to anteriorly flattened knobs. Oesophageal gland overlap 27-71 µm long. Hemizonid one annulus anterior to excretory pore which is situated 53-105 µm from anterior end. Ovary outstretched, not extending to oesophagus, oocytes in single row except for zone of multiplication, spermatheca inconspicuous, empty. Post vulval uterine branch 0.8-1.5 times the corresponding body width, 12-32 µm long, undifferentiated. Phasmids slightly posterior to mid tail, 13-16 annuli posterior to anus. Tail dorsally convex-conoid, bearing 17-33 ventral annuli, tail tip bluntly rounded to truncate, not striated.

Male: Very rare. Similar to female. Testis outstretched, spermatocytes in single row followed by region of multiple rows. Spicules very long (21-26 µm), arcuate. Gubernaculum trough-shaped 5-8 µm long. Phasmids slightly posterior to mid tail, not extending into bursa.

Diagnosis (Castillo & Vovlas 2007):

Pratylenchus thornei is characterised by a labial region with three annuli, not off set, labial framework extending two annuli into body, lateral field with four lines, outer bands straight or slightly crenate spermatheca inconspicuous, empty and rarity of males.

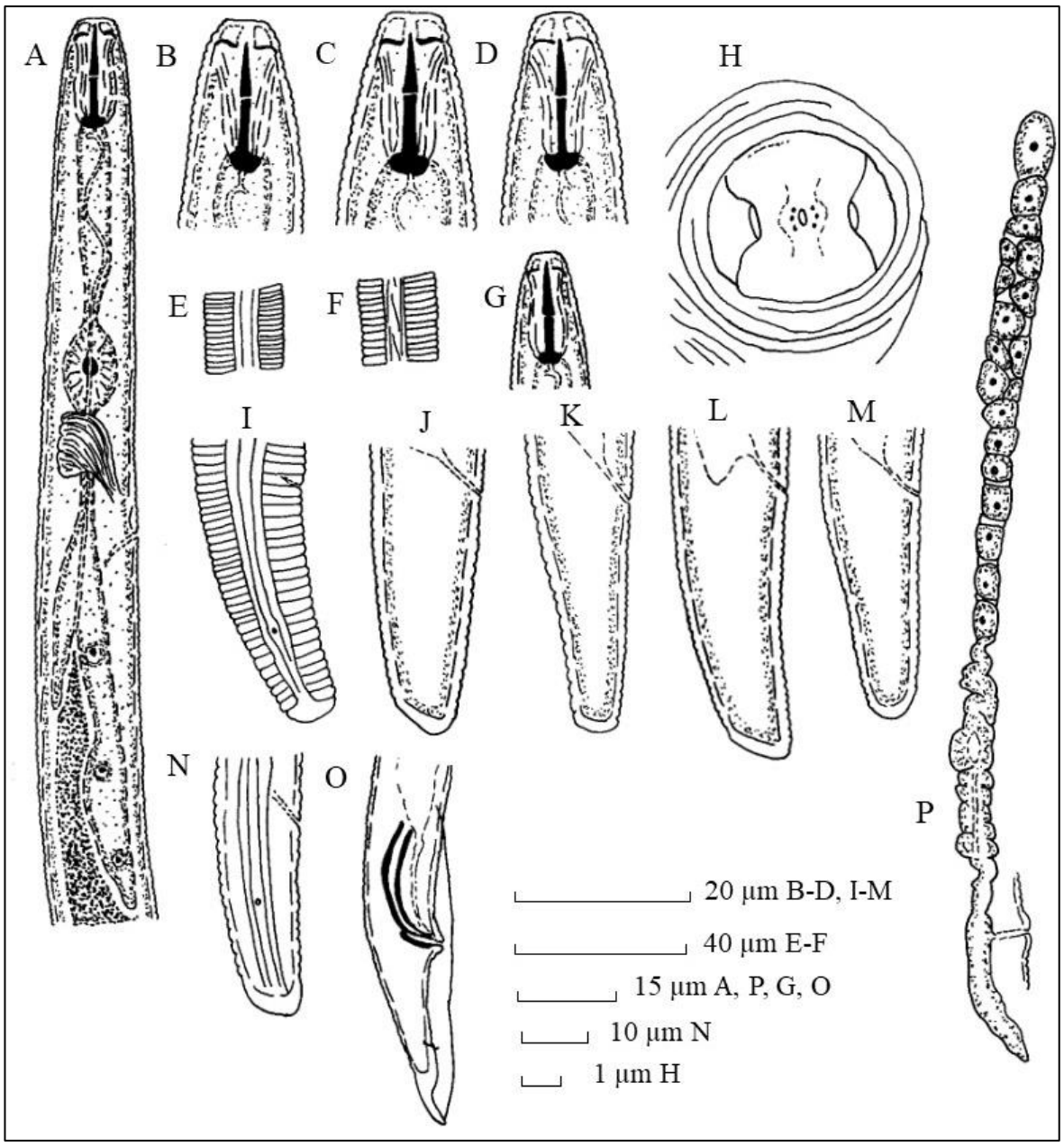


Figure 3.21: *Pratylenchus thornei* Sher & Allen, 1953 A: Female oesophageal region; B-D: Female labial region; E-F: Variation in lateral field; G: Male labial region; H: *En face* view of labial region; I-N: Variation shape of female tail; O: Male tail; P: Female genital tract (A, G, O-P redrawn from Fortuner [1977]; B-F, I-M redrawn from Pourjam *et al.* [1997]; H, N redrawn from Ryss [1988]).

3.5.2.19 *Pratylenchus vulnus* Allen & Jensen, 1951

Figure 3.22

Measurements: Table 3.12

Described from the roots of *Juglans hindsii* (Jeps.) Jeps. ex R.E. Sm. in the United States of America, *Pratylenchus vulnus* Allen & Jensen, 1951 has been reported from a wide range of hosts (over 80 plant species) in the Afrotropical, Australasian, Nearctic, Neotropical, Oriental and Palearctic biogeographic regions (Castillo & Vovlas 2007). The morphometric range of *P. vulnus* (Table 3.12) is a combination of various populations described from different localities globally including the type population by Allen & Jensen (1951) (Loof 1960; Thorne 1961; Román & Hirschmann 1969; Van den Berg 1971; Corbett 1974; Geraert *et al.* 1975; Pliansinchai & Boonduang 1978; Inserra *et al.* 1979; Doucet 1988; Ryss 1988; Lal & Khan 1990; Loof 1991; Doucet *et al.* 1996; Doucet & Lax 1997; Gao *et al.* 1997; Pourjam *et al.* 1997; Brzeski 1998; Gao *et al.* 1999; Castillo & Vovlas 2007; Geraert 2013). South African populations of *P. vulnus* were described in Van den Berg (1971). See section 3.8-3.9 for distribution and host range in South Africa (Figure 3.38, Table 3.25).

Description:

Type population (Allen & Jensen 1951): Female: L = 460-910 µm; a = 26.6-39.5; b = 5.3-7.7; c = 14.2-27.7; V = 78-84 %; stylet length = 16-18 µm.

Female: Habitus nearly straight. Lateral field marked by four lines, outer lines smooth or weakly crenate, inner band narrower than outer two, occasionally oblique lines in inner band. Labial region almost continuous with body contour, bearing three or four annuli, sclerotised labial framework extending into body for one annulus. *En face* view indicates wide subdorsal and subventral lips

separated from lateral lips by convex incisures, six small papillae surrounding oral aperture, two large amphidial apertures present laterally (Group 3, Figure 3.3). Stylet with rounded knobs, occasionally anteriorly cupped. Excretory pore opposite junction of oesophagus and intestine, 71-121 µm from anterior end. Oesophagus overlaps intestine ventrally with long lobe, 21-64 µm long. Spermatheca oblong, filled with sperm. Post-vulval uterine branch two body diameters long with rudimentary ovary. Phasmid slightly posterior to mid tail, 11-22 annuli posterior to anus. Tail with 19-34 ventral annuli, tapering, tail tip narrowly rounded to subacute, smooth, occasionally irregular or with one or two annulations.

Male: Common. Similar to female. Lateral field ending on bursa, enveloping tail. Spicules curved, cephalated, 12-23 µm long. Gubernaculum simple, 4-8 µm long.

Diagnosis (Castillo & Vovlas 2007):

Pratylenchus vulnus is characterised by slightly set off labial region, bearing three or four annuli, oesophageal overlap with long lobe, spermatheca oblong, filled with sperm, post-vulval uterine branch two corresponding body diameters long with rudimentary ovary, tail tapering, tail tip narrowly rounded to subacute, smooth, males common.

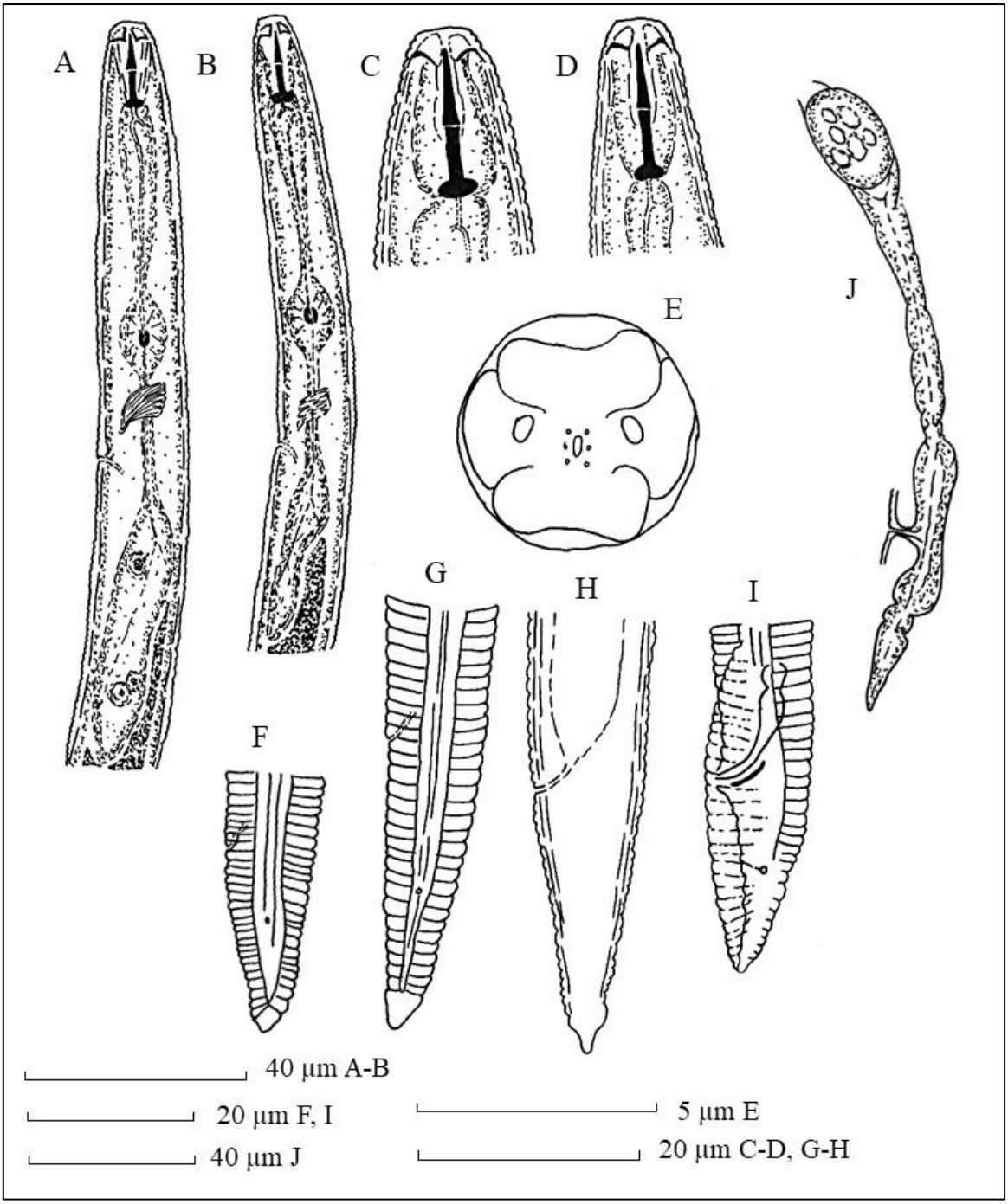


Figure 3.22: *Pratylenchus vulnus* Allen & Jensen, 1951 A: Female oesophageal region; B: Male oesophageal region; C: Female labial region; D: Male labial region; E: *En face* view of the labial region; F-H: Female tail region; I: Male tail; J: Vulval region showing spermatheca and post-vulval uterine branch (A-B, F, I-J redrawn from Pourjam *et al.* [1997]; C-E, G-H redrawn from Corbett [1974]).

Table 3.12: Morphometrics of females and males of *Pratylenchus thornei* Sher & Allen, 1953 and *Pratylenchus vulnus* Allen & Jensen, 1951. All measurements are in µm unless stated otherwise.

Species	***P. thornei* Sher & Allen, 1953**			***P. vulnus* Allen & Jensen, 1951**			
Population	**South African**	**Range**		**South African**		**Range**	
	♀	♀	♂	♀	♂	♀	♂
L	500–550	408–790	480–690	460–760	550–560	460–910	460–730
a	26.9–34.1	24.1–43.0	29.0–39.5	19.7–42.2	33.8–35.0	19.7–42.2	21.7–39.2
b	4.3–5.0	4.3–8.7	5.6–8.3	–	–	4.8–8.2	4.6–7.8
b'	–	3.3–5.6	4.2–5.9	3.6–6.5	4.3–4.5	3.2–7.1	3.5–5.7
c	16.8–22.1	11.7–29.0	18.1–26.5	13.0–27.1	20.9–27.7	13.0–31.1	17.5–29.4
c'	–	1.9–3.5	1.7–2.9	–	–	1.4–3.0	1.9–3.1
o	–	15.5–20.7	–	–	–	11–27.6	13.3–29.6
m	–	–	–	–	–	43.7–60.0	40.0–54.0
V / T	75–78	70–82	30–56	72–82	–	70–85	31–66
OV1	24	24–48	–	–	–	22–56	–
Number of annuli in labial region	3	3	3	3	3	3	3
Stylet length	16–19	12–19	16–17	13–16	14	13–19	13–18
Dorsal gland opening from base of stylet knobs	–	2.0–3.0	–	–	–	1.9–3.6	1.4–3.6
Oesophageal gland overlap length	–	27–71	–	–	–	30–45	15–35
Excretory pore from anterior end	53–77	53–105	–	71–104	–	71–121	68–96
Oesophagus length	–	105–140	–	–	–	110–193	103–132
Post–vulval uterine branch length	16–22	12–32	–	21–64	–	21–64	–
Spicule length	–	–	21–26	–	18–19	–	12–23
Gubernaculum length	–	–	5–8	–	–	–	4–8
Number of annuli from anus to phasmid	13–16	13–16	–	11–22	–	11–22	–
Ventral tail annuli	24–30	17–33	–	24–30	–	19–34	–
Tail length	25–31	17–34	–	21–43	20–27	21–43	20–34

– Indicates structure not present or value not available.

3.5.2.20 *Pratylenchus zeae* Graham, 1951

= *Pratylenchus cubensis* Razjivin & O'Relly, 1976

= *Pratylenchus zeae* Steiner in Clayton & McMurtrey, 1950 (= *nomen nudum*)

= *Pratylenchus impar* Khan & Singh, 1975

= *Pratylenchus jordanensis* Hashim, 1984

Figure 3.23

Measurements: Table 3.13

Pratylenchus zeae was described from the roots of *Z. mays* in the United States of America and is one of the most widely distributed species with an extensive host range found in all the biogeographic regions except the Antarctic (Castillo & Vovlas 2007). The morphometrical range of *P. zeae* (Table 3.13) is a combination of the type description and additional population descriptions (Graham 1951; Sher & Allen 1953; Taylor & Jenkins 1957; Thorne 1961; Román & Hirschmann 1969; Merny 1970, Van den Berg 1971; Khan & Singh 1975; Fortuner 1976; Nath *et al.* 1976; Pliansinchai & Boonduang 1978; Hashim 1984; Ryss 1988; Loof 1991; Troccoli *et al.* 1996; Doucet & Cagnolo 1998; Van den Berg & Quénéhervé 2000; Castillo & Vovlas 2007; Geraert 2013). South African populations of *P. zeae* (Table 3.13) were described in Van den Berg (1971). See section 3.8-3.9 for distribution and host range in South Africa (Figure 3.38, Table 3.25).

Description:

Neotype (Fortuner 1976): Female: L = 470 µm; a = 26.0; b = 5.9; c = 21.0; V = 70 %; stylet length = 16 µm.

Female: Habitus almost straight. Cuticular annulation fine. Lateral field with four lines extending beyond phasmids, inner band with slight irregularities in mid body. Labial region continuous with body contour, bearing three annuli, occasionally three on one side and four on the other, heavily sclerotised labial framework extending into body for one annulus. *En face* view indicates first lip annulus with no differentiation (Group 1, Figure 3.3). Stylet with broad, anteriorly flattened knobs. Hemizonid just anterior to excretory pore which is situated 52-94 µm from anterior end. Oesophageal gland overlap 18-40 µm long. Ovary with oocytes in double row, except for last two or three, not extending to oesophagus, spermatheca round, small, empty. Post-vulval uterine branch 1.0-2.0 diameters long, 15-48 µm, with rudimentary ovary. Intestine with short post-rectal extension, dorsally. Phasmids slightly posterior to mid tail, 8-15 annuli posterior to anus. Tail tapering, 16-28 annuli on ventral side, terminus smooth variable in shape, mostly almost pointed, narrowly rounded or subacute.

Male: Rare, similar to female. Spicules 14-15 µm long, slender, ventrally arcuate. Gubernaculum 4-5 µm long. Bursa with faint crenate margins.

Diagnosis (Castillo & Vovlas 2007):

Pratylenchus zeae is characterised by a labial region not set off, bearing three annuli, spermatheca round, small and empty, vulva generally at 68-76 % of body length, tail conoid, terminus smooth variable in shape, mostly almost pointed, narrowly rounded or subacute.

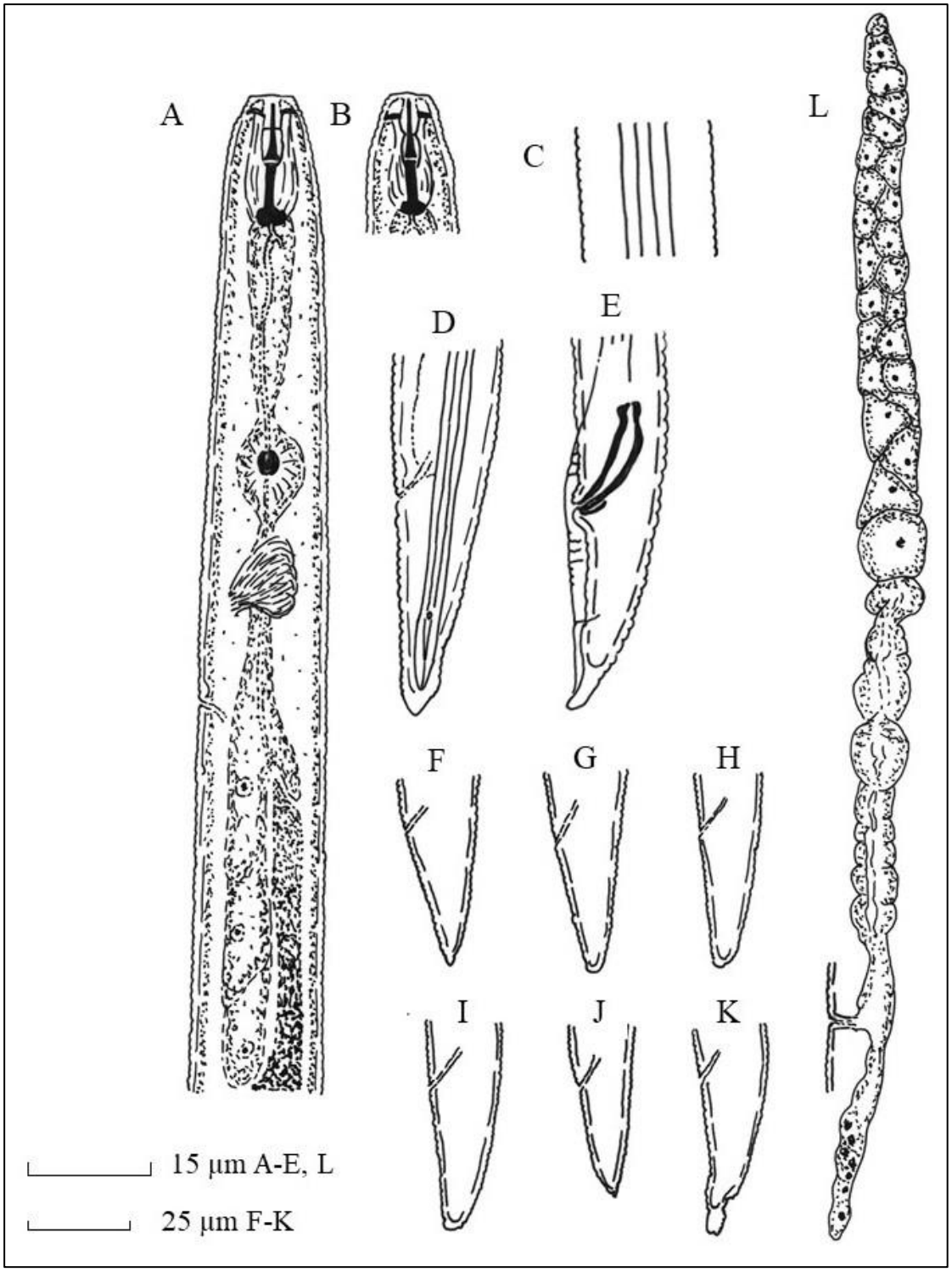


Figure 3.23: *Pratylenchus zeae* Graham, 1951 A: Female oesophageal region; B: Male labial region; C: Lateral field; D: Female tail showing end of lateral field; E: Male tail; F-K: Variation in shape of female tail shape; L: Female genital tract (A-E, L redrawn from Fortuner [1976]; F-K redrawn from Taylor & Jenkins [1957]).

Table 3.13: Morphometrics of females and males of *Pratylenchus zeae* Graham, 1951. All measurements are in µm unless stated otherwise.

Species	***P. zeae* Graham, 1951**		
Population	**South African**	**Range**	
	♀	♀	♂
L	340–590	340–640	400–420
a	12.9–29.9	12.9–35.0	27.0–32.0
b	–	3.3–9.6	3.6–5.0
b'	3.1–6.0	3.1–6.1	–
c	10.8–21.6	10.8–25.0	17.0–21.0
c'	–	2.1–4.0	–
o	–	–	–
m	–	–	–
V / T	68–79	[65]68–76[80]	30[a]
OV1	20–52	20–52	–
Number of annuli in labial region	3	3	3
Stylet length	14–16	15–18	15[a]
Oesophagus length	–	–	–
Dorsal gland opening from base of stylet knobs	–	3.0	–
Oesophageal gland overlap length	–	18–40	–
Excretory pore from anterior end	52–94	52–94	–
Post–vulval uterine branch length	16–48	15–48	–
Tail length	20–37	20–37	–
Number of annuli from anus to phasmid	8–15	8–15	–
Ventral tail annuli	19–28	16–28	–
Spicule length	–	–	14–15
Gubernaculum length	–	–	4–5

– Indicates structure not present or value not available.
[a] Indicates range not available.
Values in [] indicated values found in extreme cases.

3.5.2.21 Diagnostic keys to the species of *Pratylenchus* Filipjev, 1936 in South Africa

The polytomous key to the species of *Pratylenchus* in South Africa (Table 3.15 and 16) is based on the morphology of the female unless stated otherwise. The characters used are mostly morphological in nature due to the extensive overlapping morphometrical data of these species. Twenty-two characters were used and selected from work done by Ryss (2002a) and Castillo & Vovlas (2007). These characters were assigned a character state (Table 3.14) and each species of the genus found in South Africa was assigned a character state for each of the selected morphological characters (Table 3.15 and 16). Some

species are morphologically extremely similar and dichotomous keys are provided to these species in Notes 1-7.

Table 3.14: Characters used in the polytomous key to the species of *Pratylenchus* Filipjev, 1936 (Table 3.15 and 3.16).

Character	**Character state: Description**
A: Number of lip annuli (Figure 3.24)	1: Two annuli 2: Three annuli 3: Four annuli
B: Tail tip annulation (Figure 3.24)	1: Smooth 2: One or two crenations, giving an irregular shape 3: Distinctly annulated
C: Presence of males / sperm cells	1: Present, spermatheca filled with sperm 2: Absent: spermatheca empty 3: Rare: spermatheca empty, males in sample
D: Post-vulval uterine branch (PUB) differentiation (Figure 3.24)	1: PUB with rudimentary ovary 2: PUB without rudimentary ovary
E: Position of vulva (V)	1: <75 % 2: 76 – 80 % 3: 81 – 85 % 4: > 85 %
F: Lines in lateral field at mid body (Figure 3.24)	1: Four 2: Four with broken lines in central band giving the impression of fifth line 3: Five 4: Six
G: Shape of spermatheca (Figure 3.24)	1: Filled with sperm, oval in shape 2: Filled with sperm, round shape 3: Filled with sperm, rectangular in shape 4: Empty, distinct and offset 5: Indistinct

Table 3.14 (continued): Characters used in the polytomous key to the species of *Pratylenchus* Filipjev, 1936 (Table 3.15 and 3.16).

Character	**Character state: Description**
H: Aerolation of lateral field (Figure 3.24)	1: No aerolation 2: Aerolation in tail region 3: Aerolation in mid body
I: Labial region (Figure 3.25)	1: Offset from body contour 2: Slightly offset from body contour 3: Continuous with body contour
J: Lines in lateral field between phasmid and tail tip (Figure 3.25)	1: One 2: Two 3: Three 4: Four
K: Tail shape (Figure 3.25)	1: Conical 2: Subcylindrical 3: Cylindrical
L: Shape of tail tip (Figure 3.25)	1: Pointed 2: Conically rounded 3: Rounded 4: Spherical 5: Truncate 6: Obliquely truncate
M: Central band of lateral field (Figure 3.26)	1: Narrower than outer two bands 2: Equal to outer two bands 3: Wider than outer two bands
N: Shape of stylet knobs (Figure 3.26)	1: Sloping backwards 2: Anteriorly indented 3: Rounded 4: Flattened on anterior margin 5: Tulip-shaped
O: Length of PUB	1: Less than one body diameter long 2: One of more body diameters long

Table 3.14 (continued): Characters used in the polytomous key to the species of *Pratylenchus* Filipjev, 1936 (Table 3.15 and 3.16).

Character	**Character state: Description**
P: Number of ventral tail annuli	1: < 20 2: > 20
Q: Sclerotization of labial framework (Figure 3.26)	1: Light sclerotization 2: Moderate sclerotization 3: Strong sclerotization
R: Spicule length in male	1: < 20 µm 2: > 20 µm 3: Males absent
S: Stylet length	1: < 15 µm 2: 16 – 20 µm 3: > 21 µm
T: Oesophageal overlap length	1: Less one body diameter long 2: One to two body diameters long 3: Longer than two body diameters
U: Shape of median bulb (Figure 3.26)	1: Oval 2: Round
V: Shape of labial region in *en face* view (Figure 3.3)	1: Group 1 2: Group 2 3: Group 3

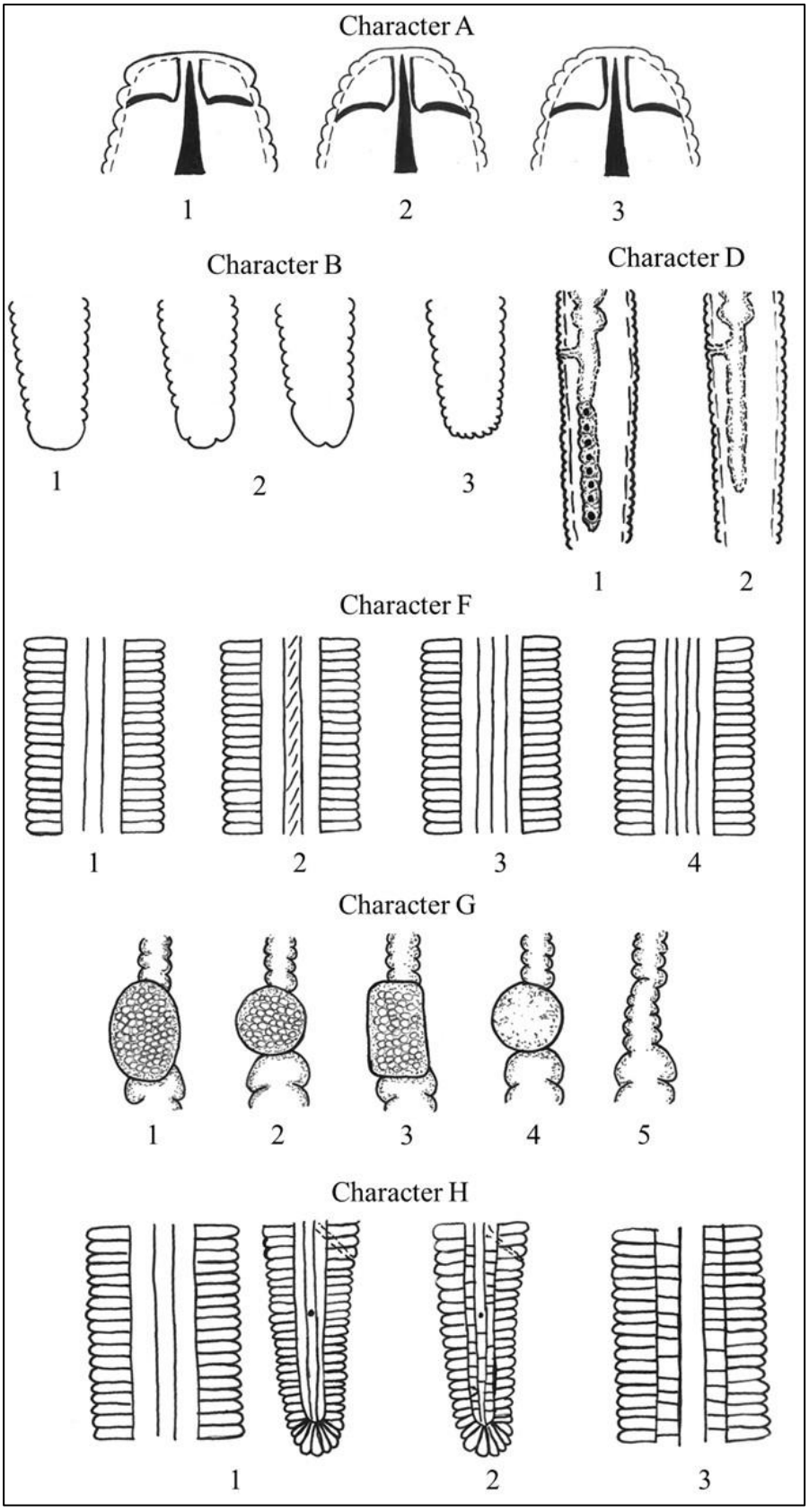


Figure 3.24: Illustrations of characters A; B; D; F; G and H with each of the states discussed in Table 3.14. Redrawn and adapted from Ryss (2002a).

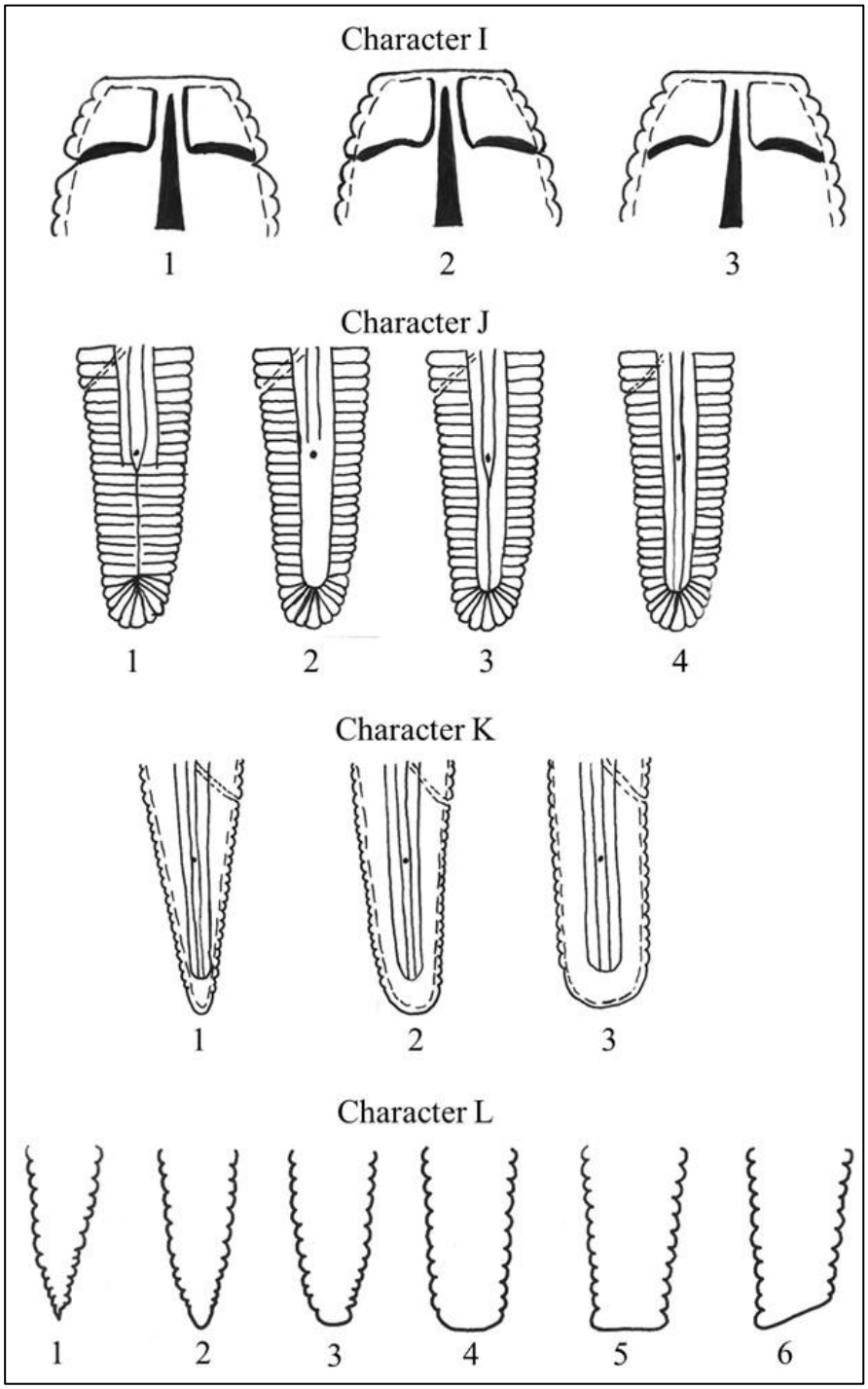


Figure 3.25: Illustrations of characters I-L with each of the states discussed in Table 3.14. Redrawn and adapted from Ryss (2002a).

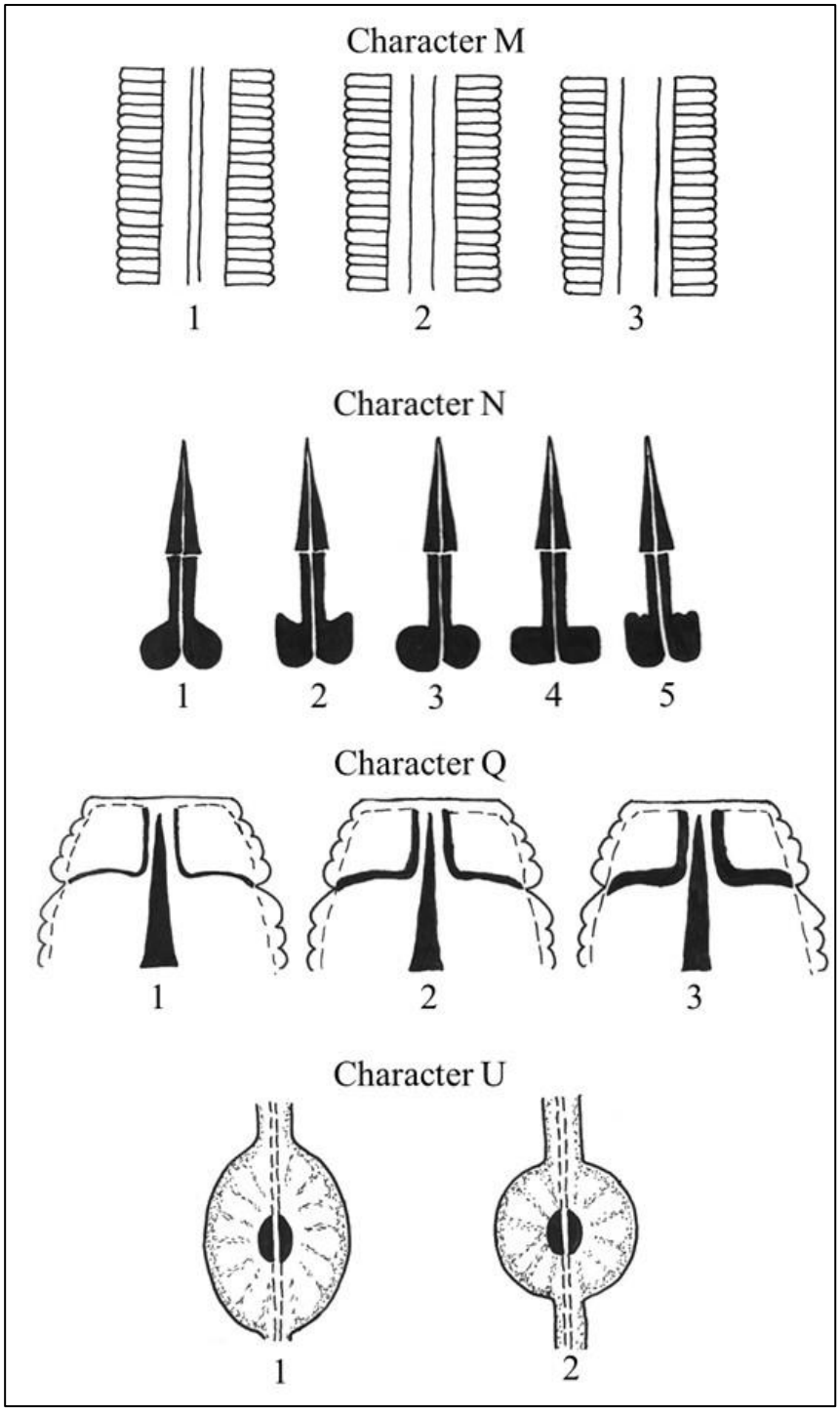


Figure 3.26: Illustrations of characters M; N; Q and U with each of the states discussed in Table 3.14. Redrawn and adapted from Ryss (2002a).

Table 3.15*:* Polytomous key to the species of *Pratylenchus* Filipjev, 1936 in South Africa, arranged by species.

Character state	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**	**M**	**N**	**O**	**P**	**Q**	**R**	**S**	**T**	**U**	**V**
P. bolivianus	2(3)	1	2	2	3(2)	1(2)	5	3(2)	2(3)	4	1(2)	3(2)	3	3	2	1	3(2)	3	2	2	1	2
P. brachyurus	1	1	2	2	3(4)	1(2)	5	1	1	2(3)	2(1)	3(4)(5)	2	3	1	1	2	3	2(3)	2(3)	2	1
P. coffeae	1	1(2)	1	1	3(2)	1(2)	2(1)	2(3)	2	2(3)	2	3	2	3	2	1(2)	2	1	(1)2	2(3)	1	1
P. crenatus	2	3	2(3)	2	3(2)	4	4(5)	3(2)	3	4(2)	2	3	2	2	2	2	2	3(1)	2(1)	2	2	1
P. delattrei	2	1	2	2	2(3)	1	4(5)	1	3	4	2(1)	2(3)	2	4(3)	2	1	3	3	2(1)	2	2(1)	0
P. fallax	2	2(3)	1	1(2)	2(3)	1(2)	2	1	3	3(2)	2	3	2	2	2	2(1)	2	1	2(1)	2	1	3
P. flakkensis	1	2(3)	1	2	2(3,1)	1	2(3)	2	2	4(2)	1(2)	3(4)	2	2	1(2)	1(2)	3	1	2(1)	1	2	0
P. goodeyi	3	1	1	2	1(2)	1	3(1)	2(3)	2	3	1	1(2)	0	4	1(2)	2	2	1(2)	2(1)	2	1	1
P. hexincisus	1	1	2	2	2(1)	4	5	1	2(1)	4	2	2	2	3	2(1)	1(2)	2	3	1(2)	2	2	3
P. loosi	1	1	1	1	3(2)	1(2)	1	1	1	3	1	2(1)	2	3	2	2	2	1	2(1)	3(2)	1	1
P. neglectus	1	1	2(3)	2	3(4)	1(2)	4(5)	2	2	3(2)	2	3(5,2,6)	2	2(3)	2(1)	1(2)	2	0	2	2	2	2
P. penetrans	2	1	1	2(1)	2(3)	1	2	2(3)	2	3(4)	1(2)	2(3)	2	3(2)	2(1)	2(1)	3	1	2	2	2	3
P. pratensis	2	3	1	2	2(1)	1	1(3)	1	3(2)	2	1(2)	2(1)	2	4(3)	2	2	2	1	1(2)	1(2)	1	3
P. pseudopratensis	2	3(2)	1	1	2	1(2)	3	1	2	3	2	3(4,5,1)	2	4	2	1(2)	2	1	2	2	2(1)	1
P. scribneri	1	1	2(3)	2	2(1)	1	4(5)	1	2	4(3)	1(2)	3(2)	2	3(4)	2(1)	1(2)	2	1	2	2	1	3
P. tenuis	1	0(1)	2	2	2	1	5	1	1	2	2	4	2	5	1	0	1	3	1(2)	3	0	0
P. teres	2	3	2	2	2	4	5(4)	3	2	4(2)	2	3	2	2	2	2	2	3	2	3	1	2
P. thornei	2	1	2(3)	2	2(3)	1(2)	5(4)	2	3	4(3)	3	5(6)	2	3(4)	2	2	2	2	2	2(3)	1	2
P. vulnus	2(3)	1(2)	1	1	2(3)	1(2)	1	1(2)	3(2)	3	1	2(1)	1	3(2)	2	2	3	1(2)	2(1)	2	1	3
P. zeae	2	1	2(3)	1	1	1	4(5)	1	3	4(3)	1	2(1)	2	4	2	2	3	1	2	2(3)	2	1

Table 3.16: Polytomous key to the species of *Pratylenchus* Filipjev, 1936 in South Africa arranged by increasing values of different characters.

Character state	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V
P. loosi	1	1	1	1	3(2)	1(2)	1	1	1	3	1	2(1)	2	3	2	2	2	1	2(1)	3(2)	1	1
P. tenuis [1]	1	0(1)	2	2	2	1	5	1	1	2	2	4	2	5	1	0	1	3	1(2)	3	0	0
P. scribneri [1]	1	1	2(3)	2	2(1)	1	4(5)	1	2	4(3)	1(2)	3(2)	2	3(4)	2(1)	1(2)	2	1	2	2	1	3
P. hexincisus [1]	1	1	2	2	2(1)	4	5	1	2(1)	4	2	2	2	3	2(1)	1(2)	2	3	1(2)	2	2	3
P. brachyurus [2]	1	1	2	2	3(4)	1(2)	5	1	1	2(3)	2(1)	3(4)(5)	2	3	1	1	2	3	2(3)	2(3)	2	1
P. neglectus [2]	1	1	2(3)	2	3(4)	1(2)	4(5)	2	2	3(2)	2	3(5,2,6)	2	2(3)	2(1)	1(2)	2	0	2	2	2	2
P. coffeae [3]	1	1(2)	1	1	3(2)	1(2)	2(1)	2(3)	2	2(3)	2	3	2	3	2	1(2)	2	1	(1)2	2(3)	1	1
P. flakkensis [3]	1	2(3)	1	2	2(3,1)	1	2(3)	2	2	4(2)	1(2)	3(4)	2	2	1(2)	1(2)	3	1	2(1)	1	2	0
P. vulnus [4]	2(3)	1(2)	1	1	2(3)	1(2)	1	1(2)	3(2)	3	1	2(1)	1	3(2)	2	2	3	1(2)	2(1)	2	1	3
P. penetrans [4]	2	1	1	2(1)	2(3)	1	2	2(3)	2	3(4)	1(2)	2(3)	2	3(2)	2(1)	2(1)	3	1	2	2	2	3
P. zeae	2	1	2(3)	1	1	1	4(5)	1	3	4(3)	1	2(1)	2	4	2	2	3	1	2	2(3)	2	1
P. delattrei [5]	2	1	2	2	2(3)	1	4(5)	1	3	4	2(1)	2(3)	2	4(3)	2	1	3	3	2(1)	2	2(1)	0
P. thornei [5]	2	1	2(3)	2	2(3)	1(2)	5(4)	2	3	4(3)	3	5(6)	2	3(4)	2	2	2	2	2	2(3)	1	2
P. bolivianus [5]	2(3)	1	2	2	3(2)	1(2)	5	3(2)	2(3)	4	1(2)	3(2)	3	3	2	1	3(2)	3	2	2	1	2
P. fallax	2	2(3)	1	1(2)	2(3)	1(2)	2	1	3	3(2)	2	3	2	2	2	2(1)	2	1	2(1)	2	1	3
P. pseudopratensis [6]	2	3(2)	1	1	2	1(2)	3	1	2	3	2	3(4,5,1)	2	4	2	1(2)	2	1	2	2	2(1)	1
P. pratensis [6]	2	3	1	2	2(1)	1	1(3)	1	3(2)	2	1(2)	2(1)	2	4(3)	2	2	2	1	1(2)	1(2)	1	3
P. crenatus [7]	2	3	2(3)	2	3(2)	4	4(5)	3(2)	3	4(2)	2	3	2	2	2	2	2	3(1)	2(1)	2	2	1
P. teres [7]	2	3	2	2	2	4	5(4)	3	2	4(2)	2	3	2	2	2	2	2	3	2	3	1	2
P. goodeyi	3	1	1	2	1(2)	1	3(1)	2(3)	2	3	1	1(2)	0	4	1(2)	2	2	1(2)	2(1)	2	1	1

[1]Note 1; [2]Note 2; [3]Note 3; [4]Note 4; [5]Note 5; [6]Note 6; [7]Note 7.

Note 1:

Three species that are morphologically similar are P. *hexincisus*, *P. scribneri* and *P, tenuis* based on characters A to E (Table 3.14). However, these species can be separated by the following key:

1 Lateral field with four lines**……2**
Lateral field with six lines**…… *P. hexincisus***

2 Two lines in lateral field between phasmid and tail tip, stylet knobs tulip shaped**……..……. *P. tenuis***
Three to four lines in lateral field between phasmid and tail tip, stylet knobs rounded**……...*P. scribneri***

Note 2:

Two species that are morphologically similar are *P. brachyurus* and *P. neglectus* based on characters A to F (Table 3.14). However, these species can be separated by the following key:

1 Anterior margin of labial region angular, stylet knobs round, *En face* view of labial region undivided with fusion of labial disc and first labial annulus**……... *P. brachyurus***
Anterior margin of labial region bluntly rounded, second annulus wider than first, stylet knobs typically indented anteriorly, *en face* view of labial region submedian segments separated from lateral segments **…….*P. neglectus***

Note 3:

Two species that are morphologically similar are *P. coffeae* and *P. flakkensis* based on characters A to J (Table 3.14). However, these species can be separated by the following key:

1 Post-vulval uterine branch with rudimentary ovaries, longer than one body diameter, two lines in lateral field between phasmid and tail tip, stylet knobs rounded, female tail pointed**……..*P. coffeae***
Post-vulval uterine branch without rudimentary ovaries, shorter than one body diameter, four lines in lateral field between phasmid and tail tip, stylet knobs anteriorly indented, female tail conically rounded**……*P. flakkensis***

Note 4:

Two species that are morphologically similar are *P. vulnus* and *P. penetrans* based on characters A to F (Table 3.14). However, these species can be separated by the following key:

1 Spermatheca oval, central band of lateral field narrower than outer bands**……*P. vulnus***
Spermatheca round, central band of lateral field equal to outer bands **……*P. penetrans***

Note 5:

Three species that are morphologically similar are *P. delattrei, P. thornei* and *P. bolivianus* based on characters A to K (Table 3.14). However, these species can be separated by the following key:

1 Lateral field not aerolated **……...*P. delattrei***
Lateral field aerolated or partially aerolated in tail region **…….2**
Tail cylindrical, tail tip truncate, central band of lateral field equal to outer bands, less than 20 ventral
2 tail annuli **……...……...*P. thornei***
Tail conical to subcylindrical, tail tip rounded to conically rounded, central band of lateral field wider than outer bands, more than 20 ventral tail annuli **……..*P. bolivianus***

Note 6:

Two species that are morphologically similar are *P. pseudopratensis* and *P. pratensis* based on characters A to I (Table 3.14). However, these species can be separated by the following key:

1 Post-vulval uterine branch with rudimentary ovaries, three lines in lateral field between phasmid and tail tip, *en face* view of labial region undivided with fusion of labial disc and first labial annulus **……*P. pseudopratensis***
Post-vulval uterine branch without rudimentary ovaries, two lines in lateral field between phasmid and tail tip, *en face* view of labial region with distinct dumb-bell shaped pattern of submedian segments **……..……*P. pratensis***

Note 7:

Two species that are morphologically similar are *P. crenatus* and *P. teres* based on characters A to I (Table 3.14). However, these species can be separated by the following key:

1 Spermatheca distinct but empty, lateral field with four lines between phasmid and tail tip, oesophageal overlap short, not more than one body diameter **……*P. crenatus***
Spermatheca indistinct, lateral field with one or two lines between phasmid and tail tip, oesophageal overlap long, more than two body diameters **……*P. teres***

3.5.3 Zygotylenchus Siddiqi, 1963

According to Geraert (2013) the genus *Zygotylenchus* consist of only four valid species i.e. *Z. guevarai* (Tobar Jiménez, 1963) Braun & Loof, 1966, *Z. gansuensis* Wang, Zhou & Liao, 2014, *Z. natalensis* Van den Berg & Tiedt, 2003 and *Z. taomasinae* (De Guiran, 1964) Braun & Loof, 1966. The species *Z. guevarai, Z. natalensis* and *Z. taomasinae* have been reported in South Africa.

Diagnosis (Siddiqi 1963; Siddiqi 2000; Geraert 2013).

Pratylenchinae: Small, less than 1000 μm. No marked sexual dimorphism in anterior region. Cuticle finely annulated. Lateral field with four incisures, not aerolated, deirids absent. Labial region low, slightly tapering, anteriorly truncate or rounded. Lip region continuous, annulated. Framework strongly sclerotized, outer margins extending into body, vestibulum extension tubular. Stylet strong, knobs rounded. Isthmus short. Oesophageal gland overlaps intestine mostly ventrally, subventral glands asymmetrical, oesophageal-intestinal junction indistinct, ventral to main body of glands, about one body width posterior to median bulb, anterior to gland nuclei. Vulva slightly posterior to mid-body. Didelphic, amphidelphic. Ovaries outstretched, rarely reflexed, oocytes arranged in single row.

Spermatheca round, filled with round sperm. Phasmids near middle of tail. Female tail cylindrical or tapering, terminus smooth or annulated. Male tail tapering to pointed terminus, bursa peloderan. Spicules cephalated, arcuate, with ventral sub-terminal pore. Gubernaculum trough-shaped, fixed.

3.5.4 Species of *Zygotylenchus* Siddiqi, 1963 reported in South Africa:

3.5.4.1 *Zygotylenchus guevarai* (Tobar Jiménez, 1963) Braun & Loof, 1966

= *Pratylenchoides guevarai* Tobar Jiménez, 1963

= *Zygotylenchus browni* Siddiqi, 1963

=*Mesotylus gallicus* De Guiran, 1964

=*Zygotylenchus gallicus* (De Guiran, 1964) Braun & Loof, 1966

Figure 3.27

Measurements: Table 3.17

Zygotylenchus guevarai was described in Spain and according to Geraert (2013) reported from the Afrotropical (South Africa) and Palearctic (Mediterranean) biogeographical regions. In South Africa this species has been reported in South Africa from various host plants (Table 3.25, Figure 3.39). The morphometrical range of this species (Table 3.17) consists of the type (as described by Siddiqi [1975]) and other population descriptions (Siddiqi 1963; Tobar Jiménez 1963; De Guiran 1964; Siddiqi 1975; Van den Berg 1989; Loof 1991; Ryss 1992; Pourjam *et al.* 2000; Geraert 2013). The South African population of this species (Table 3.17) was described by Van den Berg (1989). According to Siddiqi (1975) *Z.* guevarai was described as a new taxon almost simultaneously by three authors: as *Pratylenchoides guevarai* n. sp. by Tobar Jiménez (1963); as *Z. browni* n.g., n. sp. by Siddiqi (1963) and as *Mesotylus gallicus* n.g., n.sp. by De Guiran (1964) with priority in the date of publication in that

order. The diagnosis of *P. guevarai* Tobar Jiménez, 1963 could not be located and the descriptions of *Z. browni* Siddiqi, 1963 and *M. gallicus* De Guiran, 1964 did not include a diagnosis for the species as these papers were the type descriptions of the genera *Zygotylenchus* and *Mesotylus*, respectively. Therefore in the present compendium a differential diagnosis for *Z. guevarai* is given based on characters unique to the species.

Decsription:

Holotype (Tobar Jiménez, 1963): Female: L = 530 µm; a = 28.1; b = 4.3; c = 20.1; V = 63 %; stylet length = 16 µm.

Female: Body slender, slightly curved ventrally. Cuticle finely annulated. Lateral field with four lines, inner band wider than outer bands, irregular areolation at mid-body and tail. Labial region flattened anteriorly, moderately developed labial framework, four to five annuli. *En face* view with rounded oral disc, partially incised submedian and subventral segments separated from lateral segments by two incisures forming an obtuse angle. Second lip annule rarely marked by longitudinal incisures. Stylet strong, knobs rounded. Dorsal gland opening 1.1-3.7 µm from the base of stylet knobs. Oesophageal gland overlaps intestine ventrally, overlap 22-81 µm long. Deirids absent. Excretory pore anterior to the oesophageal-intestinal junction, 75-111 µm from anterior end. Spermatheca rounded, filled with round sperms. Phasmids minute, at posterior half of tail. Tail cylindrical, 12-27 annuli on ventral side, terminus smooth, broadly rounded to truncate, rarely with small ventral projection or cleft.

Male: Similar to female except higher and narrower lip region. Cloacal opening with distinct hypotygmata. Tail tapering to a pointed terminus, bursa peloderan. Spicules 16-27 µm long. Gubernaculum 5-9 µm, not protruding.

Differential diagnosis:

Zygotylenchus guevarai is characterised by a labial region with four to five annuli, stylet 14-20 µm long, lateral field with four lines, the inner band wider than the outer two bands, anterior and posterior genital branches equal in length, spermatheca round, filled with sperm cells, tail cylindrical to subcylindrical, bearing 12-27 annuli on ventral side, tail terminus smooth, broadly round to truncate, occasionally with small ventral projection or cleft.

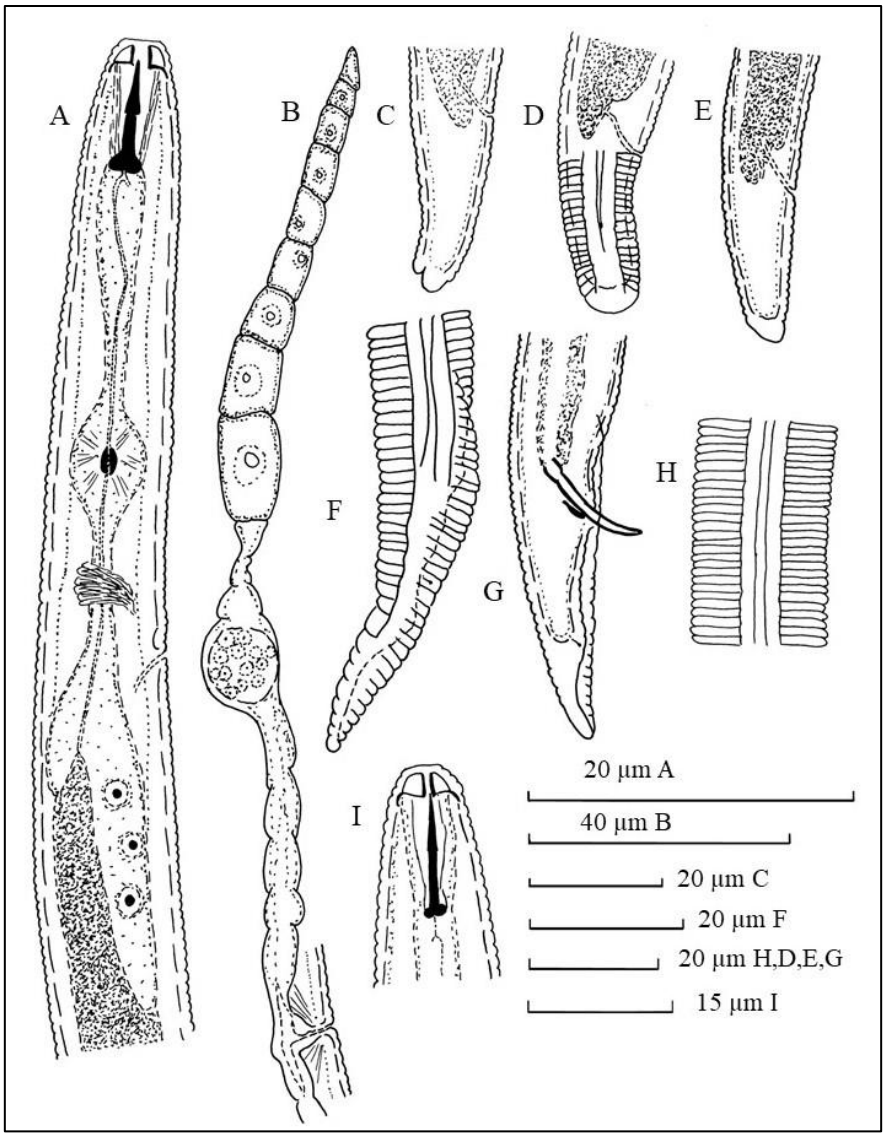


Figure 3.27: *Zygotylenchus guevarai* (Tobar Jiménez, 1963) Braun & Loof, 1966 A: Female oesophageal region; B: Female anterior genital tract showing spermatheca; C-E: Variation in female tail region; F-G: Male tails; H: Lateral field; I: Male labial region (A-C redrawn from Pourjam et al. [2000]; F redrawn from Van den Berg [1989]; D-E, G-I redrawn from Siddiqi [1975]).

3.5.4.2 *Zygotylenchus natalensis* Van den Berg & Tiedt, 2003

Figure 3.28

Measurements: Table 3.17

Zygotylenchus natalensis was described from South Africa in the KwaZulu-Natal Province in the Afrotropical biogeographical region and has not been reported since (Van den Berg & Tiedt 2003). Therefore the morphometrical range of this endemic species (Table 3.17) is from the type and only description of the species (Van den Berg & Tiedt 2003)

Description:

Holotype (Van den Berg & Tiedt 2003): Female: L = 523 µm; a = 34.7; b = 5.7; b' = 4.3; c = 17.1; c' = 3.0; V = 65 %; stylet length = 16 µm.

Female: Habitus curved irregular, rarely dorsally curved into an open C. Cuticle finely annulated, annuli 0.7-1.5 µm at mid-body. Lateral field with four lines, outer two lines slightly crenate, Scanning Electron Microscopy (SEM) shows slight aerolations in all three bands, becoming more crenate on tail where outer lines disappear near tail terminus and inner lines merge with phasmid gradually fading out before tail terminus. Labial region flattened anteriorly, slightly set off with three annuli. *En face* view shows the dorsal and ventral median segments are separated dorsally and ventrally to form separate segments resembling the blades of a fan, lateral segments not distinctly separated. Stylet well-developed, knobs anteriorly flattened or slightly indented anteriorly. Opening of dorsal gland 2.0-4.5 µm posterior to stylet knobs. Oesophageal gland overlaps intestine ventrally, rarely laterally and in one specimen dorsally, overlap 13-38 µm long. All three gland nuclei situated posterior to oesophagus-intestinal junction. Excretory pore situated from just posterior to median bulb to opposite

junction of isthmus and basal bulb, 64-89 μm from anterior end. Deirids absent. Vulva without prominent lips. Posterior branch of genital tract shorter and less developed than anterior branch Spermatheca of posterior branch reduced (rarely inconspicuous), small and empty or contains only one or two sperm cells, spermatheca of anterior branch large, rounded to elongated, filled with sperm or empty. Phasmids near middle of tail, 9-18 annuli posterior to anus. Tail conoid, bearing 27-35 ventral annuli, gradually tapering to an irregularly annulated terminus with ventral projection, hyaline portion 2-6 μm long.

Male: Similar to female except in posterior end. Spicules 15-19 μm long. Gubernaculum 4-6 μm long. Phasmid near middle of tail. Tail with narrow, finger-like tip. Bursa narrow, enveloping tail.

Diagnosis (Van den Berg & Tiedt 2003):

Zygotylenchus natalensis is characterised by slightly set off labial region with three distinct annuli, lateral field faintly aerolated, posterior genital tract less developed than anterior branch, tail irregularly annulated bearing 27-35 annuli on ventral side, tail tip with distinct ventral projection and a hyaline tip of 2-6 μm, no marked sexual dimorphism in males, spicules 15-19 μm, male tail with fring-like hyaline tip 7-11 μm long.

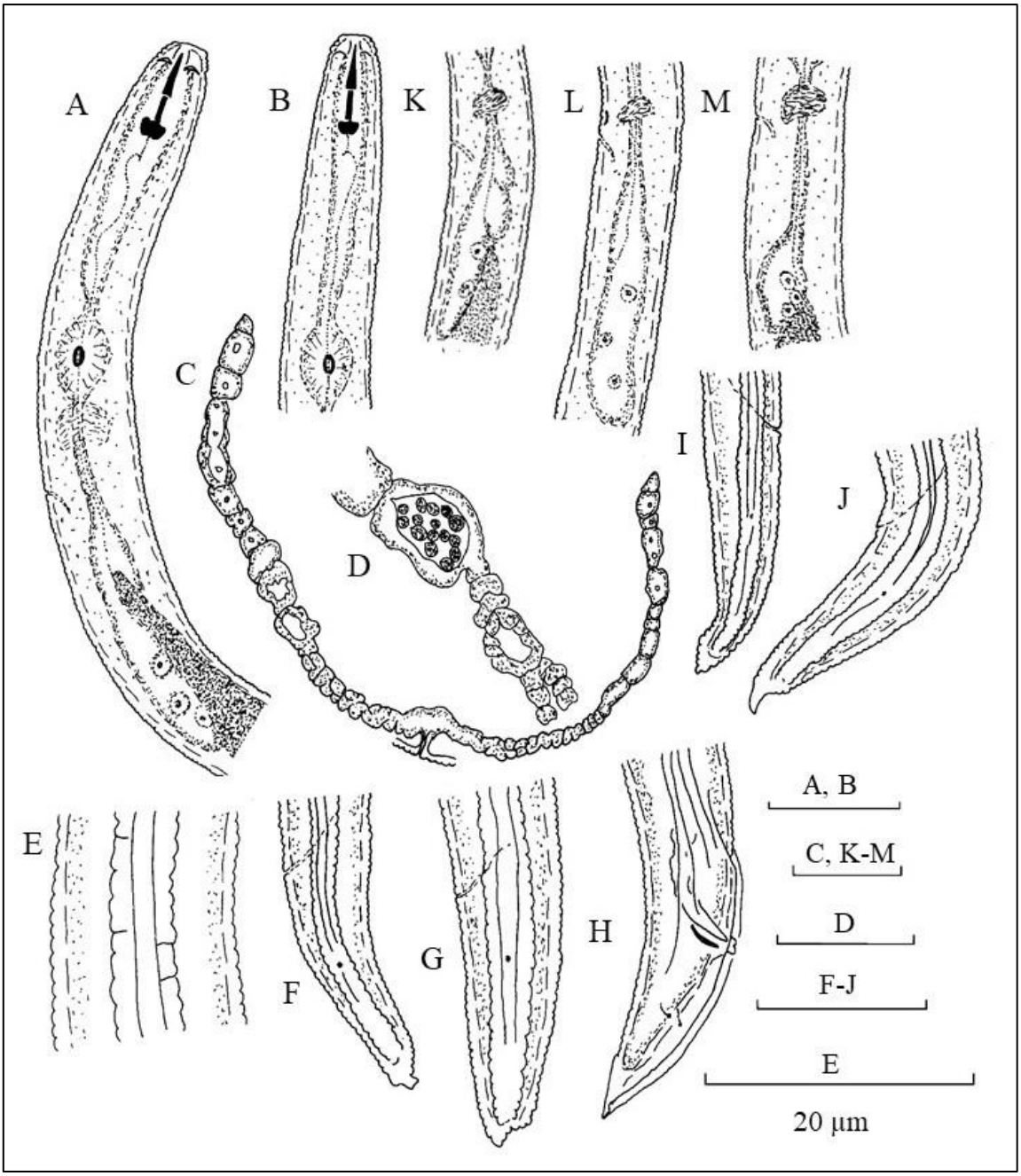


Figure 3.28: *Zygotylenchus natalensis* Van den Berg & Tiedt, 2003 A: Female oesophageal region; B: Male anterior region; C: Female genital tract; D: Spermatheca in anterior genital branch; E: Lateral field; F-G, I-J: Variation in shape of female tail; H: Mail tail; K-M: Variation of oesophageal gland overlap in females (redrawn from Van den Berg & Tiedt [2003]).

Table 3.17: Morphometrics of females and males of *Zygotylenchus guevarai* (Tobar Jiménez, 1963) Braun & Loof, 1966 and *Zygotylenchus natalensis* Van den Berg & Tiedt, 2003. All measurements are in µm unless stated otherwise.

Zygotylenchus	***Z. guevarai* (Tobar Jiménez, 1963) Braun & Loof, 1966**				***Z. natalensis* Van den Berg & Tiedt, 2003**	
	South African		**Range**		**Type, SA and range**	
	♀	♂	♀	♂	♀	♂
L	440–696	482–619	390–770	430–660	500–669	492–632
a	23.9–32.5	27.3–31.7	23.0–34.7	23.8–37.2	29.5–40.5	32.5–44.1
b	4.1–5.3	3.8–5.6	3.6–8.1	3.7–7.1	5.4–7.1	5.4–6.7
b'	5.5–7.7	5.0–6.8	3.5–7.7	3.6–6.8	4.1–5.4	4.1–5.2
c	15.8–22.3	13.6–25.7	15.8–27.7	13.6–25.7	16.5–20.6	17.1–21.2
c'	2.1–3.1	1.9–3.5	1.1–3.1	1.9–3.5	2.8–3.3	2.3–2.8
o	7.9–23.3	6.8–22.9	7.9–23.3	6.8–22.9	11.6–26.7	16.7–30.0
m	44–53	44–51	44–53	44–51	44–50	–
V / T	60–65	–	53–67	25–49	58–65	–
OV1	12–29	–	12-29	–	15–25	–
OV2	17–21	–	17–21	–	11–21	–
Number of annuli in labial region	3–4	3–4	3–5	3–5	3	3
Stylet length	14–18	14–17	14–20	14–18	14–17	13–15
Dorsal gland opening from base of stylet knobs	1.1–3.7	1.1–3.7	1.1–3.7	1.1–3.7	2.0–4.5	3.0–4.5
Oesophageal gland overlap length	25–41	27–36	22–81	26–40	13–38	16–34
Excretory pore from anterior	84–111	85–99	75–111	76–100	64–89	73–87
Oesophagus length	109–138	109–154	100–170	96–154	94–140	104–131
Spicule length	–	18–22	–	16–27	–	15–19
Gubernaculum length	–	5–7	–	5–9	–	4–6
Hyaline length	3–6	7–18	3–6	7–18	2–6	7–11
Number of annuli from anus to phasmid	8–14	–	8–14	–	9–18	–
Ventral tail annuli	16–27	–	12–27	–	27–35	–
Tail length	23–38	22–39	23–38	20–39	25–38	27–32

– Indicates structure not present or value not available.

3.5.4.3 *Zygotylenchus taomasinae* (De Guiran, 1964) Braun & Loof, 1966

= *Mesotylus taomasinae* De Guiran, 1964

= *Pratylenchoides taomasinae* (De Guiran, 1964) Tarjan & Weischer, 1965

Figure 3.29

Measurements: Table 3.18

Zygotylenchus taomasinae was described from Madagascar and has since only been reported from South Africa from *Nicotiana tabacum* L. in the Western Cape Province (Van den Berg, 1986). The distribution of this species seems limited to the Afrotropical biogeographical region. The morphometrical range (Table 3.18) is a combination of the type description and the South African population (De Guiran 1964; Van den Berg 1986; Geraert 2013). The South African population of *Z. taomasinae* (Table 3.18) was described by Van den Berg (1986). As in the case of *Z. guevarai* the diagnosis of *Z. taomasinae* was not given in the original description by De Guiran (1964). Therefore a differential diagnosis of the species based on characters unique to the species is given.

Description:

Holotype (De Guiran 1964): Female: L = 840 µm; a = 34.0; b = 4.5; c = 14.0; V = 56 %;

Female: Habitus ranging from slightly curved ventrally to an open C. Cuticle distinctly annulated, annuli 1.1-1.6 µm at mid-body. Lateral field with four lines, in some specimens with fainter broken lines in the central band, outer two lines slightly crenate over entire body, but distinctly crenate on tail. Labial region flattened anteriorly, slightly set off with three annuli, framework heavily sclerotized, basal plate extending 1 annulus backwards. Stylet slender, anterior margin of knobs ranging from slightly indented to slightly sloping posteriorly. Opening of dorsal gland 1.5-2.6 µm. Oesophageal

gland overlaps intestine ventrally in most specimens, 56-68 μm long. All three gland nuclei situated posterior to oesophageal-intestinal junction. Excretory pore opposite anterior end of oesophageal lobe, 72-110 μm from anterior end. Vulva with prominent lips. Spermatheca ranges from indistinct, round, small and empty to sub-spherical and filled with rounded sperm. Phasmids near middle of tail, 15-19 annuli posterior to anus. Tail gradually tapering to an narrowly rounded, unstriated or partially striated terminus with 30-45 annuli on ventral side, inner lines of lateral field continuing past the phasmid then coalescing and continuing as a single line for a distance along the tail, but not as far posteriorly as the outer two lines.

Male: Similar to female except less developed oesophageal lobes. Tail tapering to a slender, rounded hyaline peg, with the long, narrow bursa ending at the base of the peg. Spicules 18-27 μm long. Gubernaculum 4-9 μm long.

Differential diagnosis:

Zygotylenchus taomasinae is characterised by labial region with three distinct annuli, slightly set off from body contour, stylet 13-18 μm long, lateral field with four lines, outer lines slightly crenate over entire length of body, distinctly crenate in tail region, inner band occasionally with faint broken lines, genital branches equal in length, spermatheca indistinct, empty, tail bearing 30-45 ventral annuli, tapering gradually to narrowly rounded, smooth or partly annulated terminus.

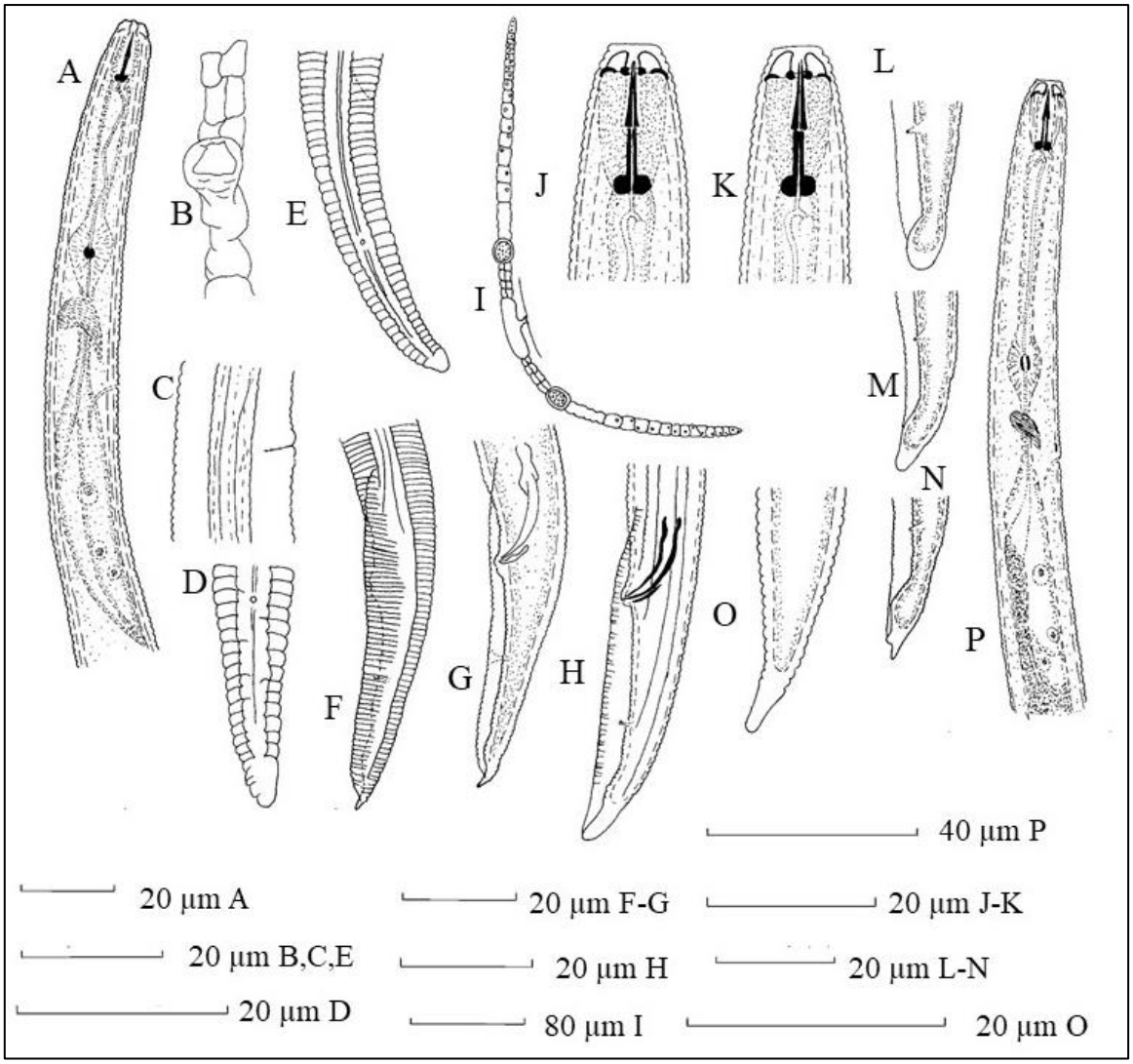


Figure 3.29: *Zygotylenchus taomasinae* (De Guiran, 1964) Braun & Loof, 1966 A, P: Female oesophageal region; B: Shape of spermatheca; C: Lateral field at vulval region; D-E, O: Variation in shape of female tail; F-H, L-N: Variation in shape of male tail; I: Female genital tract; J: Female labial region; K: Male labial region (A-G redrawn from Van den Berg [1986]; H-P redrawn from De Guiran [1964]).

Table 3.18: Morphometrics of females and males of *Zygotylenchus taomasinae* (De Guiran, 1964) Braun & Loof, 1966. All measurements are in µm unless stated otherwise.

Species	***Z. taomasinae* (De Guiran, 1964) Braun & Loof, 1966**			
	South African		**Range**	
	♀	♂	♀	♂
L	528–624	514–647	528–840	514–680
a	27–36.7	32.3–40.6	27.0–41.0	32.8–48
b	3.2–5	4.4–6.1	3.2–5.3	4.3–6.1
b'	4.1–7	5.7–8.3	4.1–7.0	5.7–8.3
c	11.9–15.5	13.1–16.7	11.9–16.0	13.0–16.7
c'	3.7–4.5	2.7–4.2	3.0–4.5	2.7–4.2
o	10.2–18.9	12.2–15.7	10.2–18.9	12.2–15.7
m	50–55	50–55	50–55	50–55
V / T	56–59	–	56–60	33–46
OV1	14–25	–	14–25	–
OV2	17–23	–	14–23	–
Number of annuli in labial region	3	3	3	3
Stylet length	13.2–14.7	13.3–16.2	13–18	13–17
Dorsal gland opening from base of stylet knobs	1.5–2.6	1.8–2.2	1.5–2.6	1.8–2.2
Oesophageal gland overlap length	51–68	27–38	51–68	27–38
Excretory pore from anterior	72–89	74–99	72–110	74–99
Oesophagus length	119–162	99–142	1119–162	99–142
Spicule length	–	22–25	–	18–27
Gubernaculum length	–	6–9	–	4–9
Hyaline length	–	–	6[a]	5–6
Number of annuli posterior to phasmid	–	–	6–9	–
Number of annuli from anus to phasmid	15–19	–	15–19	–
Ventral tail annuli	30–34	–	30–45	–
Tail length	40–45	35–45	40–45	35–45

– Indicates structure not present or value not available.
[a] Indicates range not available.

3.5.4.4 *Key to the species of Zygotylenchus in South Africa*

Table 3.19: Key to *Zygotylenchus* Siddiqi, 1963

1	Female tail with annulated terminus, posterior branch of genital tract less developed than anterior **…… *Z. natalensis***
	Female tail with smooth or partially annulated terminus, posterior and anterior branches of genital tracts equally developed…..**……2**
2	Female tail sub-cylindrical, 12-27 ventral tail annuli **……*Z. guevarai***
	Female tail conical, tip finely rounded, 30-45 ventral tail annuli**…….……*Z. taomasinae***

3.6 Subfamily Hirschmanniellinae Fotedar & Handoo, 1978

Diagnosis (Geraert 2013)

Pratylenchidae. Moderate to large and slender. No marked sexual dimorphisms in anterior region. Cuticle finely annulated. Lateral field with four incisures, reducing towards extremities. Labial region continuous, usually hemispheroidal, labial framework heavily sclerotized. *En face* of labial region with no differentiation between lip segments and oral disc. Stylet large. Oesophageal glands elongated, overlapping intestine mostly ventrally. Didelphic, amphidelphic. Phasmids in posterior third of tail. Tail of both sexes similar, elongate-conoid, often with a mucro. Bursa large, sub-terminal. Spicules with dorsal sub-terminal pore. Gubernaculum fixed, simple. Phasmids in posterior third of tail.

3.6.1 *Hirschmanniella* Luc & Goodey, 1964

According to Geraert (2013), the genus *Hirschmanniella* consists of 37 valid species. Members of this genus prefer warm moist habitats and more than half of the species have been reported from *Oryza sativa* L. (Loof 1991; Castillo *et al.* 2012). According to Castillo *et al.* (2012) some species have also been reported from aquatic plants such *Hydrophyllum* L. and *Sagittaria subulata* (L.) Buch. i.e. *H.*

caudacrena Sher, 1968 and *H. oryzea* (Van Breda De Haan, 1902) Luc & Goodey, 1964. In South Africa, two species of the genus *Hirschmanniella* have been reported.

Diagnosis (Luc & Goodey 1962; Geraert 2013):

Hirschmanniellinae. Long (1000-4000 μm) and slender. No sexual dimorphism in anterior region. Cuticle distinctly annulated. Four incisures in lateral field, often with partial areolation and rarely with complete areolation. Labial region low, flattened with rounded edges or higher, hemispherical. Three to seven lip annuli, continuous with body contour. Stylet strong, three to five times the body width at the base of head in length, knobs large. Median bulb well developed. Oesophageal glands in tandem, forming a long, ventrally overlapping lobe. The mid-intestine does not fill the whole body cavity, between mid-intestine and body wall transverse strands are present giving the impression of a row of cells on either side, called thorneian cells. Female genital tract paired, equally developed. Male with bursa extending over two-thirds of tail. Spicules tylenchoid, gubernaculum variable in shape. Phasmids in posterior third of tail. Tails in both sexes elongate, tapering to terminus baring a mucro, rarely two, in some species a ventral notch just anterior to terminus.

3.6.2 Species of *Hirschmanniella* reported in South Africa:

3.6.2.1 *Hirschmanniella spinicaudata* (Schuurmans Stekhoven, 1944) Luc & Goodey, 1964

= *Tylenchorhynchus spinicaudatus* Schuurmans Stekhoven, 1944

= *Hirschmannia spinicaudata* (Schuurmans Stekhoven, 1944) Luc & Goodey, 1962

= *Radopholus lavabri* Luc, 1957

= *Hirschmanniella lavabri* (Luc, 1957) Sher, 1968

Figure 3.30

Measurements: Table 3.20.

Hirschmanniella spinicaudata (Schuurmans Stekhoven, 1944) Luc & Goodey, 1964 is the type species of the genus and was described from the Democratic Republic of the Congo. The species has since been reported from various hosts in the Afrotropical, Nearctic and Neotropical biogeographical regions (Geraert 2013). Populations of this species have been described by various authors and the morphometrical range of the species (Table 3.20) is a combination of these descriptions (Schuurmans Stekhoven 1944; Luc 1957; Luc & Goodey 1962; Sher 1968a; Luc 1975; Van den Berg *et al.* 2009; Geraert 2013). The South African population of *H. spinicaudata* (Table 3.20) was described from a wetland in KwaZulu-Natal, South Africa (Van den Berg *et al.* 2009).

Description:

Lectotype (Luc & Goodey 1964): Female: L = 2130 µm; a = 42.0; b = 13.0; b' = 5.2; c = 17.0; c' = 3.6; V = 57 %; stylet length = 40 µm.

Female: Annuli 3 µm wide at mid-body. Lateral field with four lines, 17 µm wide, aerolated to incompletely aerolated. Labial region hemispherical, 10 µm high and 21 µm wide with four to five annuli. SEM observations show plain face with four papillae. Stylet knobs massive, round, 7.5 µm high and 9 µm wide. Dorsal gland opening 2-4 µm posterior to stylet knobs. Median bulb 26.5 long µm and 23 µm wide. Excretory pore 178-227 µm from anterior end. Intestine distinctly overlapping rectum. Two genital tracts equal in length, spermatheca round, filled with sperm. Phasmid 14-24 annuli anterior to tail tip. Tail with 60 ventral annuli, terminus pointed to rounded, often with an elongated terminus.

Male: Similar to female except with shorter stylet. Spicules, arcuate, cephalated, flanges on the inner edges, 41-54 µm long. Gubernaculum curved, simple, 13-20 µm long. Bursa crenate, extending

from distal end of spicules to posterior third of tail. Phasmids situated on posterior third of tail. Tail elongate-conoid, tail tip as in female.

Diagnosis (Sher 1968a):

Hirschmanniella spinicaudata is characterised by a long styket (40 µm or longer), the intestine distinctly overlapping the rectum and aerolation of the lateral field.

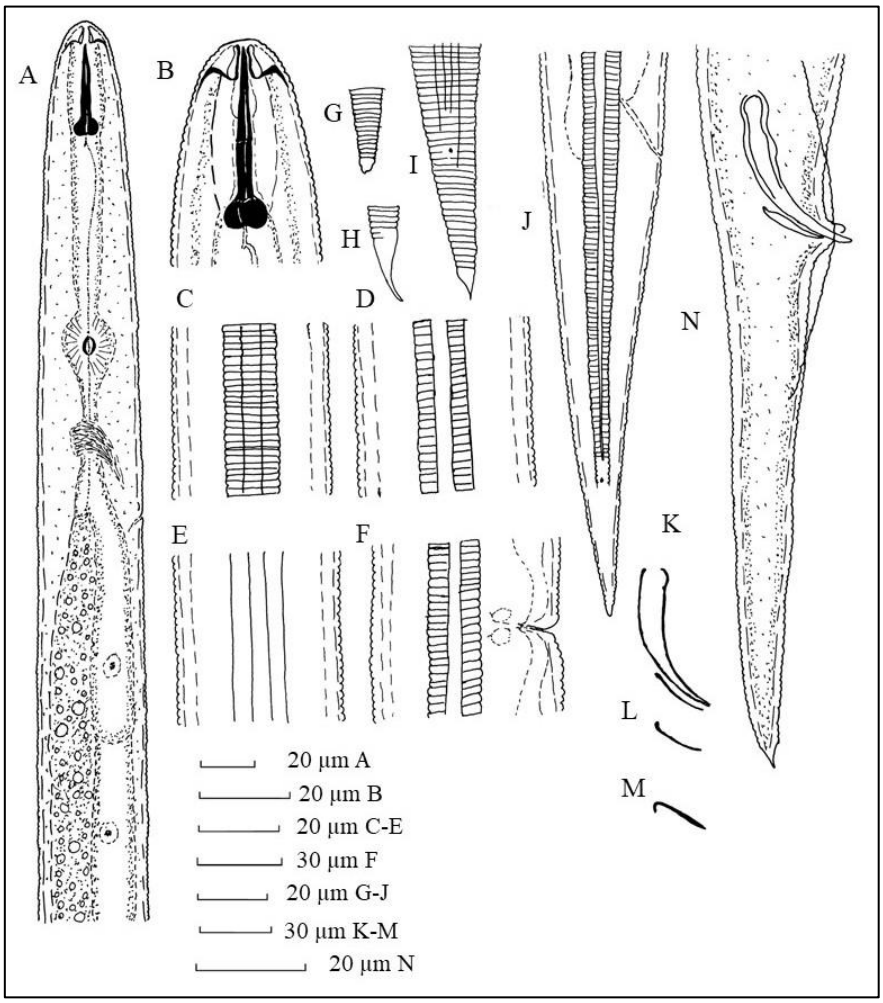


Figure 3.30: *Hirschmanniella spinicaudata* (Schuurmans Stekhoven, 1944) Luc & Goodey, 1964 A: Female oesophageal region; B: Female labial region; C-E: Lateral field at mid body; F: Lateral field at vulval region; G-J: Female tail and variation in tail tip; K: Spicule shape; L-M: Shape of gubernaculum; N: Male tail [A-E, G-M redrawn from Sher (1968); F, N redrawn from Van den Berg *et al*. (2009)].

Table 3.20: Morphometrics of females and males of *Hirschmanniella spinicaudata* (Schuurmans Stekhoven, 1944) Luc & Goodey, 1963. All measurements are in µm unless stated otherwise.

Species	***H. spinicaudata* (Schuurmans Stekhoven, 1944) Luc & Goodey, 1963**			
Population	**South African**		**Range**	
	♀	♂	♀	♂
L	3436	2770	1860–4020	2109–3150
a	52.2	62.8	39.9–74.0	43.9–62.8
b	–	–	5.5–19.0	4.3–17.0
b'	5.6	–	4.0–9.5	4.2–9.2
c	24.7	19.9	16.4–28.3	17.0–24.0
c'	3.9	4.6	3.0–4.7	3.0–5.8
o	8.5	6.8	3.0–9.0	3.0–9.0
m	–	–	50–58	51–57
V / T	54	–	50–59	34[a]
OV1	–	–	15	–
OV2	–	–	17	–
Number of annuli in labial region	6	6	4–6	6
Annulus width	3	3	2–3	3[a]
Lip region height	10	8	10[a]	8[a]
Lip region width	21	17	21[a]	15–17
Lateral field width	17	12	17[a]	12[a]
Stylet length	48	43	40–50	38–47
Dorsal gland opening from stylet knobs	4	3	2–4	3[a]
Median bulb length	27	32	27[a]	32[a]
Median bulb width	23	21	23[a]	21[a]
Oesophageal gland overlap length	–	–	–	–
Excretory pore from anterior	227	–	178–227	–
Oesophagus length	610	–	385–610	–
Number of annuli posterior to phasmid	14	–	14–24	–
Ventral tail annuli	60	–	60[a]	–
Testis length	–	–	–	–
Spicule length	–	54	–	41–54
Gubernaculum length	–	19	–	13–20
Tail length	139	139	120–139	139[a]

– Indicates structure not present or value not available.
[a] Indicates range not available.

3.6.2.2 *Hirschmanniella kwazuna* Van den Berg, Subbotin, Handoo & Tiedt, 2009

Figure 3.31

Measurements: Table 3.21

Hirschmanniella kwazuna Van den Berg, Subbotin, Handoo & Tiedt, 2009 was described from wetlands in KwaZulu-Natal, South Africa (Afrotropical biogeographical region) and is considered endemic (Van den Berg *et al.* 2009; Geraert 2013). The morphometric range of this species (Table 3.21) consists of the type description by Van den Berg *et al.* (2009) only.

Description:

Holotype (Van den Berg *et al.* 2009): Female: L = 1803 µm; a = 64.5; b = 4.8; b' = 13.0; c = 17.6; c' = 5.1; V = 53 %; stylet length = 22 µm.

Female: Body slender, ranging from slightly arcuate ventrally (rare) to highly irregular. cuticle annulated, width of annuli 1.5-2.0 µm. Beginning of lateral field near labial region starting a body diameter posterior to stylet knobs with four lines. Aerolated along entire length of body, more distinct in anterior and posterior region, SEM showing anastomoses and broken striations in middle band. Labial region 3 -5 µm high and 10-13 µm wide, rounded, not set off, with four or five annuli, indistinct in some specimens with no longitudinal lines on lip annuli. In e*n face* view, labial disc oblong, slightly raised above labial plate laterally and fused with labial plate dorsally and ventrally. Amphidial openings forming two slits on lateral sides of labial disc. Labial framework moderately sclerotized, extending posteriorly for two to three annuli from basal plate. Stylet knobs 2.0-3.5 µm high and 4.0-5.0 µm wide, rounded posteriorly, varying from sloping to indented. Opening of dorsal gland 3-4 µm from posterior to end of stylet knobs. Excretory pore position highly variable 69-151 µm from anterior end. Median

bulb 15-21 µm long and 11-18 µm wide, valve 4.0-5.5 µm long. Oesophageal glands slender, overlapping on ventral side, overlap 141-297 µm. Intestine not, or only slightly, overlapping rectum. Vacuoles observed more clearly in some specimens. In most specimens, crystal-like inclusions were noticed, varying in shape and size. Spermatheca round to oblong, large, filled with large, rounded sperm cells. Small, round to oval vaginal glands present. Phasmids situated 12-24 annuli anterior to tail tip but not exactly opposite each other. Tail with 62-81 ventral annuli, tapering to a narrow terminus, more rounded dorsally with a ventral mucro with a knob-like tip.

Male: Oesophagus as in female. Spicules arcuate ventrad, 28-35 µm long. Gubernaculum 10-16 µm long, simple with variously hooked or curved distal end, not protruding. Phasmids situated 17-32 annuli anterior to tail tip. Tail with 68-82 ventral annuli, strongly curved dorsad in most specimens with a ventral mucro.

Diagnosis (Van den Berg *et al.* 2009):

Hirschmanniella kwazuna is characterised by irregular habitus, 1522-2049 µm long, low, rounded labial region bearing four or five annuli, stylet 18-23 µm long, excretory pore position highly variable, ranging from just anterior to median bulb to opposite anterior end of oesophageal lobe, lateral field aerolated along entire body, spermatheca filled with sperm, tail with 62-81 ventral annuli, narrowing to terminus with a ventral mucro, angular crystal-like inclusions in body cavity and the phasmids situated 12-24 annuli from terminus, males similar to females, including crystal-like inclusions and with a dorsally curved tail with a ventral mucro.

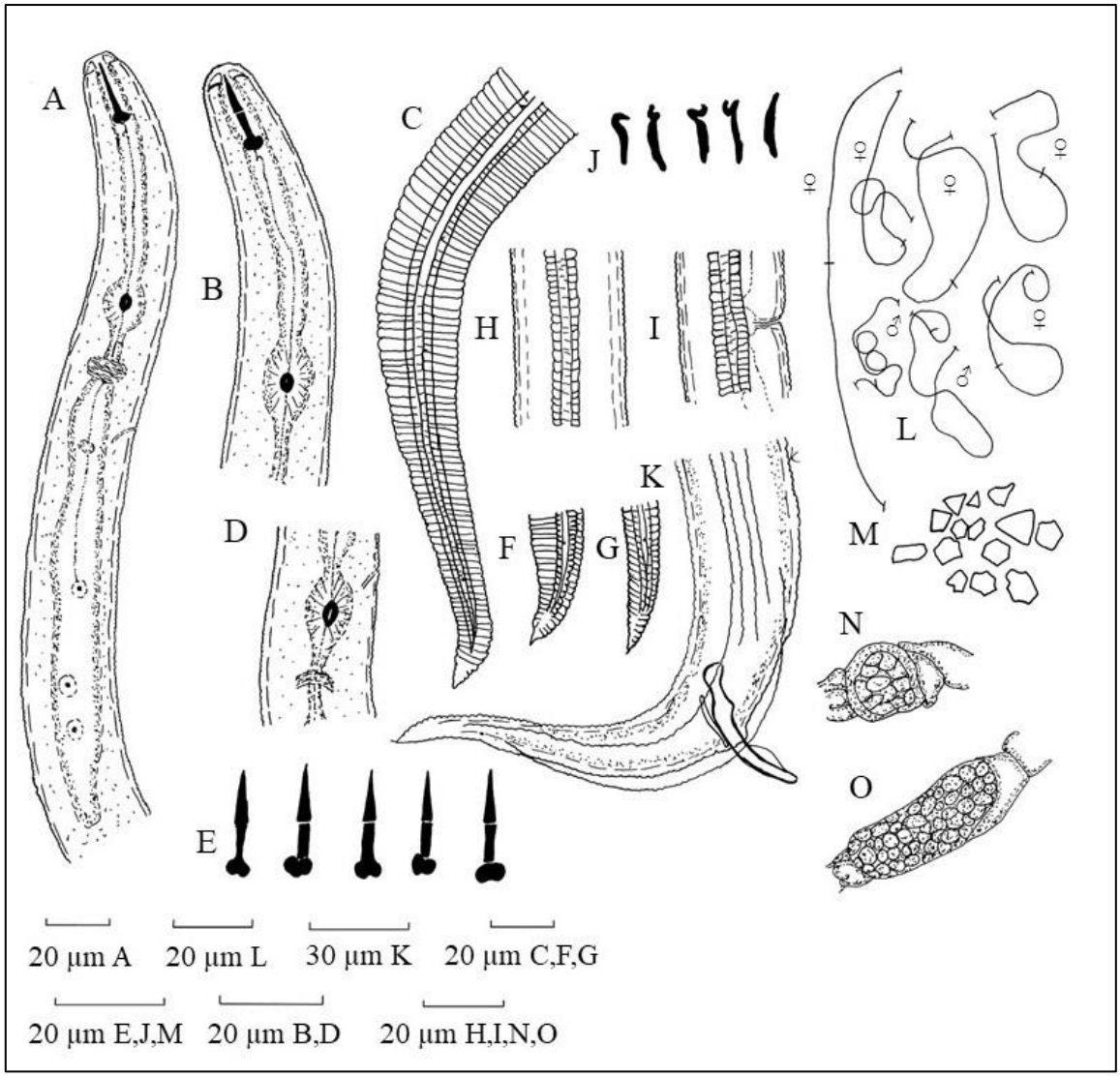


Figure 3.31: *Hirschmanniella kwazuna* Van den Berg, Subbotin, Handoo & Tiedt, 2009 A: Female oesophageal region; B: Male anterior region; C, F-G: Female tails; D: Female median buld showing position of excretory pore; E: Variation of shape of the stylet knobs; H: Lateral field at mid body; I: Lateral field in vulval region; J: Variation in the shape of the gubernaculum distal end; K: Male tail; L: Variation of the habitus of males and females; M: Variation in shape and size of crystalline inclusions in the body; N-O: Variation in shape of spermatheca (Redrawn from Van den Berg et al. [2009]).

Table 3.21: Morphometrics of females and males of *Hirschmanniella kwazuna* Van den Berg, Subbotin, Handoo & Tiedt, 2009. All measurements are in µm unless stated otherwise.

Species	***H. kwazuna* Van den Berg, Subbotin, Handoo & Tiedt, 2009**	
Population	**Type, SA and range**	
	♀	♂
L	1522–2049	1314–1960
a	56.2–72.2	54.0–78.0
b	3.7–7.8	4.3–7.1
b'	9.7–16.1	11.4–14.8
c	17.6–26.4	16.7–25.2
c'	2.9–5.2	3–4.9
o	14.0–21.6	13.5–21.6
m	46–53	44–51
V / T	46–59	39–40
OV1	22–34	–
OV2	19–29	–
Number of annuli in labial region	4–5	4–5
Annulus width	1.5–2.0	1.5–2.0
Lip region height	4–5	3–5
Lip region width	10–13	10–13
Lateral field width	7–12	6–12
Stylet length	18–23	19–23
Dorsal gland opening from stylet knobs	3–4	3–4
Median bulb length	15–21	14–18
Median bulb width	11–18	10–16
Excretory pore from anterior	69–151	66–121
Oesophageal gland overlap length	141–297	180–246
Oesophagus length	233–437	220–373
Number of annuli posterior to phasmid	12–24	17–32
Ventral tail annuli	62–81	68–82
Testis length	–	514–776
Spicule length	–	28–35
Gubernaculum length	–	10–16
Tail length	68–107	52–93

– Indicates structure not present or value not available.

3.6.2.3 Key to the species of Hirschmanniella in South Africa

Table 3.22: Key to the species of *Hirschmanniella* Luc & Goodey, 1964 in South Africa

1	Female stylet 18-23 µm, spicule 28-35µm, crystal-like inclusions in body, tail tip with ventral mucro**……*H. kwazuna***
	Female stylet 40-50 µm, spicules 41-54µm no crystal-like inclusions in body, tail tip pointed to rounded **……..*H. spinicaudata***

3.7 Subfamily Radopholinae Allen & Sher, 1967

= Hoplotylinae Khan, 1969

Diagnosis (Geraert 2013)

Pratylenchidae. Small to medium sized and vermiform. Cuticle distinctly annulated. Lateral fields with 3-7 incisures. In most genera there is a sexual dimorphism in the anterior region: 1) labial region in juveniles and females low, hemispherical or slightly conoid, continuous or offset, framework strongly sclerotized, in males high arched and often offset, with reduced sclerotization, labial disc indistinct; 2) stylet well developed in juveniles and females, in males generally reduced, may be rudimentary; 3) oesophagus well developed in females and reduced in males. Oesophageal glands overlapping intestine mostly dorsally and dorso-laterally. Tails usually more than twice anal body width long, often with distinct hyaline terminal portion.

3.7.1 *Radopholus* Thorne, 1949

Members of the genus *Radopholus* are some of the species within the Pratylenchidae that are considered economically important in the agricultural sector (Loof 1991). The genus consists of 23 species (Geraert 2013) of which the most well-known species is probably *R. similis*, which has also been reported from several localities in South Africa (Table 3.25, Figure 3.41). More than 250 plant

species are known to be hosts of *R. similis* of which *Musa* is the most important. This nematode species is known to cause "toppling over disease in banana and "yellows" in pepper and is therefore a well studied species in this genus (Loof 1991).

Diagnosis (Geraert 2013):

Radopholinae. Female usually slender, vermiform. Lateral field with three to six incisures. Labial region barely off set, low with strong sclerotization of labial framework. Stylet short, stout with well developed stylet knobs. Median bulb well developed with distinct valve. Oesophageal gland overlaps the intestine with a long dorsal lobe. Deirids absent. Vulva situated at 50-62% of body length. Anterior and posterior genital branches equally developed and functional. Tail elongate-conoid with narrowly rounded terminus, often shorter tails and cylindrical tails also occur. Sexual dimorphism in anterior region. Males with an off set labial region, knob shaped, not sclerotised. Stylet thin with rudimentary or absent stylet knobs. Median bulb, valves and oesophageal lobe poorly developed. Bursa not extending to tail tip in most species. Gubernaculum slightly protrusible.

3.7.2 Species of *Radopholus* reported in South Africa:

3.7.2.1 *Radopholus similis* (Cobb, 1893) Thorne, 1949

= *Tylenchus similis* Cobb, 1893

= *Anguillulina similis* (Cobb, 1893) Goodey, 1932

= *Rotylenchus similis* (Cobb, 1893) Filipjev, 1936

= *Tylenchus granulosus* Cobb, 1893

= *Anguillulina granulosus* (Cobb, 1893) Goodey, 1932

= *Tetylenchus granulosus* (Cobb, 1893) Filipjev, 1936

= *Radopholus granulosus* (Cobb, 1893) Siddiqi, 1986

= *Tylenchus acutocaudatus* Zimmerman, 1898

= *Anguillulina acutocaudatus* (Zimmerman, 1898) Goodey, 1932

= *Tylenchorhynchus acutocaudatus* (Zimmerman, 1898) Filipjev, 1934

= *Radopholus acutocaudatus* (Zimmerman, 1898) Siddiqi, 1986

= *Tylenchus biformis* Cobb, 1909

= *Anguillulina biformis* (Cobb, 1909) Goodey, 1932

= *Radopholus acutocaudatus* (Cobb, 1909) Siddiqi, 1986

= *Radopholus citrophilus* Huettel, Dickson & Kaplan, 1984

= *Radopholus similis citrophilus* (Huettel, Dickson & Kaplan, 1984) Siddiqi, 1986

Figure 3.32

Measurements: Table 3.23

Radopholus similis is the type species of the genus and was described from *Musa* sp. in Fiji. This species is common in tropical and subtropical areas and has been associated with various crops, especially *Musa* sp. (Geraert 2013). Populations of *R. similis* were described by various authors and the morphometric range (Table 3.23) of this species is a combination of these reports (Sher 1968b; Pliansinchai & Boonduang 1978; Loof 1991; Ryss & Wouts 1997; Elbadri *et al.* 1999a; Elbadri *et al.* 1999b; Geraert 2013). The South African population of *R. similis* was described by Elbadri *et al*. (1999b) from Mpumalanga. Although this species is frequently found in South Africa, this was the only population morphologically described.

Description:

Topotypes (Sher 1968): Female: L = 520-880 µm; a = 22.0-30.0; b = 4.7-7.4; b' = 3.5-5.2; c = 8.0-13.0; c' = 2.9-4.0; V = 55-61 %; stylet length = 17-20 µm.

Female: Habitus straight or slightly ventrally curved. Cuticular annulation 1.1-1.8 µm wide at mid body. Lateral field with four incisures, occasionally incompletely aerolated on tail, reduced to three incisures at level of phasmid. Labial region set off from body contour, hemispherical, rarely flattened with rounded edges, bearing three to six annuli. Stylet moderately strong, stylet knobs anteriorly flattened, rarely rounded. Dorsal gland opening 2.0-7.5 µm posterior to stylet knobs. Median bulb oval. Oesophageal gland lobe 50-204 µm long. Excretory pore at level of junction of oesophagus and intestine, 59-103 µm from anterior end. Both genital branches equally developed, spermatheca round to oval, filled with rod-like sperm. Post-rectal intestinal sac present. Phasmids distinct, 7-17 annuli posterior to anus. Tail conical, seldom sub-cylindrical, 27-65 ventral annuli. Terminus finely annulated to smooth, rarely regular annulated, narrow, conically rounded, rarely with mucronate tip, hyaline part of tail 8-23 µm long.

Male: Habitus curved ventrally. Sexual dimorphism in anterior region. Lateral field with four incisures. Labial region knob-like, set off from body contour, smooth or rarely annulated, bearing four to five annuli. Stylet slender, stylet knobs fused in a single round base. Excretory pore located at base of oesophagus, 65-109 µm from anterior end, oesophageal lobe reduced, rarely normally developed. Tail tapering to rounded, almost pointed terminus. Bursa not enveloping tail. Testis with rod-like sperm. Post-rectal sac absent. Spicules 16-24 µm long, gubernaculum 8-14 µm long.

Diagnosis (Sher 1968b):

Radopholus similis is characterised by female with three to four annuli in labial region, stylet generally 17-20 µm long, long hyaline portion of tail terminus, 9-17 µm, and male with long, narrow tail (c' generally 4.8-6.7).

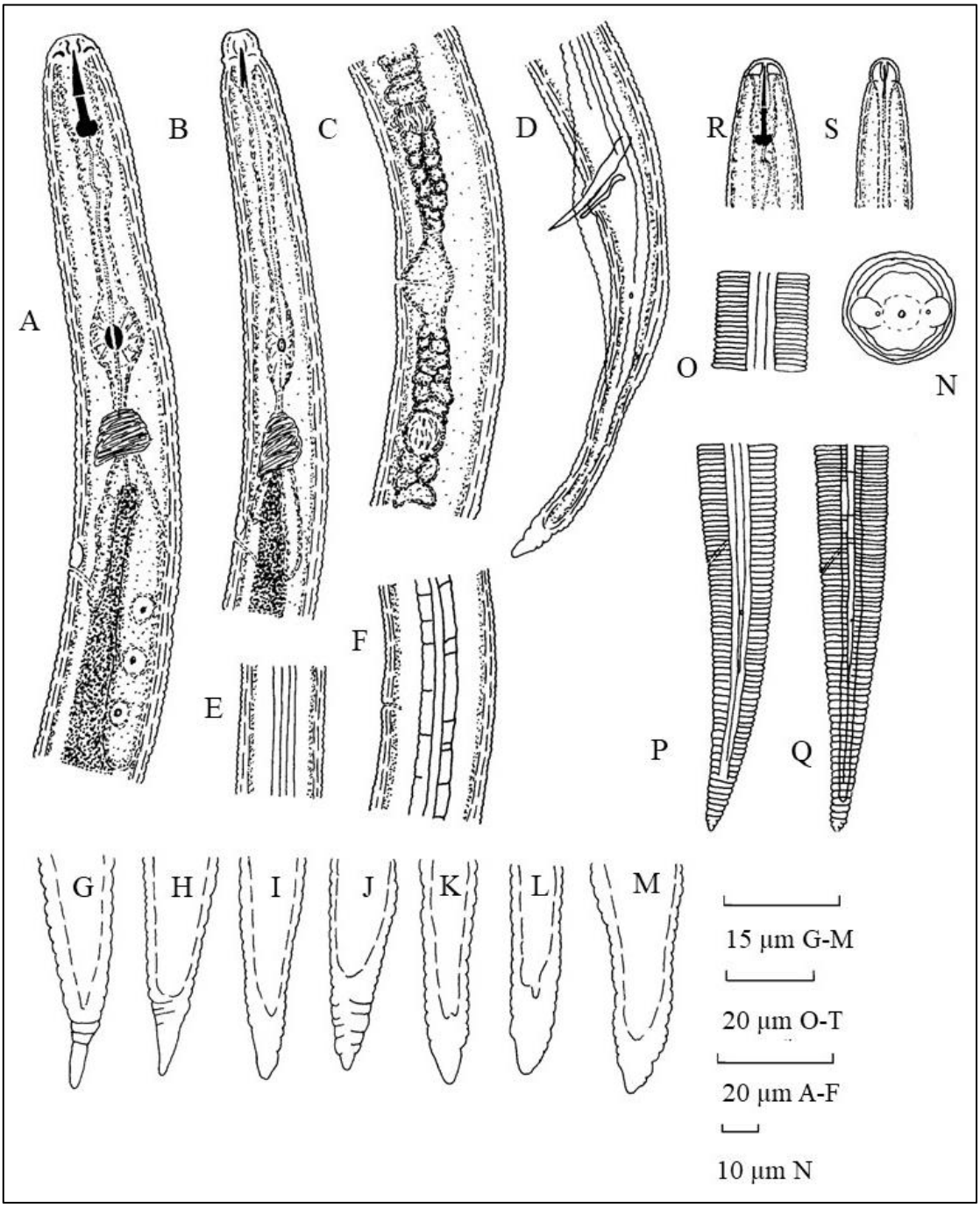


Figure 3.32: *Radopholus similis* (Cobb, 1893) Thorne, 1949 A: Female oesophageal region; B: Male oesophageal region; C: Vulval region; D: Male tail; E, O: Lateral field at mid body; F: Lateral field at vulval region; G-M, P-Q: Female tail showing variation of tail tip; N: *En face* view of labial region; R: Female labial region; S: Male labial region (A-F redrawn from Murukesan *et al.* [2005]; G-M redrawn from Elbadri *et al.* [1999b]; N redrawn from Xu *et al.* [2014]; O-S: redrawn from Sher [1968b]).

Table 3.23: Morphometrics of females and males of *Radopholus similis* (Cobb, 1893) Thorne, 1949. All measurements are in μm unless stated otherwise.

	***R. similis* (Cobb, 1893) Thorne, 1949**			
Population	**South African**		**Range**	
	♀	♂	♀	♂
L	540–695	527–630	520–880	450–723
a	23–30.7	25.7–38.0	18.0–36.0	24.5–44.0
b	6.7–9.6	7.0–8.6	4.7–10.0	4.7–9.6
b'	3.7–4.9	4.9–6.5	3.5–5.2	3.9–6.9
c	8.6–11.1	7.5–9.6	6.8–13.0	6.8–10.2
c'	3.3–4.8	3.7–5.6	2.4–6.3	3.7–6.9
o	–	–	12.0–25.0	–
m	–	–	–	–
V / T	55–59	26–39	50–67	26–39
OV1	–	–	19–41	–
OV2	–	–	17–38	–
Number of annuli in labial region	4–5	4–5	3–6	3–5
Stylet length	17–21	12–16	16–23	10–17
Excretory pore from anterior	68–92	71–101	59–103	65–109
Oesophagus length	69–92	68–83	67–132	55–123
Dorsal gland opening from stylet knobs	3.0–6.0	–	2.0–7.5	4.5–5.5
Oesophageal gland overlap length	130–167	90–127	50–204	18–138
Number of annuli from anus to phasmid	–	–	7–17	–
Ventral tail annuli	–	–	27–65	–
Hyaline length	–	–	8–23	7–12
Spicule length	–	18–23	–	16–24
Gubernaculum length	–	9–12	–	8–14
Tail length	54–74	56–80	50–100	52–96

– Indicates structure not present or value not available.

3.7.3 *Apratylenchoides* Sher, 1973

According to Geraert (2013) *Apratylenchoides* is a very rare genus and consists of only three valid species *A. belli* Sher, 1973, *A. homoglans* Siddiqi, Khan & Reddy, 1991 and *A. joenssoni* Ryss, Boström & Sohlenius, 2005. The type species *A. belli* was described from soil around *Artiplex confertifola* Wats in Utah, United States of America (Sher 1973). *Apratylenchoides homoglans* and *A. joenssoni* were described from India and East Antarctica, respectively (Siddiqi *et al.* 1991; Ryss *et al*. 2005). Apart from their type localities *A. belli* and *A. homoglans* was also reported from *Vicia faba* L. in Iran (Azimi *et al*. 2014). No other reports exist for any of the above-mentioned species. In South

Africa, the genus has been recorded, however no species have been identified (SAPPNS, Table 3.25, Figure 3.42).

Diagnosis (Geraert 2013):

Radopholinae. Habitus straight or slightly arcuate. Body small-sized (< 600 µm) and slender. Sexual dimorphism in anterior region poorly defined. Lateral field with four incisures, not aerolated. Labial region low, flat, not set off from body contour, bearing two to three annuli, labial framework strongly sclerotised in both sexes. Stylet strong but small, metenchium about half the length of stylet, stylet knobs round, may be flattened anteriorly. Median bulb spheroidal, muscular. Oesophageal gland overlaps intestine mostly dorsally and laterally, junction of oesophagus and intestine with small oval valve. Deirids present. Vulva situated at 77-82% of body length, ovary single, anteriorly outstretched, posterior branch rudimentary. Spermatheca round, axial, with round sperm when fertilised. Female tail tapering, two times anal body diameter in length. Male tail conical, over two cloacal body widths long, bursa crenate, enveloping tail tip. Phasmidial pseudoribs present in bursa. Spicules cephalated, small, gubernaculum small. Phasmids situated just behind mid tail.

3.7.4 Radopholoides De Guiran, 1967

The genus *Radopholoides* consists of five valid species (Geraert 2013). This genus was synonymised with *Radopholus* by Luc (1987) and with *Hoplotylus* by Andrássy (2007). However, these synonimisations were not accepted by Sturhan (2008) and Geraert (2013) and is here considered as a valid genus. *Radopholoides antoni* (Van den Berg, Heyns & Tiedt, 2000) Geraert, 2013 is the only species of the genus reported from South Africa and was described from natural vegetation in the Eastern Cape Province (Van den berg *et al.* 2000).

Diagnosis (Geraert 2013):

Radopholinae. Sexual demorphism in anterior region well defined. Small sized nematodes, 300-600 µm in length. Lateral field with three to six lines. Labial region in females low, not set off, annulated, labial framework heavily sclerotised, in males labial region high and round, knob-like with slight sclerotisation. Stylet 12-20 µm long, stylet knobs rounded, not fully ad-pressed to shaft. Oesophageal gland overlaps intestine dorsally, overlap two to three times body width long, nuclei in tandem. Vulva situated at 65-73 % of body length, posterior ovary degenerate, not functional. Female tail 1.9-4.4 anal body diamjeters long, tapering to small round terminus, terminus striated or smooth, hyaline portion short, less than 25 % of tail length. Phasmids situated in anterior region of tail. In males, bursa peloderan, spicules cephalated, almost straight, pointed. Gubernaculum half spicule length, protrusible. Cloacal lips raised, rounded.

3.7.5 Species of *Radopholoides* reported in South Africa:

3.7.5.1 *Radopholoides antoni* (Van den Berg, Heyns & Tiedt, 2000) Geraert, 2013

=*Radopholus antoni* Van den Berg, Heyns & Tiedt, 2000

Figure 3.33

Measurements: Table 3.24

Radopholoides antoni was described from South Africa (Afrotropical biogeographical region) as *Radopholus antoni* Van den Berg, Heyns, Tiedt, 2000. The morphometrics (Table 3.24) are from the type description of the species (Van den Berg *et al.,* 2000) and to our knowledge, the only report of this species globally.

Description:

Holotype (Van den Berg *et al.* 2000): Female: L = 486 µm; a = 29.0; b = 4.0; b' = 6.0; c = 12.5; c' = 3.2; V = 69 %; stylet length = 17 µm.

Female: Habitus almost straight to slightly arcuate. Cuticular annulation 1 µm wide at mid body, wider in tail region. Lateral field with four lines, becoming three just anterior or posterior to anus, outer bands aerolated in anterior region and on tail, occasionally crenate on rest of the body. Labial region rounded, not off set, bearing three annuli, labial framework moderately developed, extending to second body annulus. *En face* view with labial disc fused with first annulus, only the lateral sectors of the lip annulus developed, leaf like in form and reaching posteriad to third lip annulus. Stylet strong, stylet knobs slightly indented anteriorly. Dorsal gland opening 1.5-3.0 µm posterior to base of stylet knobs. Oesophagus with three gland nuclei situated posterior to the junction of oesophagus and intestine, 29-74 µm dorsal overlap. Excretory pore situated 53-83 µm from anterior end. Deirid distinct, posterior to excretory pore. Vulval lips not protruding, anterior genital branch more than twice the length of posterior branch, with a distinct, rounded spermatheca, filled with sperm. Spermatheca not seen in posterior branch. Phasmids 5-9 annuli posterior to anus. Tail tapering gradually to rounded, crenate terminus, tail bearing 23-30 annuli on ventral side, hyaline portion of tail 6-8 µm long, some specimens showed a slight indent dorsally and ventrally near terminus, giving the tail a digitate appearance.

Male: Marked sexual dimorphism in anterior region. Lateral field with four lines. Labial region rounded, set off from body contour, bearing five to six indistinct annuli. Stylet weakly developed. Deirid not seen. Tail 33 µm long with finger-like projection, 7 µm long. Bursa as for genus. Spicules 14 µm long. Gubernaculum 9 µm long. Phasmid in posterior half of tail.

Diagnosis (Van den Berg *et al.* 2000):

Radopholoides antoni is characterised by four lines in the lateral field, stylet 16-18 µm long, longer dorsal stylet knob, reduced length of posterior ovary, without a spermatheca, distinct deirid, posterior vulva (V=66-72 %), tail with crenate terminus and short hyaline part (6-8 µm), c' = 2.6-3.8, male with degenerate oesophagus and faint stylet, metenchium more distinct than telenchium, gubernaculum protrusible.

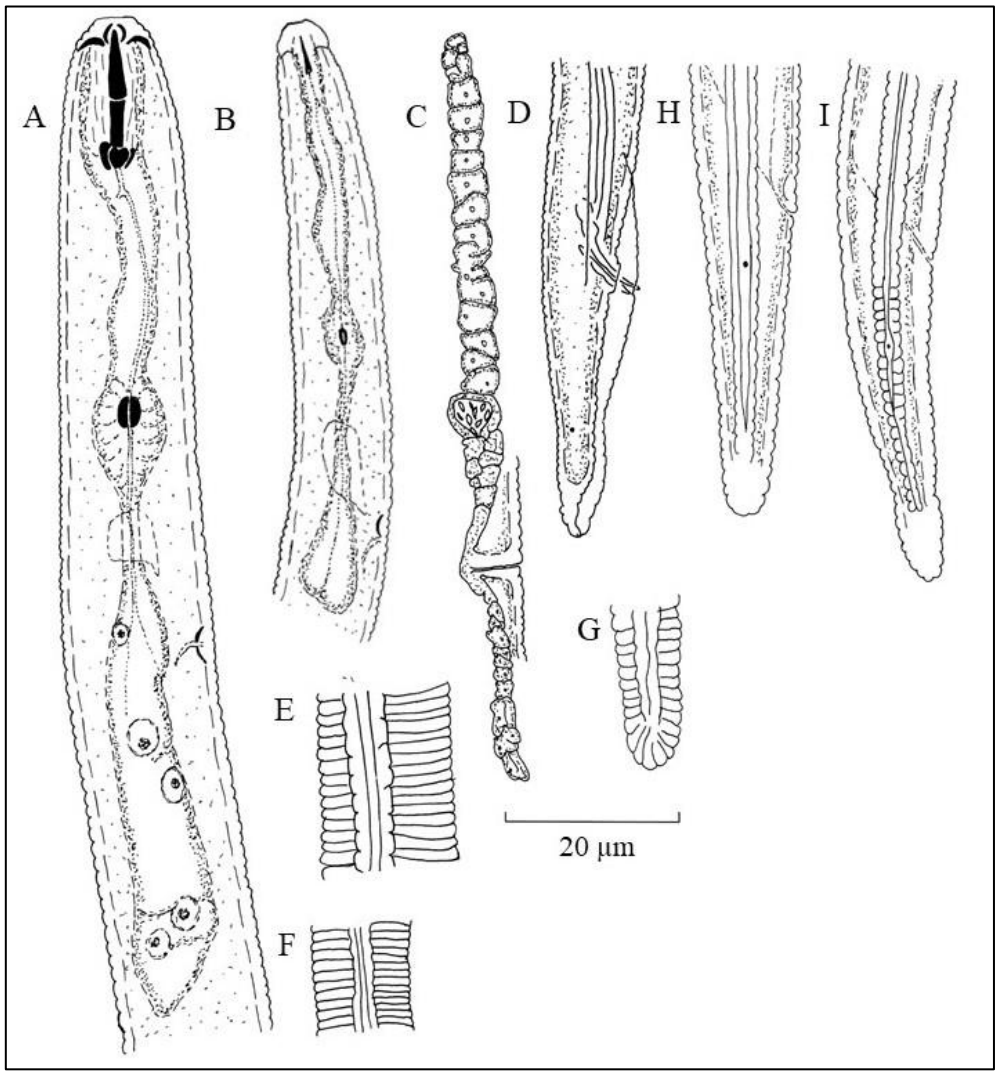


Figure 3.33: *Radopholoides antoni* (Van den Berg, Heyns & Tiedt, 2000) Geraert, 2013 A: Female oesophageal region; B: Male oesophageal region; C: Female genital tract; D: Male tail; E: Lateral field at mid body; F: Male lateral field; G-I: Female tails (Redrawn from Van den Berg *et al*. [2000]).

Table 3.24: Morphometrics of females and males of *Radopholoides antoni* (Van den Berg, Heyns & Tiedt, 2000) Geraert, 2013. All measurements are in µm unless stated otherwise.

	***R. antoni* (Van den Berg, Heyns & Tiedt, 2000) Geraert, 2013**	
Population	**Type, SA and range**	
	♀	♂
L	353–492	345
a	21.0–29.0	28.0
b	2.5–4.0	–
b'	4.0–6.0	5.0
c	10.5–14.0	10.0
c'	2.6–3.8	3.3
o	9.0–24.0	–
m	–	–
V / T	66–72	–
OV2	17–25	–
OV1	8–12	–
Number of annuli in labial region	4	5/6
Stylet length	16–18	–
Dorsal gland opening from stylet knobs	1.5–3.0	–
Excretory pore from anterior	53–83	64
Oesophageal gland overlap length	29–74	–
Oesophagus length	96–153	72
Number of annuli from anus to phasmid	5–9	–
Ventral tail annuli	23–30	–
Hyaline length	6–8	7
Testis length	–	–
Spicule length	–	14
Gubernaculum length	–	9
Tail length	26–44	33

– Indicates structure not present or value not available.

3.8 Distribution of members of the family Pratylenchidae Thorne, 1949 in South Africa

3.8.1 *Pratylenchus* Filipjev, 1936

According to the SAPPNS, *P. zeae* is the most widely distributed species in South Africa (865 records) from all nine provinces (Figure 3.38); is followed by *P. brachyurus* (294 records) (Figure 3.34) and *P. neglectus* (235 records) (Figure 3.36) also reported from all nine provinces. Other species with a countrywide distribution (reported from all provinces) include *P. delattrei* (46 records) (Figure 3.35), *P. penetrans* (105 records) (Figure 3.36), *P. scribneri* (109 records) (Figure 3.37) and *P. thornei* (130 records) (Figure 3.38).

Some species currently seem to have a more limited distribution in South Africa. For example *P. loosi* has only been reported from Limpopo, North West and Western Cape provinces – Figure 3.36); *P. fallax* (records from the Northern Cape and Western Cape provinces) (Figure 3.35), *P. hexincisus* (two records from the Limpopo and Western Cape provinces, respectively) (Figure 3.36). Whereas *P. bolivianus* (Western Cape Province – Figure 3.36); *P. goodeyi* (Mpumalanga Province) (Figure 3.35), and *P. tenuis* (Eastern Cape Province) (Figure 3.37) have only been reported once. The map of *P. pseudopratensis* is not given, as the report in the present thesis is the first record of this species for the country from the Telperion Nature Reserve, Mpumalanga Province.

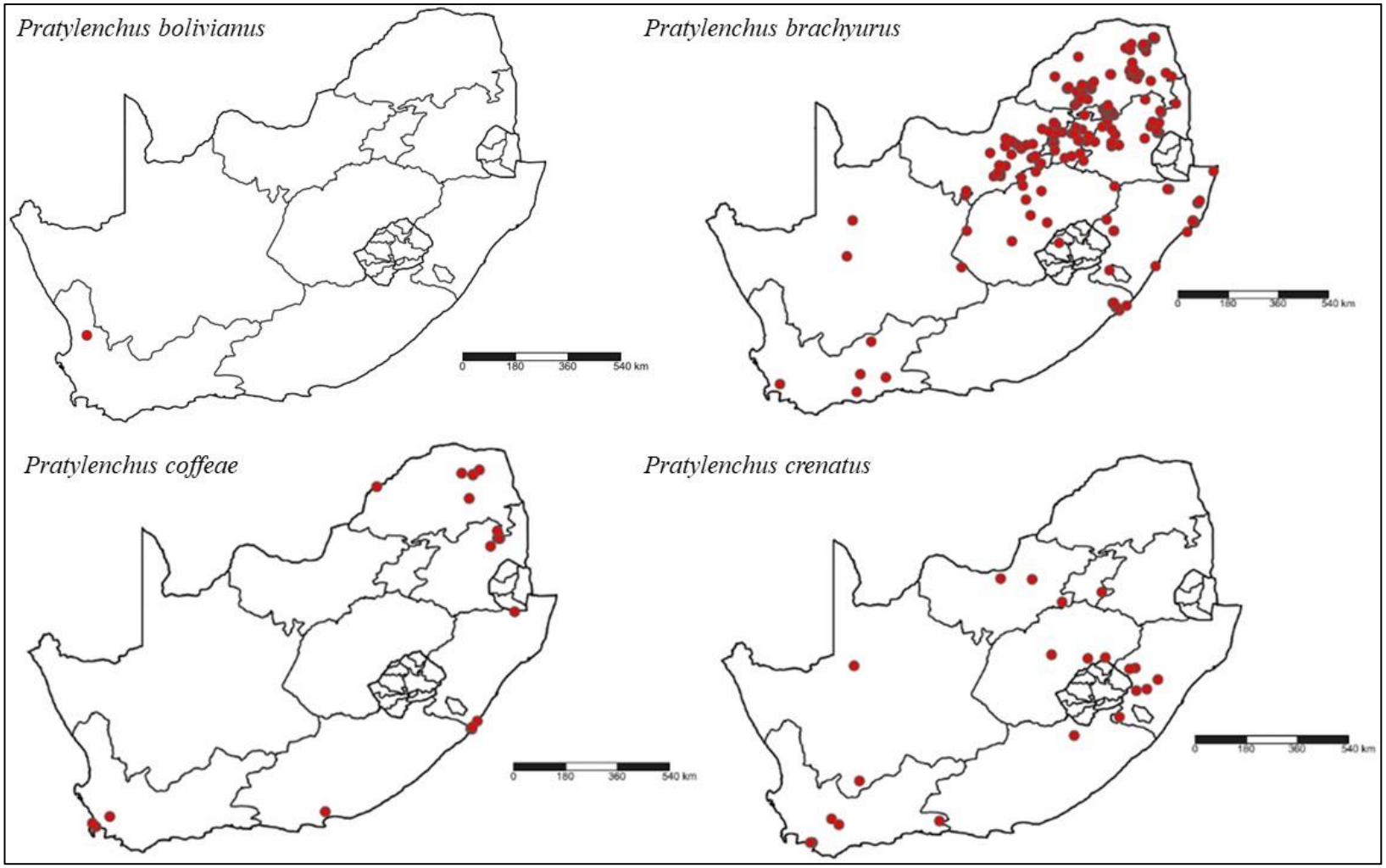


Figure 3.34: Geographical distribution of *Pratylenchus* Filipjev, 1936 in South Africa. Names of provinces illustrated in Figure 3.2 (SAPPNS; Shorthouse 2010).

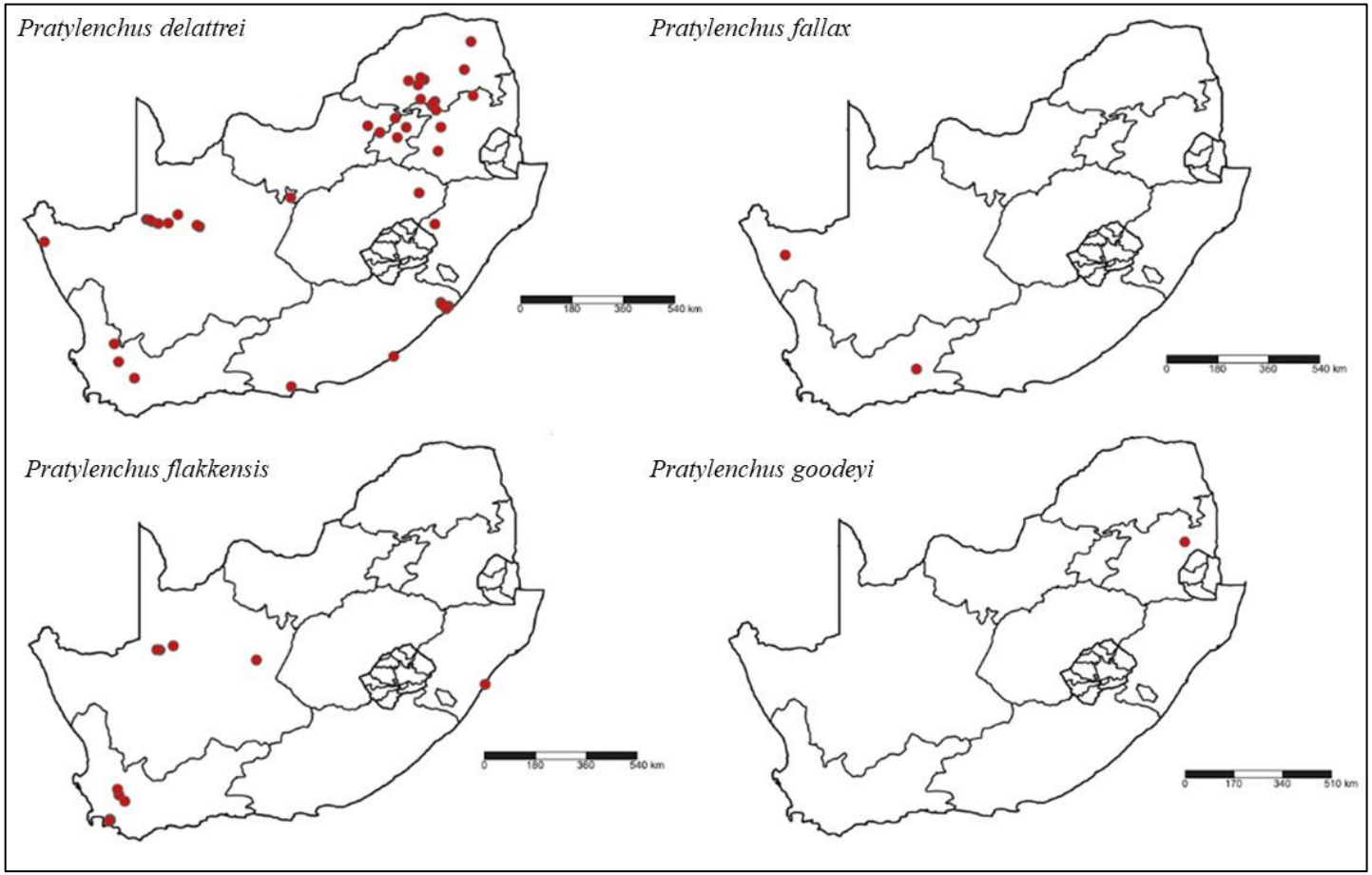


Figure 3.35: Geographical distribution of *Pratylenchus* Filipjev, 1936 in South Africa. Names of provinces illustrated in Figure 3.2 (SAPPNS; Shorthouse 2010).

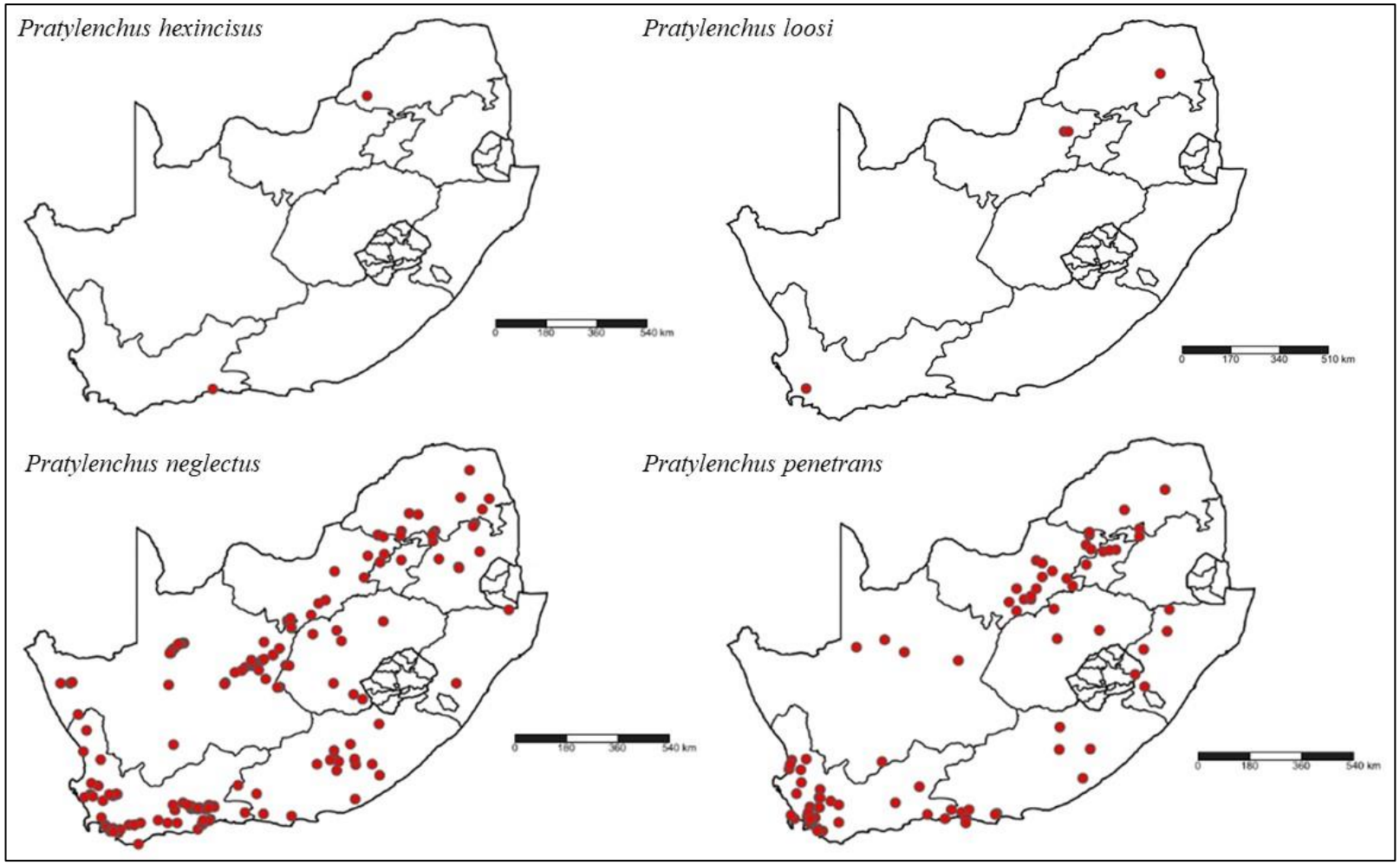


Figure 3.36: Geographical distribution of *Pratylenchus* Filipjev, 1936 in South Africa. Names of provinces illustrated in Figure 3.2 (SAPPNS; Shorthouse 2010).

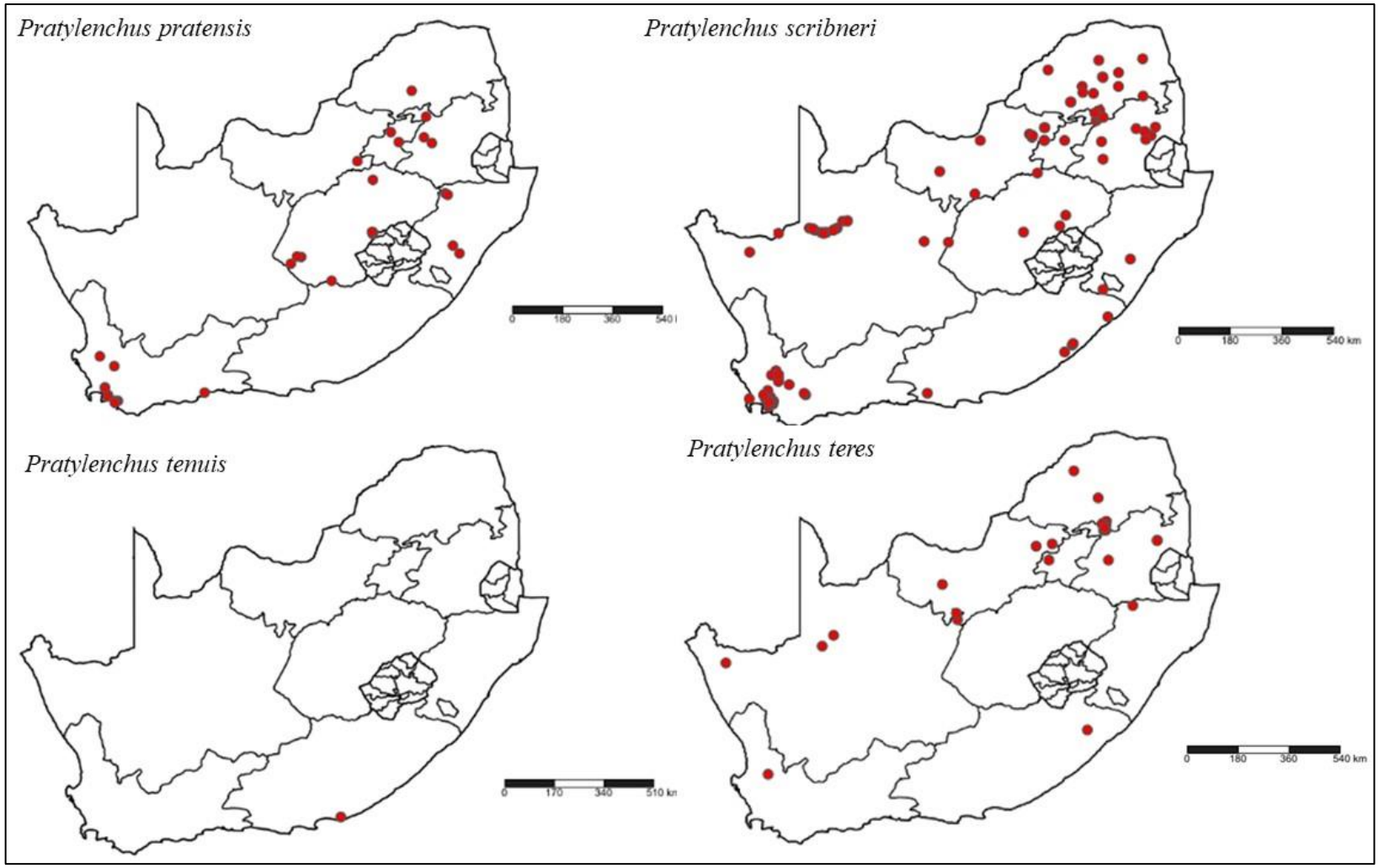


Figure 3.37: Geographical distribution of *Pratylenchus* Filipjev, 1936 in South Africa. Names of provinces illustrated in Figure 3.2 (SAPPNS; Shorthouse 2010).

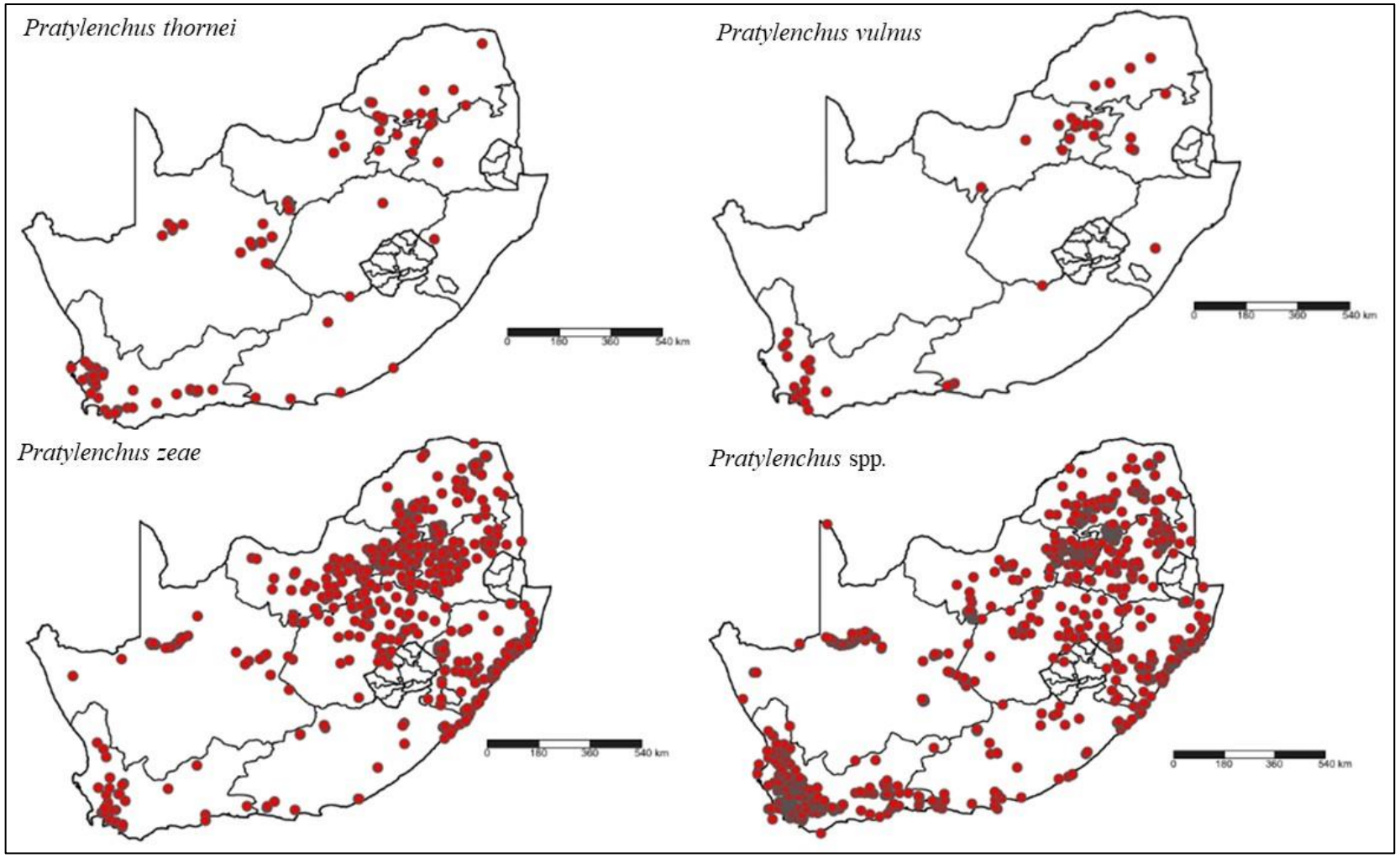


Figure 3.38: Geographical distribution of *Pratylenchus* Filipjev, 1936 in South Africa. Names of provinces illustrated in Figure 3.2 (SAPPNS; Shorthouse 2010).

3.8.2 *Zygotylenchus* Siddiqi, 1963

Three species of *Zygotylenchus* have been reported from South Africa (Figure 3.39). The endemic *Zygotylenchus natalensis* described from the KwaZulu-Natal Province (Van den Berg & Tiedt 2003) and recorded again from a wetland in the same province (SAPPNS). The most common species found in South Africa being *Z. guevarai* recorded from the Mpumalanga, Western Cape and Northern Cape provinces. *Zygotylenchus taomasinae* has only been recorded in South Africa once in the Western Cape. Several other *Zygotylenchus* specimens have been found in South Africa, however no species were identified (SAPPNS, Figure 3.39, Table 3.25) as of yet.

3.8.3 *Hirschmanniella* Luc & Goodey, 1964

Hirschmanniella kwazuna and *H. spinicaudata* are the two species of the genus reported from South Africa (Figure 3.40). *Hirschmanniella kwazuna* was described in South Africa from unidentified grasses in a wetland, KwaZulu-Natal Province (Van den Berg *et al.* 2009) and later found again in *S. officinarum*, the same province (Van den Berg *et al.* 2017). *Hirschmanniella spinicaudata* was recorded from one locality in the KwaZulu-Natal Province from a wetland (SAPPNS; Van den Berg *et al.* 2009). Apart from the species recorded in KwaZulu-Natal Province, two more records of unidentified *Hirschmanniella* species were from the Eastern and Western Cape provinces respectively. However, the species were not identified (SAPPNS, Figure 3.40).

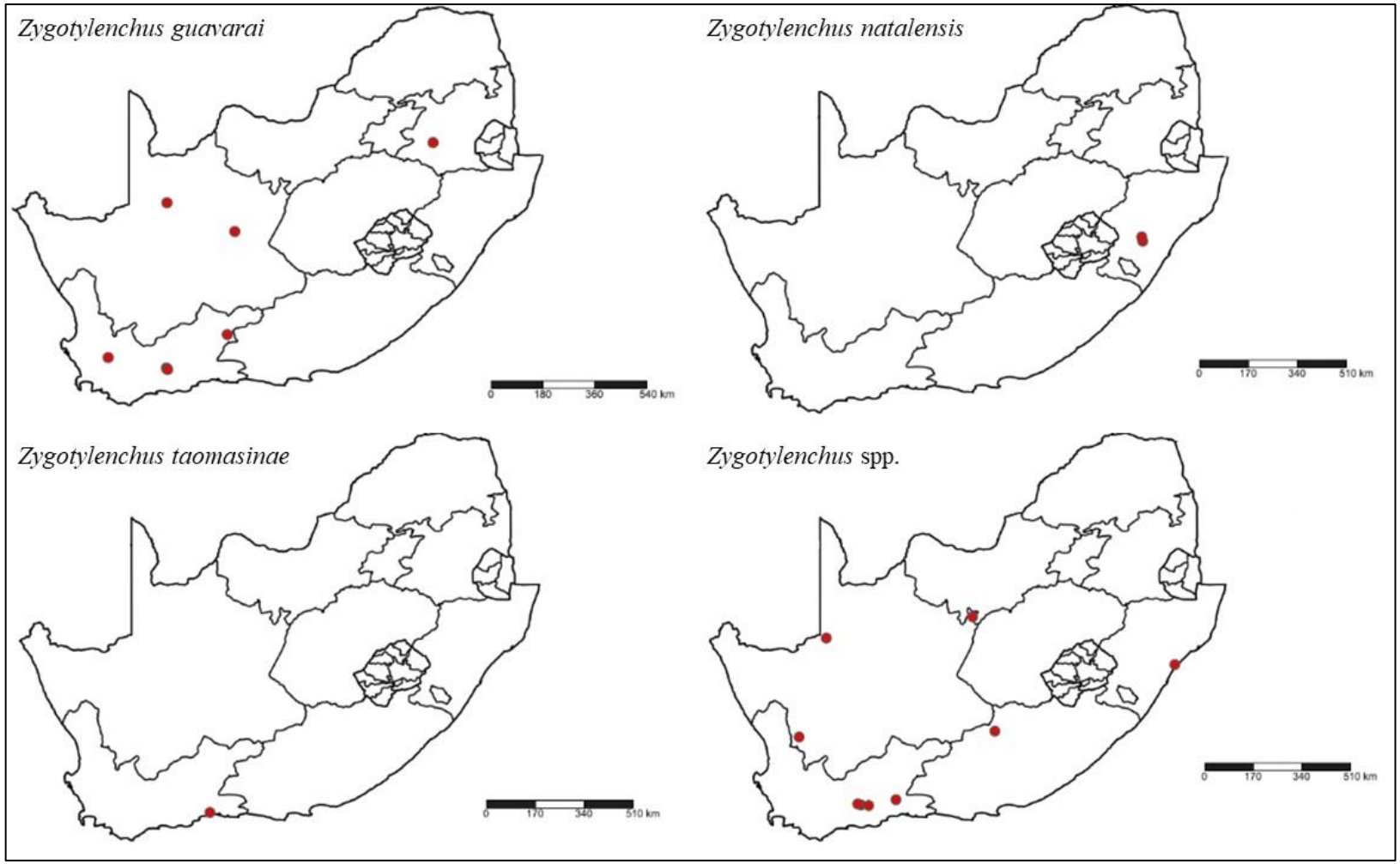


Figure 3.39: Geographical distribution of *Zygotylenchus* Siddiqi, 1963 in South Africa. Names of provinces illustrated in Figure 3.2 (SAPPNS; Shorthouse 2010).

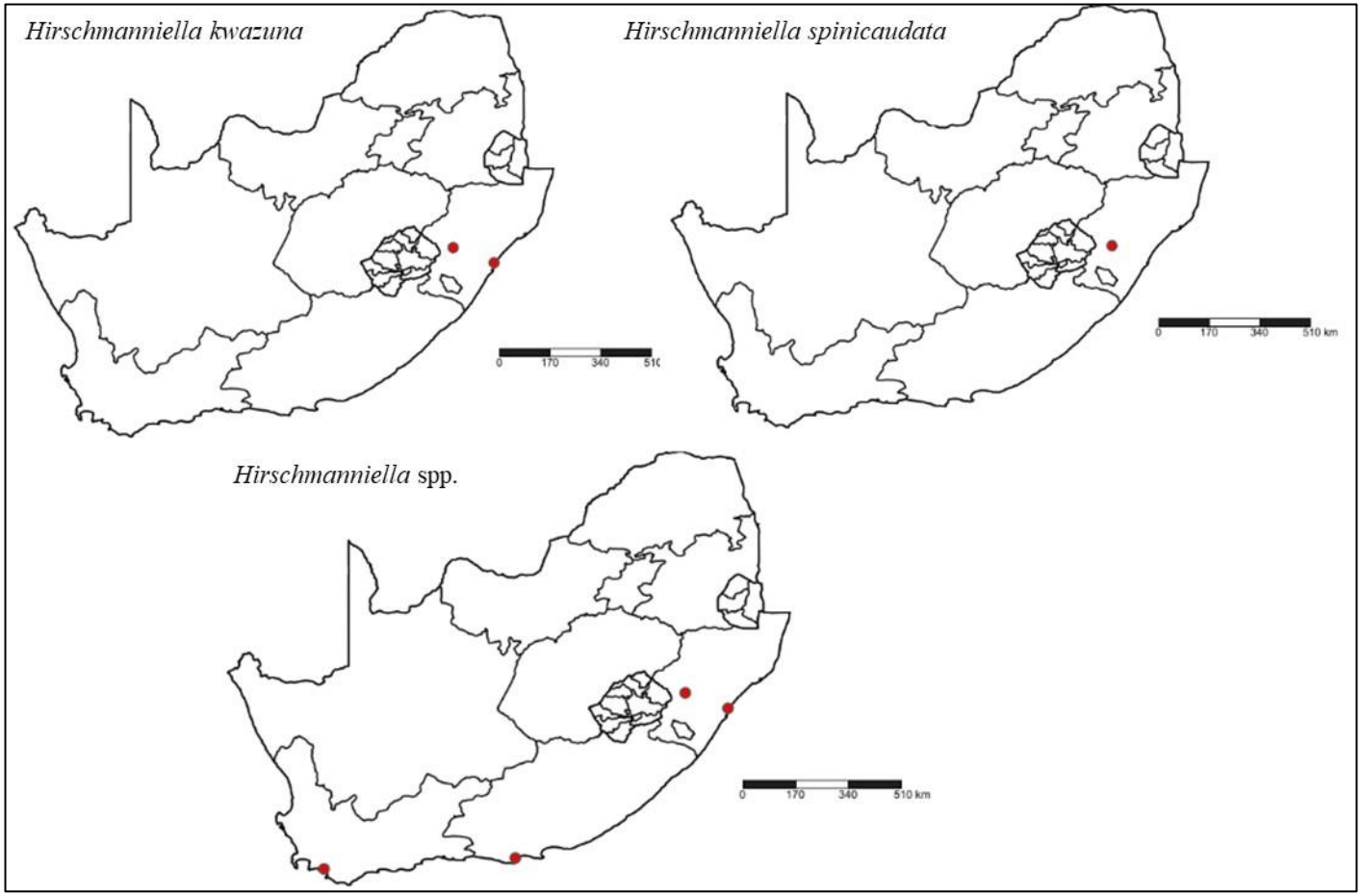


Figure 3.40: Geographical distribution of *Hirschmanniella* Luc & Goodey, 1964 in South Africa. Names of provinces illustrated in Figure 3.2 (SAPPNS; Shorthouse 2010).

3.8.4 *Radopholus* Thorne, 1949

Radopholus similis has been recorded from four provinces in South Africa (Figure 3.41). Several unidentified specimens of *Radopholus* have also been reported from the KwaZulu-Natal Province (Figure 3.41, SAPPNS).

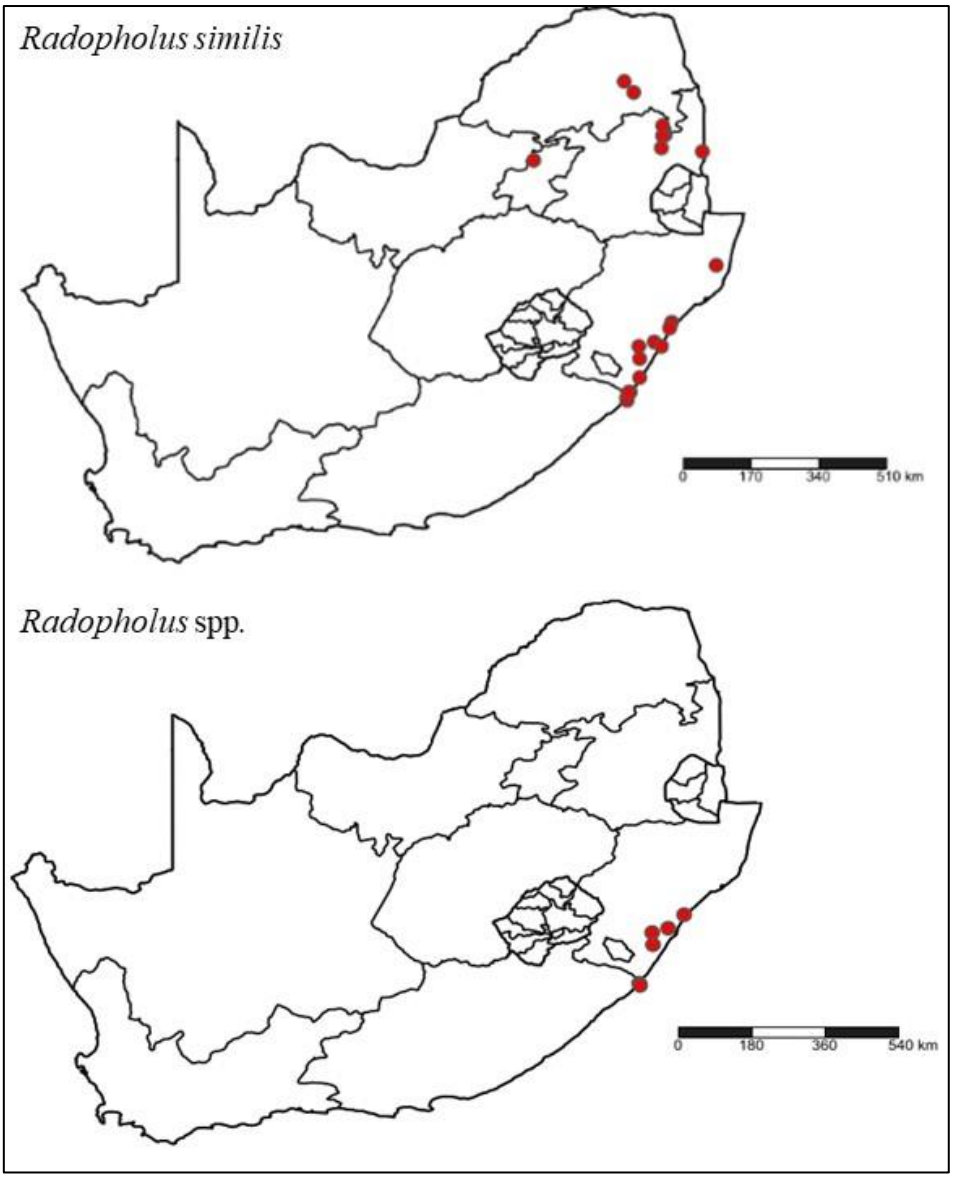


Figure 3.41: Geographical distribution of *Radopholus* Thorne, 1949 in South Africa. Names of provinces illustrated in Figure 3.2 (SAPPNS; Shorthouse 2010).

3.8.5 *Apratylenchoides* Sher, 1973

In South Africa, an unidentified species of the genus *Apratylenchoides* was reported from *Gazania linearis* (Thumb.) Druce in the Eastern Cape Province (Kleynhans *et al.* 1996; SAPPNS), this being the only report of the genus in South Africa (SAPPNS, Figure 3.42).

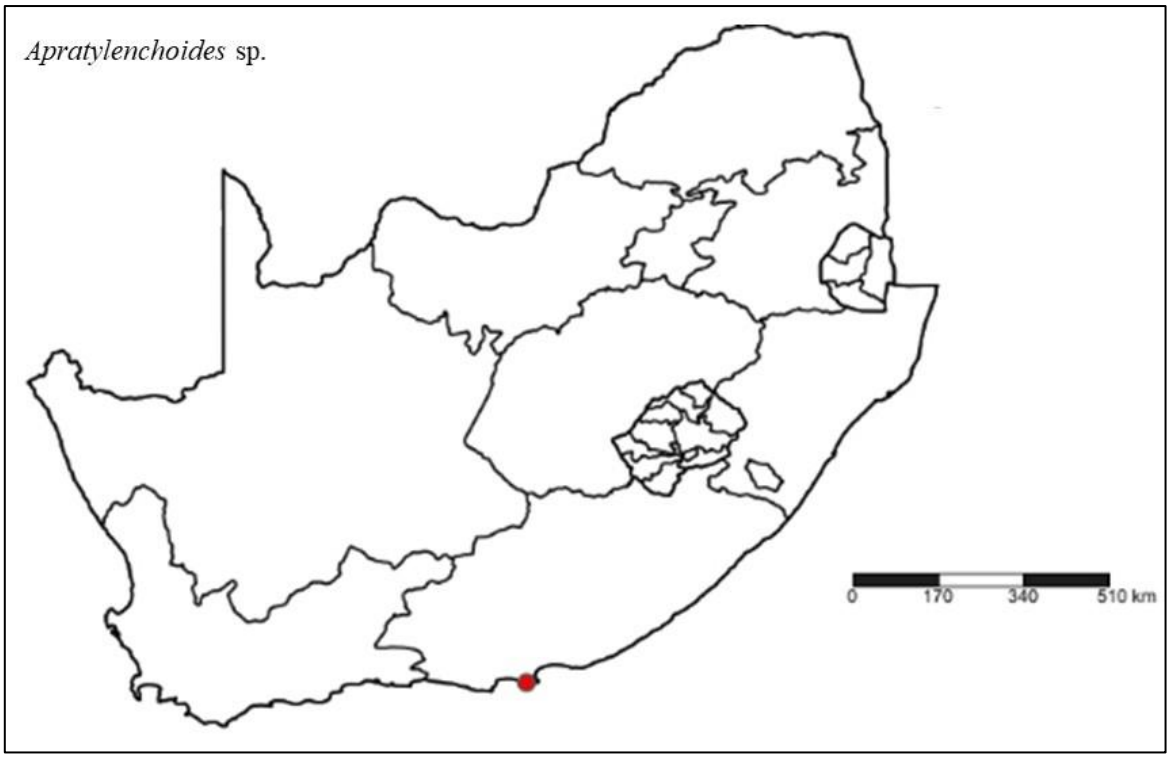


Figure 3.42: Geographical distribution of *Apratylenchoides* Sher, 1973 in South Africa. Names of provinces illustrated in Figure 3.2 (SAPPNS; Shorthouse 2010).

3.8.6 *Radopholoides De* Guiran, 1967

Radopholoides antoni was described from unidentified grasses in East London, Eastern Cape Province (Van den Berg *et al.* 2000) and recorded from a second locality from natural veld also in the Eastern Cape Province (Figure 3.43, SAPPNS).

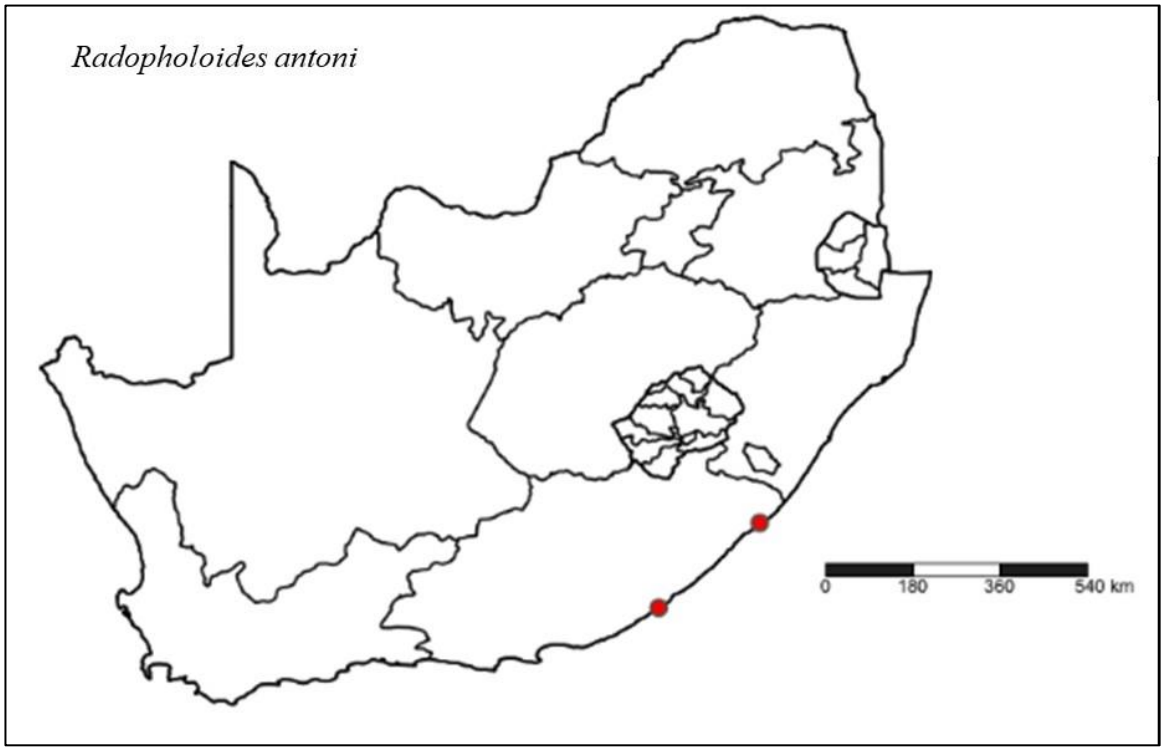


Figure 3.43: Geographical distribution of *Radopholoides antoni* (Van den Berg, Heyns & Tiedt, 2000) Geraert, 2013 in South Africa. Names of provinces illustrated in Figure 3.2 (SAPPNS; Shorthouse 2010).

3.9 Host range of Pratylenchidae Thorne, 1949 in South Africa

It is important to have knowledge of the host range of members of the family Pratylenchidae due to their economic importance and damage they cause to various crops. According to the SAPPNS, species of the family Pratylenchidae have been recorded from 176 different plant species. There were 1903 records of the family Pratylenchidae in the SAPPNS, with *Z. mays* the most common host followed by *Vitis* spp. (Table 3.25). Based on host range the most common species, *P. zeae*, also seems to have the widest host range (105 host plants), including crops and uncultivated areas (Table 3.25).

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Table 3.25: Host range of species of the family Pratylenchidae Thorne, 1949 reported from South Africa.

Genus	***Pratylenchus***																				***Zygotylenchus***			***Hirschmanniella***		***Radopholus***	***Apratylenchoides***	***Radopholoides***
Species	*P. bolivianus* (1)[a]	*P. brachyurus* (283)	*P. coffeae* (22)	*P. crenatus* (20)	*P. dellatrei* (44)	*P. fallax* (6)	*P. flakkensis* (20)	*P. goodeyi* (1)	*P. hexincisus* (1)	*P. loosi* (4)	*P. neglectus* (234)	*P. penetrans* (100)	*P. pratensis* (28)	*P. scribneri* (107)	*P. subrajani* (1)	*P. tenuis* (1)	*P. teres* (23)	*P. thornei* (123)	*P. vulnus* (50)	*P. zeae* (795)	*Z. guevarai* (4)	*Z. natalensis* (2)	*Z. taomasinae* (1)	*H. kwazuna* (2)	*H. spinicaudata* (1)	*R. similis* (26)	*Apratylenchoides* sp. (1)	*R. antoni* (2)
Host																												
Crops (unidentified) [16][b]		11								1	2				1					1								
Dune Vegetation [3]		1											1						1									
Fallow [7]		1		1	1													1		3								
Fynbos and Sandbos [6]											1	2							2	1								
Grass [56]		7	1	1	2						1	2	1	1			1	4		33	1							1
Indigenous Forest [11]					1							9								1								
Mixed pastures [6]											4									2								
Natural veld, ferns and mountain veld [100]		11	1	4	2	3					21	11	5				2	1	2	36								1
Wetland Vegetation [5]		1											1									1		1	1			
Abelmoschus esculentus (L.) Moench [5]		2			1															2								
Actinidia deliciosa (A.Chev.) C.F.Liang & A.R.Ferguson [1]								1																				
Agave americana L. [1]											1																	
Agrostis L.[1]																				1								
Allium cepa L. [6]		1									2							1	1	1								
Aloe ferox Mill. [3]		1												1						1								
Alsophila capensis (L.f.) J.Sm. [1]												1																
Amaranthus L. [2]																				2								
Amaryllis L. [1]											1																	
Anacardium occidentale L. [1]																				1								
Ananas comosus var. *comosus* (L.) Merr. [4]		3																								1		
Anthurium Schott [2]																										2		
Arachis hypogaea L. [32]		13			1						2			1					1	14								
Aspalathus linearis (Burm.f.) R.Dahlgren [3]	1																			2								

[a] Numbers in square brackets indicate number of records for each host plant (SAPPNS).

[b] Numbers in round brackets indicate number of records from South Africa for each nematode species (SAPPNS).

Table 3.25 (continued): Host range of species of the family Pratylenchidae Thorne, 1949 reported from South Africa.

Genus	***Pratylenchus***																			***Zygotylenchus***			***Hirschmanniella***		***Radopholus***	***Apratylenchoides***	***Radopholoides***
Species	*P. bolivianus* (1)[a]	*P. brachyurus* (283)	*P. coffeae* (22)	*P. crenatus* (20)	*P. dellatrei* (44)	*P. fallax* (6)	*P. flakkensis* (20)	*P. goodeyi* (1)	*P. hexincisus* (1)	*P. loosi* (4)	*P. neglectus* (234)	*P. penetrans* (100)	*P. pratensis* (28)	*P. scribneri* (107)	*P. subrajani* (1)	*P. tenuis* (1)	*P. teres* (23)	*P. thornei* (123)	*P. vulnus* (50)	*P. zeae* (795)	*Z. guevarai* (4)	*Z. natalensis* (2)	*Z. taomasinae* (1)	*H. kwazuna* (2)	*H. spinicaudata* (1)	*R. similis* (26)	*Apratylenchoides* sp. (1)	*R. antoni* (2)
Host																												
Avena sativa L. [4][b]				1								1								2								
Avena strigosa Schreb. [1]																				1								
Beta vulgaris L. [4]											1							1		2								
Betula L. [1]												1																
Bidens L. [1]														1														
Brassica napus L. [3]											2							1										
Brassica oleracea var. *italica* Plenck [3]					1							1								1								
Brassica rapa subsp. *pekinensis* (Lour.) Hanelt [2]																				2								
Brassica rapa subsp. *rapa* L. [1]																				1								
Brassica L. [15]		2			1						2	1		1				1		7								
Bryophyta [1]														1														
Camellia sinensis (L.) Kuntze [1]																				1								
Cannabis sativa L. [6]		1															2			3								
Capsicum baccatum L. [1]																		1										
Capsicum L. [3]		1																1		1								
Carissa bispinosa (L.) Desf. ex Brenan [1]												1																
Carpobrotus edulis (L.) N.E.Br. [1]												1																
Carpobrotus N.E.Br. [2]											2																	
Carya illinoinensis (Wangenh.) K.Koch [2]											1									1								
Chenopodium L. [1]														1														
Chlorophytum Ker Gawl. [1]												1																
Chrysanthemum L. [3]		2												1														

[a] Numbers in square brackets indicate number of records for each host plant (SAPPNS).

[b] Numbers in round brackets indicate number of records from South Africa for each nematode species (SAPPNS).

Table 3.25 (continued): Host range of species of the family Pratylenchidae Thorne, 1949 reported from South Africa.

Genus	***Pratylenchus***																			***Zygotylenchus***			***Hirschmanniella***		***Radopholus***	***Apratylenchoides***	***Radopholoides***
Species	*P. bolivianus* (1)[a]	*P. brachyurus* (283)	*P. coffeae* (22)	*P. crenatus* (20)	*P. dellatrei* (44)	*P. fallax* (6)	*P. flakkensis* (20)	*P. goodeyi* (1)	*P. hexincisus* (1)	*P. loosi* (4)	*P. neglectus* (234)	*P. penetrans* (100)	*P. pratensis* (28)	*P. scribneri* (107)	*P. subrajani* (1)	*P. tenuis* (1)	*P. teres* (23)	*P. thornei* (123)	*P. vulnus* (50)	*P. zeae* (795)	*Z. guevarai* (4)	*Z. natalensis* (2)	*Z. taomasinae* (1)	*H. kwazuna* (2)	*H. spinicaudata* (1)	*R. similis* (26)	*Apratylenchoides* sp. (1)	*R. antoni* (2)
Host																												
Cichorium intybus L. [1][b]																1												
Citrullus lanatus (Thunb.) Matsum. & Nakai [1]																				1								
Citrus L. [12]		1	2								1			1			1	1		5								
Clitoria ternatea L. [2]																				2								
Colocasia esculenta (L.) Schott [1]																				1								
Combretum glutinosum Perr. ex DC. [1]																				1								
Commelina L. [1]														1														
Crotalaria juncea L. [1]																				1								
Cucumis melo L. [1]														1														
Cucumis sativus L. [7]		1												1			2		1	2								
Cucurbita maxima Duchesne [1]											1																	
Cucurbita maxima subsp. *maxima* Duchesne [2]																		1		1								
Cucurbita pepo L. [1]											1																	
Cucurbita L. [2]		1																		1								
Cyclopia Vent. [1]				1																								
Cydonia oblonga Mill. [2]																				2								
Cynodon Rich. [1]											1																	
Daucus carota subsp. *sativus* (Hoffm.) Schubl. & G.Martens [45]		5									2	1					2		2	33								
Dianthus caryophyllus L. [1]																		1										
Dietes iridioides (L.) Sweet ex Klatt [1]												1																
Dolichos L. [1]																				1								
Eragrostis curvula (Schrad.) Nees [2]																				2								

[a] Numbers in square brackets indicate number of records for each host plant (SAPPNS).

[b] Numbers in round brackets indicate number of records from South Africa for each nematode species (SAPPNS).

Table 3.25 (continued): Host range of species of the family Pratylenchidae Thorne, 1949 reported from South Africa.

Genus	***Pratylenchus***																			***Zygotylenchus***			***Hirschmanniella***		***Radopholus***	***Apratylenchoides***	***Radopholoides***
Species	*P. bolivianus* (1)[a]	*P. brachyurus* (283)	*P. coffeae* (22)	*P. crenatus* (20)	*P. dellatrei* (44)	*P. fallax* (6)	*P. flakkensis* (20)	*P. goodeyi* (1)	*P. hexincisus* (1)	*P. loosi* (4)	*P. neglectus* (234)	*P. penetrans* (100)	*P. pratensis* (28)	*P. scribneri* (107)	*P. subrajani* (1)	*P. tenuis* (1)	*P. teres* (23)	*P. thornei* (123)	*P. vulnus* (50)	*P. zeae* (795)	*Z. guevarai* (4)	*Z. natalensis* (2)	*Z. taomasinae* (1)	*H. kwazuna* (2)	*H. spinicaudata* (1)	*R. similis* (26)	*Apratylenchoides* sp. (1)	*R. antoni* (2)
Host																												
Drosanthemum curtophyllum L.Bolus [1][b]					1																							
Eleusine Gaertn. [1]																				1								
Englerophytum magalismontanum (Sond.) T.D.Penn. [1]		1																										
Erica chloroloma Lindl. [1]																				1								
Eucalyptus L'Her. [8]		3										1								4								
Euphorbia dregeana E.Mey. ex Boiss. [1]											1																	
Ficus carica L. [1]																				1								
Fragaria × *ananassa* Duchesne ex Rozier [6]		1																	1	4								
Galenia africana L. [2]		1									1																	
Gardenia volkensii K.Schum. [2]		2																										
Gazania linearis (Thumb.) Druce [1]																											1	
Glycine max (L.) Merr. [16]		1									4	1		1				5		4								
Gomphocarpus fruticosus (L.) Aiton f. [1]																			1									
Gossypium L. [28]		2			3						3	2	1	1			1	10		5								
Grubbia tomentosa (Thunb.) Harms [1]																	1											
Helianthus annuus L. [12]											3							6		3								
Hibiscus cannabinus L. [2]				1																1								
Hordeum vulgare L. [2]											2																	
Hypericum L. [4]														1						3								
Ipomoea batatas var. *batatas* (L.) Lam. [8]		1																1		6								
Juglans L. [2]																			1	1								
Kalmia L. [1]																				1								

[a] Numbers in square brackets indicate number of records for each host plant (SAPPNS).

[b] Numbers in round brackets indicate number of records from South Africa for each nematode species (SAPPNS).

Table 3.25 (continued): Host range of species of the family Pratylenchidae Thorne, 1949 reported from South Africa.

Genus	***Pratylenchus***																				***Zygotylenchus***			***Hirschmanniella***		***Radopholus***	***Apratylenchoides***	***Radopholoides***
Species	*P. bolivianus* (1)[a]	*P. brachyurus* (283)	*P. coffeae* (22)	*P. crenatus* (20)	*P. dellatrei* (44)	*P. fallax* (6)	*P. flakkensis* (20)	*P. goodeyi* (1)	*P. hexincisus* (1)	*P. loosi* (4)	*P. neglectus* (234)	*P. penetrans* (100)	*P. pratensis* (28)	*P. scribneri* (107)	*P. subrajani* (1)	*P. tenuis* (1)	*P. teres* (23)	*P. thornei* (123)	*P. vulnus* (50)	*P. zeae* (795)	*Z. guevarai* (4)	*Z. natalensis* (2)	*Z. taomasinae* (1)	*H. kwazuna* (2)	*H. spinicaudata* (1)	*R. similis* (26)	*Apratylenchoides* sp. (1)	*R. antoni* (2)
Host																												
Lablab purpureus subsp. *purpureus* (L.) Sweet [1][b]																				1								
Lactuca sativa L. [2]		1									1																	
Lantana L. [2]		1																		1								
Lessertia frutescens (L.) Goldblatt & J.C.Manning [1]					1																							
Limeum L. [1]																				1								
Limonium Mill. [1]																			1									
Lolium L. [3]											2									1								
Lupinus L. [3]											1							2										
Macadamia integrifolia Maiden & Betche [4]		1																		3								
Malus Mill. [74]				1	1		14			1	7	11	5	17				1	8	8								
Mangifera indica L. [2]		1																		1								
Manihot esculenta Crantz [2]		1																		1								
Medicago polymorpha L. [6]											3							3										
Medicago sativa subsp. *sativa* L. [95]		8		1							52	1		2				21		8	2							
Medicago L. [3]											1							2										
Mesembrynthemaceae [2]						1					1																	
Musa L [46]		5	17							1	1									2						20		
Nasturtium W.T.Aiton [2]										1										1								
Nicotiana tabacum L. [28]		2									3			4			3	2	1	12			1					
Olea europaea L. [1]																				1								
Olea L. [1]																										1		
Oncosiphon grandiflorum (Thunb.) Källersjö [2]											1	1																

[a] Numbers in square brackets indicate number of records for each host plant (SAPPNS).

[b] Numbers in round brackets indicate number of records from South Africa for each nematode species (SAPPNS).

Table 3.25 (continued): Host range of species of the family Pratylenchidae Thorne, 1949 reported from South Africa.

Genus	***Pratylenchus***																			***Zygotylenchus***			***Hirschmanniella***		***Radopholus***	***Apratylenchoides***	***Radopholoides***
Species	*P. bolivianus* (1)[a]	*P. brachyurus* (283)	*P. coffeae* (22)	*P. crenatus* (20)	*P. dellatrei* (44)	*P. fallax* (6)	*P. flakkensis* (20)	*P. goodeyi* (1)	*P. hexincisus* (1)	*P. loosi* (4)	*P. neglectus* (234)	*P. penetrans* (100)	*P. pratensis* (28)	*P. scribneri* (107)	*P. subrajani* (1)	*P. tenuis* (1)	*P. teres* (23)	*P. thornei* (123)	*P. vulnus* (50)	*P. zeae* (795)	*Z. guevarai* (4)	*Z. natalensis* (2)	*Z. taomasinae* (1)	*H. kwazuna* (2)	*H. spinicaudata* (1)	*R. similis* (26)	*Apratylenchoides* sp. (1)	*R. antoni* (2)
Host																												
Oncosiphon piluliferum (L.f.) Källersjö [1][b]																												
Ornithogalum thyrsoides Jacq. [1]												1																
Oryza sativa L. [1]																				1								
Paspalum L. [1]																				1								
Passiflora edulis Sims [4]		2									1									1								
Pennisetum clandestinum Hochst. ex Chiov. [4]											1									3								
Pennisetum glaucum (L.) R.Br. [1]														1														
Persea americana Mill. [2]		1																		1								
Phaseolus L. [43]		12		1	4						3			1				2		20								
Phoenix dactylifera L. [2]											1							1										
Phragmites Adans. [1]																				1								
Pinus patula Schltdl. & Cham. [2]																				2								
Pinus L. [1]		1																										
Pisum sativum L. [15]		1										1		1						12								
Platylophus trifoliatus (L.f.) D.Don [1]												1																
Plectranthus esculentus N.E.Br. [1]																				1								
Polygala fruticosa P.J.Bergius [1]												1																
Poncirus trifoliata (L.) Raf. [1]																				1								
Protea repens (L.) L. [1]												1																
Protea L. [3]											1									2								
Prunus armeniaca L. [6]					1						1	1	1	1						1								
Prunus avium (L.) L. [1]													1															

[a] Numbers in square brackets indicate number of records for each host plant (SAPPNS).

[b] Numbers in round brackets indicate number of records from South Africa for each nematode species (SAPPNS).

Table 3.25 (continued): Host range of species of the family Pratylenchidae Thorne, 1949 reported from South Africa.

Genus	***Pratylenchus***																			***Zygotylenchus***			***Hirschmanniella***		***Radopholus***	***Apratylenchoides***	***Radopholoides***
Species	*P. bolivianus* (1)[a]	*P. brachyurus* (283)	*P. coffeae* (22)	*P. crenatus* (20)	*P. dellatrei* (44)	*P. fallax* (6)	*P. flakkensis* (20)	*P. goodeyi* (1)	*P. hexincisus* (1)	*P. loosi* (4)	*P. neglectus* (234)	*P. penetrans* (100)	*P. pratensis* (28)	*P. scribneri* (107)	*P. subrajani* (1)	*P. tenuis* (1)	*P. teres* (23)	*P. thornei* (123)	*P. vulnus* (50)	*P. zeae* (795)	*Z. guevarai* (4)	*Z. natalensis* (2)	*Z. taomasinae* (1)	*H. kwazuna* (2)	*H. spinicaudata* (1)	*R. similis* (26)	*Apratylenchoides* sp. (1)	*R. antoni* (2)
Host																												
Prunus domestica L. [10][b]		2			1														2	5								
Prunus persica (L.) Batsch [37]		9			2						1		1	1			1	2	5	15								
Prunus persica var. *nucipersica* (Suckow) C.K.Schneid. [20]		11									1	2		2						4								
Prunus L. [1]																				1								
Psidium guajava L. [4]		4																										
Pyrus L. [28]				1							3	3	5	3				1	9	3								
Quercus L. [1]																				1								
Rosa L. [9]		1			2						2			2					1	1								
Rubus L. [1]													1															
Ruscus L. [4]											1	1							2									
Saccharum officinarum L. [85]		2									1			1						80				1				
Salix babylonica L. [1]				1																								
Salsola kali L. [1]											1																	
Sclerocarya birrea subsp. *caffra* (Sond.) Kokwaro [2]																				2								
Searsia lancea (L.f.) F.A.Barkley [1]											1																	
Searsia undulata (Jacq.) T.S.Yi & al. [1]						1																						
Silybum marianum (L.) Gaertn. [1]												1																
Solanum lycopersicum L. [25]		2									3	1		5					1	13								
Solanum mauritianum Scop. [1]																				1								
Solanum tuberosum L. [96]		15			3		1		1		9	9	2	3			1	2	3	44	1	1				1		
Sorghum bicolor (L.) Moench [2]														1			1											
Sorghum bicolor subsp. *bicolor* (L.) Moench [2]																				2								

[a] Numbers in square brackets indicate number of records for each host plant (SAPPNS).

[b] Numbers in round brackets indicate number of records from South Africa for each nematode species (SAPPNS).

Table 3.25 (continued): Host range of species of the family Pratylenchidae Thorne, 1949 reported from South Africa.

Genus	***Pratylenchus***																			***Zygotylenchus***			***Hirschmanniella***		***Radopholus***	***Apratylenchoides***	***Radopholoides***
Species	*P. bolivianus* (1)[a]	*P. brachyurus* (283)	*P. coffeae* (22)	*P. crenatus* (20)	*P. dellatrei* (44)	*P. fallax* (6)	*P. flakkensis* (20)	*P. goodeyi* (1)	*P. hexincisus* (1)	*P. loosi* (4)	*P. neglectus* (234)	*P. penetrans* (100)	*P. pratensis* (28)	*P. scribneri* (107)	*P. subrajani* (1)	*P. tenuis* (1)	*P. teres* (23)	*P. thornei* (123)	*P. vulnus* (50)	*P. zeae* (795)	*Z. guevarai* (4)	*Z. natalensis* (2)	*Z. taomasinae* (1)	*H. kwazuna* (2)	*H. spinicaudata* (1)	*R. similis* (26)	*Apratylenchoides* sp. (1)	*R. antoni* (2)
Host																												
Spinacia oleracea L. [1][b]																				1								
Strelitzia alba (L.f.) Skeels [1]																										1		
Swietenia mahagoni (L.) Jacq. [1]																				1								
Tagetes minuta L. [2]												1								1								
Terminalia sericea Burch. ex DC. [2]		1																		1								
Themeda triandra Forssk. [1]																				1								
Trifolium L. [1]																		1										
Triticum L. [72]		3									21	4						30		14								
Triticum turgidum subsp. *durum* (Desf.) Husn. [1]											1																	
Tulipa gesneriana L. [1]												1																
Tulipa L. [1]																				1								
Vigna radiata var. *radiata* (L.) R. Wilczek [8]		3																		5								
Vigna subterranea (L.) Verdc. [1]																				1								
Vigna unguiculata (L.) Walp. [36]		20																		16								
Vitis L. [131]		3	1		6		5				21	3		41			3	2	2	44								
Zantedeschia Spreng. [1]																				1								
Zea mays L. [413]		92		6	9	1					24	17	3	6			2	14	2	237								

[a] Numbers in square brackets indicate number of records for each host plant (SAPPNS).

[b] Numbers in round brackets indicate number of records from South Africa for each nematode species (SAPPNS).

3.10 Conclusions

Members of the family Pratylenchidae are economically important due to the extensive crop loss caused by a number of its species. The correct identification of the species is therefore vitally important, especially when farmers must decide on control strategies. Likewise, it is also important to know the distribution and host range of these species. Due to the large number of species within the family Pratylenchidae and with the wide range of morphological and morphometrical variation, it is often difficult to identify them. This compendium is therefore a collection of selected characters which are easily recognisable under the light microscope, and which were found during the present study to be the most useful diagnostic characters within the different genera. Naturally, caution needs to be practised with the identification of the species and all available literature should be used in combination with the compendium and proposed keys. As the identification of species in the genus *Pratylenchus* is difficult even to a trained eye, the use of molecular work in combination with taxonomic expertise is imperative.

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3.12 References

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CHAPTER 4: ARTICLE 3

Checklist of the terrestrial free–living nematodes of the Telperion Nature Reserve, South Africa

Prepared for submission to **Koedoe**

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4.1 Abstract

The Grassland Biome is one of the largest and most important vegetation types in South Africa. It is considered endangered with less than three percent protected and its size decreasing due to anthropogenic activities. Nematodes form a large percentage of the mesofauna in the soil ecosystem and are considered the most abundant multicellular, eukaryotic organisms. Due to their abundance, role in ecological processes and importance in the soil food web, nematodes are frequently used as biological indicators of soil health and may be useful as a tool in future conservation of the Grassland Biome. A nematode survey was conducted in the Telperion Nature Reserve, Mpumalanga to determine the diversity of the terrestrial free–living nematodes of this reserve. The reserve is situated in a protected area of the Rand Highveld Grassland of the Grassland Biome. Soil samples were collected from 26 sites within the reserve for four consecutive seasons. The present study is the second nematode survey to be conducted protected areas in the province. During the survey, seventy nematode genera were found belonging to forty six families. Four genera are reported here for the first time in South Africa i.e. *Discomyctus, Drilocephalobus, Heterocephalobellus,* and *Seinura*.

4.2 Conservation implications

The diversity of nematodes in the Grassland Biome is high, however only a small percentage of this biome is protected. It is therefore important to determine the diversity of these organisms in protected areas for conservation purposes. Also, these organisms not only contribute to the biological diversity of these areas but can also be used as biological indicators to further support conservation strategies.

4.3 Introduction

Grasslands cover approximately 40 % of the earth's terrestrial landmass (Ayres et al. 2008). Only 2.2 % of the Grassland Biome in South Africa falls within protected areas with almost 30 % of it having been permanently transformed as a result of human activity which primarily includes, agriculture, forestry, urbanization and mining. This biome is not only threatened by human impact but also by less controllable factors such as climate change (Mucina et al. 2006). Nematodes are considered the most abundant multicellular, eukaryotic organisms and are diverse, widely distributed, linked to several key trophic levels in the soil food web, integrators of soil properties and respond rapidly to biotic and abiotic changes in the soil environment (Azmat & Akhter 2009). Freeliving nematodes constitute a large portion of the nematode communities in various habitats. It is therefore important to determine the diversity of these nematodes especially in natural habitats.

Nematode surveys that include terrestrial free–living nematodes in South Africa started with work by Heyns (1961). Many nematode surveys in this country focussing on terrestrial free–living nematodes have been done in agricultural soils, caves and freshwater habitats (Durand et al. 2012; Mobara 2014; Du Preez et al. 2015; Swart et al. 2017; Habig et al. 2018; Mbatyoti et al. 2018). Other surveys on free–living nematodes include work done in the Kruger National Park (Botha & Heyns 1990; 1993) as well as work done by the Nematology Unit (NCN, Biosystematics, Agricultural Research Council–Plant Health and Protection [ARC–PHP]) in the last decade. These surveys include the Karoo BioGaps project (SANBI 2018) and surveys conducted in the Groenkloof and Rietvlei Dam Nature Reserves in 2013, the Serene Valley as part of the green belt around the Moreleta Spruit, Garsfontein in 2014 (M. Marais [ARC–PHP] pers. comm., 12 February 2019) all in the Gauteng Province. As part of the South African Grassland Survey the Willem Pretorius, Erfenis Dam and Kalkfontein Dam Nature Reserves in the Free State Province, were also sampled in 2014 (M. Marais [ARC–PHP] pers. comm., 28 February 2019). Telperion Nature Reserve falls within the Rand Highveld Grassland of which only 1 % is conserved (Mucina et al. 2006). Based on the scarcity of information on the diversity of terrestrial free–living nematodes, the survey conducted in the Telperion Nature Reserve will not only add to our

knowledge about the diversity of these nematodes in natural habitats but can also be used as baseline data in future ecological and conservation studies. Knowledge about the diversity of these nematodes can also be used as a reference site when compared to disturbed / stressed soils.

4.4 Methods and materials

4.4.1 Telperion Nature Reserve:

The study commenced during 2015 and 2016 in the Telperion Nature Reserve, Mpumalanga Province, South Africa (Figure 4.1), which is situated on the farm Elandsfontein 493 JR, (between -25.65986, 29.00921 and -25.71322, 29.03384, North to South; and -25.6724, 29.04703 and -25.69527, 28.97136, East to West). The reserve stretches over 7 350 ha and is dominated by grassy plains, interspersed with rocky outcrops dominated by woody plant species. The area lies on the Wilge River in the Ecca and Dwyka formations of the Waterberg and Karoo groups, which were formed during the Mokolian and Palaeozoic eras, respectively, and contains three streams that originate from higher lying wetlands and sponge areas. The lithology of Telperion is dominated by Arenite–Conglomerate, producing dystrophic or mesotrophic soils with some red soils, as well as rocky areas with miscellaneous soil (Grobler 1999).

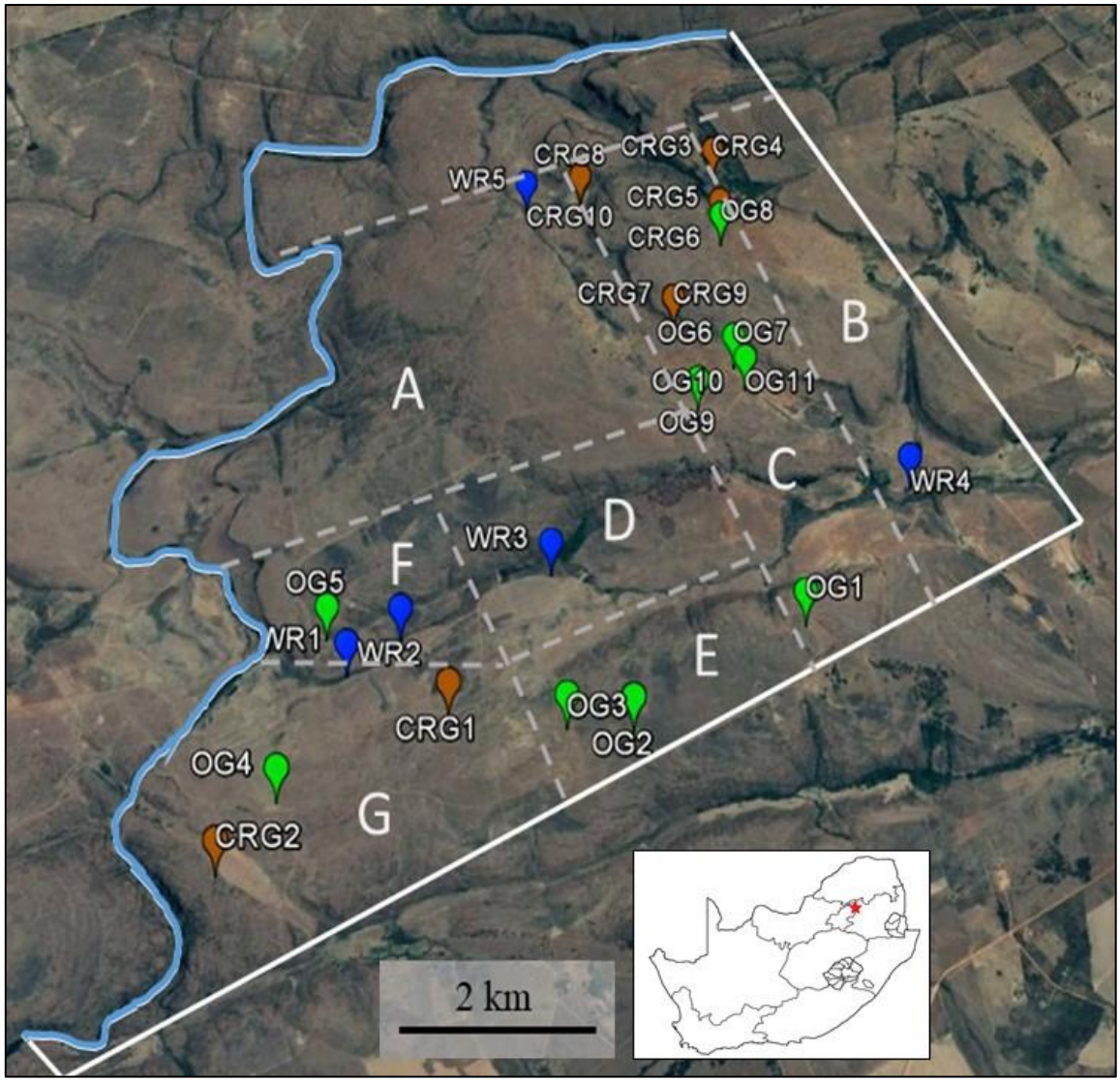


Figure 4.1: Map of Telperion Nature Reserve indicating the sampling sites as well as the various sections of the reserve. Green marks = Open Grassland sites; Blue marks = Water Related sites; Orange marks = Covered Rocky Grassland sites; A = Main house section; B = Blaauwpoort 2 section; C = Blaauwpoort 1 section; D = Nursery section; E = School section; F = Cottage section; G = Lewa section; Blue line = Wilge River. Map insert = South Africa where red star indicates location of Telperion Nature Reserve, Mpumalanga. (Shorthouse 2010).

4.4.2 Description of sites and collection of samples:

One hundred and four samples were collected from 26 sites within the reserve (Figure 4.1, Table 4.1) during 2015 and 2016 over four consecutive seasons (winter, spring, summer, autumn). Approximately 1 kg soil was collected, from the top 20–30 cm of soil at each site, using a garden trowel

which was cleaned with water between the sites to prevent contamination from one site to another. These samples were placed in plastic bags, transported to the laboratory and stored in a 10 ° C cold room until nematode extraction commenced.

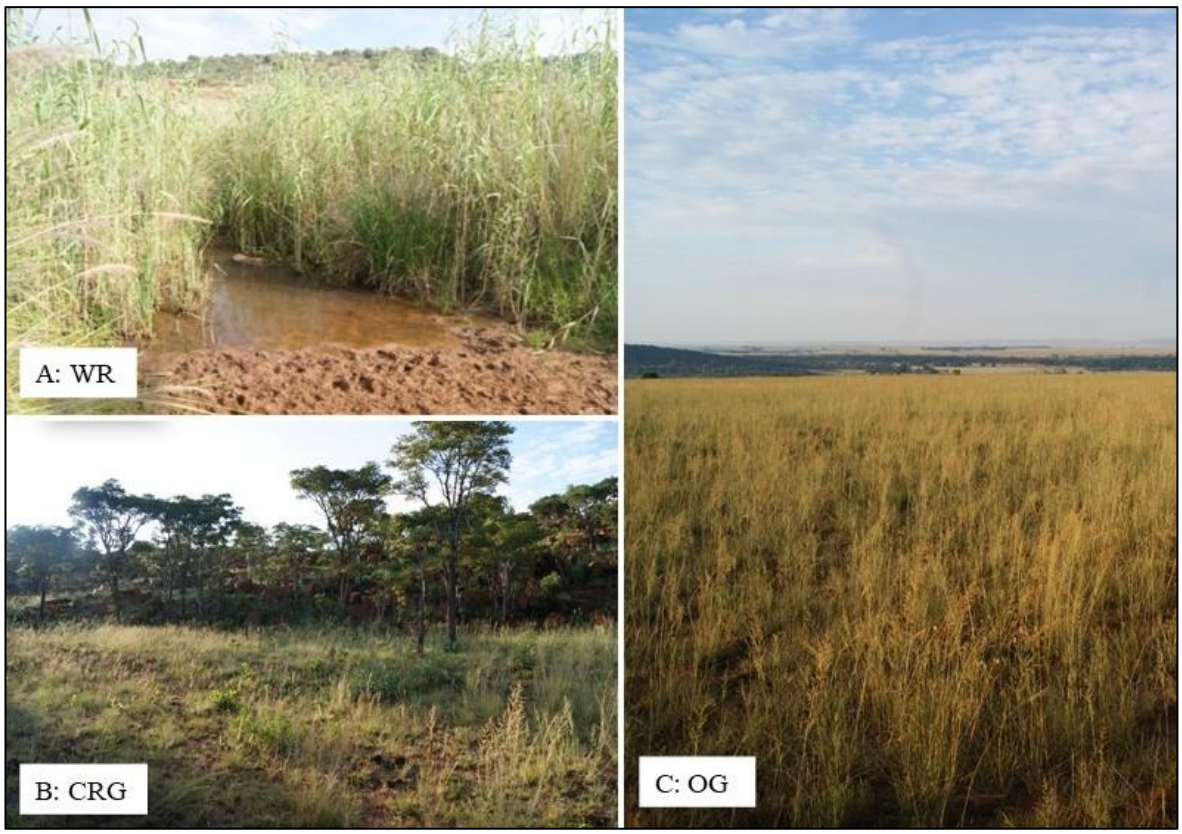


Figure 4.2: Sampling site categories A: water related site (WR); B: covered rocky grassland site (CRG); C: open grassland site (OG) in the Telperion Nature Reserve (Mpumalanga, South Africa) where nematode samples were taken during 2015 and 2016.

Site selection was based on the following: i) Vegetation types: sampling sites included most of the vegetation types included in the reserve and the two main groups selected were open grass plains and rocky areas dominated by woody plants; ii) Accessibility: some areas were restricted and / or not accessible by motor or foot; iii) Management purposes: the reserve is divided into various sections to allow proper management and therefore each section was sampled (Figure 4.1). Due to the various differences between sites based on vegetation type, exposure to sunlight and presence / absence of water the selected sites were grouped into three categories (Figure 4.2). This was done to ensure that the

selected sites were comparable and to rule out any biotic or abiotic factors that may influence results. These categories included the following: i) Water related sites (WR): any sites that were in the proximity of a water source but not classified as a freshwater site. Samples taken at these sites were usually muddy soil (during the wet season) (Figure 4.2A); ii) Open grassland (OG) sites: these sites were fully exposed to sunlight and the surrounding vegetation consisted purely of grasses (Figure 4.2C); iii) Covered rocky grassland sites (CRG): sites located under trees / shrubs and near the rocky outcrops of the reserve. These sites were not fully exposed to sunlight (Figure 4.2B).

Table 4.1: Specific site details of the three habitats sampled at the at the Telperion Nature Reserve, Mpumalanga, South Africa

Habitat	Sample name	Section	Georeference		Altitude (meters)
			Latitude	Longitude	
Open grassland	OG1	Blaauwpoort 1	-25.70071	29.04562	1451
	OG2	School	-25.71105	29.02932	1417
	OG3	School	-25.71125	29.02262	1400
	OG4	Cottage	-25.71905	28.99495	1337
	OG5	Cottage	-25.70467	28.99660	1333
	OG6	Blaauwpoort 1	-25.67612	29.03546	1482
	OG7	Blaauwpoort 2	-25.67612	29.03546	1482
	OG8	Blaauwpoort 2	-25.66286	29.03251	1480
	OG9	Nursery	-25.68080	29.03209	1466
	OG10	Blaauwpoort 1	-25.67823	29.03695	1448
	OG11	Blaauwpoort 1	-25.67823	29.03695	1448
Covered rocky grassland	CRG1	School	-25.71076	29.01050	1361
	CRG2	Cave	-25.72542	28.99062	1351
	CRG3	Blaauwpoort 2	-25.65525	29.03067	1447
	CRG4	Blaauwpoort 2	-25.65525	29.03067	1447
	CRG5	Blaauwpoort 2	-25.66123	29.03221	1467
	CRG6	Blaauwpoort 2	-25.66286	29.03251	1480
	CRG7	Blaauwpoort 2	-25.67240	29.02834	1479
	CRG8	Blaauwpoort 1	-25.65909	29.01574	1394
	CRG9	Blaauwpoort 1	-25.67240	29.02834	1479
	CRG10	Blaauwpoort 2	-25.65909	29.01574	1394
Water related	WR1	Cottage	-25.70771	28.99930	1304
	WR2	Cottage	-25.70418	29.00408	1323
	WR3	Nursery	-25.69715	29.01855	1333
	WR4	Blaauwpoort 1	-25.68675	29.05549	1383
	WR5	Main house	-25.66000	29.00957	1371

4.4.3 Nematode extraction, preservation and identification:

Nematodes were extracted from 250 cm^3 soil samples using an adapted decanting and sieving method, followed by a sugar flotation method (Marais et al. 2017b). The nematodes were then fixed in a heated 4 % formaldehyde + 1 % propionic acid (FPG) solution, dehydrated in a glycerine solution and mounted in glycerine on permanent glass slides using a wax ring method (Marais et al. 2017b). Nematodes were identified to genus level using a Zeiss Discovery V8 stereomicroscope and a Zeiss Axio Imager A2 compound microscope. Classification was based on Hunt (1993) for Aphelenchida; and Andrássy (2005; 2007; 2009) for other free–living nematodes.

4.5 Results

A total of 70 nematode genera belonging to 46 families were found during the present study (Table 4.2). The class Penetrantia Andrássy, 1974 was the most diverse with 32 genera, followed by the class Secernentia Von Linstow, 1905 (25 genera) and the class Torquentia Andrássy, 1974 (13 genera). Apart from the new reports, many of the genera found during the survey are frequently found in terrestrial habitats in South Africa. Most of these nematodes have been reported in South Africa in the last decade (Table 4.2), except for the genus *Neoactinolaimus* Thorne, 1967 for which the most recent record is 1987 (NCN). In the present survey, more than 60 % of the genera were found in all three habitat types and only 25 % were found in one habitat of the Telperion Nature Reserve (Table 4.2). The genera reported from one habitat were mostly aquatic nematodes from the water related sites. Four genera are reported for the first time from South Africa i.e. *Discomyctus* Thorne, 1939, *Drilocephalobus* Coomans & Goodey, 1965, *Heterocephalobellus* Rashid, Geraert & Sharma, 1985, and *Seinura* Fuchs, 1931. Specimens of the genus *Discomyctus* were found in the water related habitat of the reserve. *Drilocephalobus* was found in all three habitats, *Heterocephalobellus* specimens were found in the open grassland and covered rocky grassland while specimens of *Seinura* were found in the open grassland and water related habitats.

Table 4.2: Checklist of the terrestrial free–living nematodes found at the Telperion Nature Reserve, Mpumalanga, South Africa

	Habitat			Year first recorded in South Africa	Reference of record	Year of most recent record in South Africa	Reference of record
	Open grassland	Covered rocky grassland	Water related				
Class Torquentia Andrássy, 1974							
Family Aphanolaimidae Chitwood, 1936							
Genus *Aphanolaimus* De Man, 1880			x	1987	NCN	2014	Mobara (2014)
Family Chromadoridae Filipjev, 1917							
Genus *Punctodora* Filipjev, 1930		x	x	1959	NCN	2013	NCN
Family Chronogastridae Gagarin, 1975							
Genus *Chronogaster* Cobb, 1913	x	x	x	1959	NCN	2017	Swart et al. (2017)
= *Walcherenia* De Man, 1921							
Family Cyatholaimidae Filipjev, 1918							
Genus *Achromadora* Cobb, 1913	x		x	1965	NCN	2018	Habig et al. (2018)
= *Cyantholaimus* (Schromadora Cobb, 1913) Altherr, 1938							
= *Nannonchus* Cobb, 1913							
= *Cyantholaimus* (Nannonchus Cobb 1913) Micoletzky, 1922							
= *Kreisonema* Khera, 1969							
Family Metateratocephalidae Eroshenko, 1973							
Genus *Eutertocephalus* Andrássy, 1958			x	1974	NCN	2017	Swart et al. (2017)
Family Monhysteridae De Man, 1876							
Genus *Monhystera* Bastian, 1865	x	x	x	1959	NCN	2018	Habig et al. (2018)
Genus *Monhystrella* Cobb, 1918			x	2012	Durand et al. (2012)	2012	Durand et al. (2012)
= *Monhystera* (Monhystrella Cobb, 1918) Steiner, 1920							
= *Terschellingia* (Monhystrella Cobb, 1918) Micoletzky, 1922							
Family Odontolaimidae Gerlach & Riemann, 1974							
Genus *Odontolaimus* De Man, 1880			x	2015	Du Preez et al. (2015)	2015	Du Preez et al. (2015)
= *Neonchus* Cobb, 1893							
Family Plectidae Örley, 1880							
Genus *Anaplectus* De Coninck & Schuurmans Stekhoven, 1933	x	x	x	1988	NCN	2018	Habig et al. (2018)
= *Marinoplectus* Kreis, 1963							
Genus *Ceratoplectus* Andrássy, 1984				2014	Mobara (2014)	2014	Mobara (2014)
Genus *Plectus* Bastian, 1865	x	x	x	1959	NCN	2018	Habig et al. (2018)
= *Plectoides* De Man, 1904							
= *Proteroplectus* Paramonov, 1964							
Genus *Wilsonema* Cobb, 1913	x	x	x	1961	NCN	2018	Habig et al. (2018)
= *Bitholinema* De Coninck, 1931							
= *Pycnolaimus* Cobb, 1920							
= *Wilsotylus* Chawla, Khan & Prasad, 1969							
Family Rhabdolaimidae Chitwood, 1951							
Genus *Rhabdolaimus* De Man, 1880	x	x		1964	NCN	2015	Du Preez et al. 2015
= *Pseudorhabdolaimus* Soós, 1937							
Class Secernentia Von Linstow, 1905							
Family Anguinidae Nicoll, 1935 (1926)							
= Anguillulinidae Baylis & Daubney, 1926							
= Anguinidae (Paramonov, 1962) Siddiqi, 1971							
= Ditylenchidae (Golden, 1971) Fotedar & Handoo, 1978							
= Nothotylenchidae (Thorne, 1941) Jairajpuri & Siddiqi, 1969							
Genus *Ditylenchus* Filipjev, 1936	x	x	x	1959	NCN	2018	Habig et al. (2018)
= *Anguillulina* (*Ditylenchus* Filipjev) (Schneider, 1939)							
Nothotylenchus Thorne, 1941							
Boleodoroides Mathur, Khan & Prasad, 1966							
Diptenchus Khan, Chawla & Seshardi, 1969							
Safianema Siddiqi, 1981							
Orrina Brzeski, 1981							

[a] Indicates specimen that could not be identified due to poor quality of specimens or only juvenile specimens were obtained.
NCN – record information was obtained from the National Collection of Nematodes database (Marais et al. 2017a).

Table 4.2 (continued): Checklist of the terrestrial free–living nematodes found at the Telperion Nature Reserve, Mpumalanga, South Africa

	Habitat			Year first recorded in South Africa	Reference of record	Year of most recent record in South Africa	Reference of record
	Open grassland	Covered rocky grassland	Water related				
Family *Aphelenchidae* (Fuchs, 1937) Steiner, 1949							
Genus *Aphelenchus* Bastian, 1865	x	x	x	1959	NCN	2018	Habig et al. (2018)
= *Isonchus* Cobb, 1913							
= *Metaphelenchus* Steiner, 1943							
= Aphelenchus (*Anaphelenchus*) Anderson & Hooper, 1980							
Family Aphelenchoididae (Skarbilovich, 1947) Paramonov, 1953							
Genus *Aphelenchoides* Fisher, 1894	x	x	x	1959	NCN	2018	Habig et al. (2018)
= *Asteroaphelenchoides* Drozdovsky, 1967							
= *Chitinoaphelenchus* Micoletzky, 1922							
= *Paraphelenchoides* Haque, 1967							
= *Pathoaphelenchus* Cobb, 1927							
= *Pseudaphelenchoides* Drozdovsky, 1967							
Family Cephalobidae Filipjev, 1934							
= Metacrobelidae Andrássy, 1974							
Genus *Acrobeles* Von Linstow, 1877	x	x	x	1959	NCN	2018	Habig et al. (2018)
Genus *Acrobeloides* (Cobb, 1924) Thorne, 1937	x	x	x	1960	NCN	2018	Habig et al. (2018)
= *Acrobeles* (Acrobeloides) Cobb, 1924							
= *Rafiqius* Khan & Husain, 1997							
Genus *Cephalobus* Bastian, 1865	x	x	x	1964	NCN	2018	Habig et al. (2018)
= *Paracephalobus* Akhtar, 1962							
Genus *Cervidellus* Thorne, 1937	x	x	x	1962	NCN	2018	Habig et al. (2018)
= *Ypsylonellus* Andrássy, 1984							
= *Triligulla* Siddiqi, 1993							
Genus *Chiloplacus* Thorne, 1937	x	x	x	1959	NCN	2018	Habig et al. (2018)
= *Gaddinia* Penso, 1938							
= *Acrobelinema* Khera, 1967							
Genus *Eucephalobus* Steiner, 1936	x	x	x	1959	NCN	2018	Habig et al. (2018)
Genus *Heterocephalobellus* Rashid, Geraert & Sharma, 1985	x	x		First report			
Genus *Seleborca* Andrássy, 1985	x	x	x	2013	NCN	2018	Mbatyoti et al. (2018)
Genus *Zeldia* Thorne, 1937	x	x	x	1959	NCN	2018	Habig et al. (2018)
Cephalobidae[a]	x	x	x				
Family Diplogastridae Micoletzky, 1922							
Genus *Butlerius* Goodey, 1929	x	x	x	1961	Heyns (1971)	2018	Habig et al. (2018)
= *Butlerioides* Zamith & Lordello, 1959							
= *Butleriellus* Meyl, 1961							
= *Mesodiplogasteroides* Khera, 1969							
Diplogastridae[a]		x					
Family Diplogasteroididae Filipjev & Schuurmans Stekhoven, 1941							
Diplogasteroididae[a]			x				
Family Elaphonematidae Heyns, 1962							
Genus *Elaphonema* Heyns, 1962	x	x		1959	NCN	2017	Swart et al. (2017)
Family Mesorhabditidae Andrássy, 1976							
Genus *Cruznema* Artigas, 1927		x		1962	NCN	2018	Habig et al. (2018)
= *Pelodera* (*Cruznema* Artigas, 1927)							
= *Rhabditis* (*Cruznema* Artigas, 1927) Sudhaus, 1974							
= *Epimenides* Gutierrez, 1949							
Genus *Mesorhabditis* Osche, 1952	x	x	x	1961	Heyns (1971)	2018	Habig et al. (2018)
= *Rhabditis* (*Mesorhabditis)* Osche, 1952							
= *Rhabditis* (*Uniovaria*)Khera, 1968							

[a] Indicates specimen that could not be identified due to poor quality of specimens or only juvenile specimens were obtained.
NCN – record information was obtained from the National Collection of Nematodes database (Marais et al. 2017a).

Table 4.2 (continued): Checklist of the terrestrial free–living nematodes found at the Telperion Nature Reserve, Mpumalanga, South Africa

	Habitat			Year first recorded in South Africa	Reference of record	Year of most recent record in South Africa	Reference of record
	Open grassland	Covered rocky grassland	Water related				
Family Neodiplogastridae Paramonov, 1952							
Genus *Fictor* Paramonov, 1852 = *Diplogasterium* Paramonov, 1852 = *Paradiplogaster* Paramonov, 1852, nec Schuurmans Stekhoven & Teunissen, 1938 = *Holodiplogaster* Meyl, 1961		x	x	1971	Heyns (1971)	2018	Habig et al. (2018)
Genus *Koerneria* Meyl, 1960 = *Gobindonema* Khera, 1970 = *Glauxinemella* Gagarin, 1998		x	x	1971	Heyns (1971)	2018	Habig et al. (2018)
Family Neotylenchidae (Thorne, 1941) Thorne, 1949 = Hexatylidae (Skarbilovich, 1952) Paramonov, 1970 = Fergusobiidae (Goodey, 1963) Siddiqi & Goodey, 1964 = Gymnotylenchidae Siddiqi, 1980 = Phaenopisitylenchidae (Blinova & Korentchenko, 1986 Neotylenchidae[a]	x	x	x				
Family Osstellidae Heyns, 1962 Genus *Drilocephalobus* Coomans, & Goodey, 1965	x	x	x	First report			
Family Panagrolaimidae Thorne, 1937 Genus *Panagrolaimus* Fuchs, 1930	x	x	x	1959	NCN	2018	Habig et al. (2018)
Family Paraphelenchidae (T. Goodey, 1951) J.B. Goodey, 1960 Genus *Paraphelenchus* (Micoletzky, 1922) Micoletzky, 1925 = *Aphelenchus* (*Paraphelenchus*) Micoletzky, 1922		x	x	1959	NCN	2017	Swart et al. (2017)
Family Rhabditidae Örley, 1880 Genus *Rhabditis* Dujardin, 1845 = *Tribactis* Dujardin, 1845 = *Rhabditis* (*Choriorhabditis)* Osche, 1952 = *Rhabditis* (*Indororhabditis*) Chaturvedi & Khera, 1979	x	x	x	1959	NCN	2018	Habig et al. (2018)
Rhabditidae[a]	x						
Family *Seinuridae* Husain & Khan, 1967 (Baranovskaya, 1981) Genus *Seinura* Fuchs, 1931	x		x	First report			
Family Steinernematidae Filipjev, 1934 Genus *Steinernema* Travassos, 1927 = *Neoaplectana* Steiner, 1929	x	x	x	2009	NCN	2018	Habig et al. (2018)
Family Teratocephalidae Andrássy, 1958 Genus *Teratocephalus* De Man, 1876 = *Mitrephorus* Von Linstow, 1877	x	x	x	1987	NCN	2018	Mbatyoti et al. (2018)
Class Penetrantia Andrássy, 1974							
Family Actinolaimidae Thorne, 1939 Genus *Neoactinolaimus* Thorne, 1967 Actinolaimidae			x x	1964	NCN	1987	NCN
Family Alaimidae Micoletzky, 1922 Genus *Alaimus* De Man, 1880	x	x	x	1959	NCN	2013	NCN
Family Amphidelidae Andrássy, 2002 Genus *Amphidellus* Thorne, 1939		x	x	1963	NCN	1999	NCN
Family Anatonchidae Jairajpuri, 1939 Genus *Iotonchus* Cobb, 1916 = *Mononchus* (*Iotonchus*) Cobb, 1916	x	x	x	1963	NCN	2017	Swart et al. (2017)
Genus *Jensenonchus* Jairajpuri & Khan, 1982	x			1967	Coetzee (1967)	2017	Swart et al. (2017)

[a] Indicates specimen that could not be identified due to poor quality of specimens or only juvenile specimens were obtained.
NCN – record information was obtained from the National Collection of Nematodes database (Marais et al. 2017a).

Table 4.2 (continued): Checklist of the terrestrial free–living nematodes found at the Telperion Nature Reserve, Mpumalanga, South Africa

	Habitat			Year first recorded in South Africa	Reference of record	Year of most recent record in South Africa	Reference of record
	Open grassland	Covered rocky grassland	Water related				
Family Aporcelaimidae Heyns, 1965							
Genus Aporcelaimellus Heyns, 1965 = *Takamangai* Yeates, 1967	x	x	x	1968	NCN	2018	Habig et al. (2018)
Genus *Aporcelaimus* Thorne & Swanger, 1936	x	x	x	1962	NCN	2018	Habig et al. (2018)
Genus *Makatinus* Heyns, 1965	x	x		1963	NCN	2017	Swart et al. (2017)
Aporcelaimidae[a]	x	x	x				
Family Crateronematidae Siddiqi, 1969							
Genus *Lordellonema* Andrássy, 1959	x			1959	NCN	1990	NCN
Family Diphtherophoridae Micoletzky, 1922							
Genus *Diphtherophora* De Man, 1880 = *Chaolaimus* Cobb, 1893 = *Archionchus* Cobb, 1913	x	x	x	1960	NCN	2013	NCN
Family Dorylaimidae De Man, 1876							
Genus *Dorylaimus* Dujardin, 1845	x	x	x	1959	NCN	2018	Habig et al. (2018)
Genus *Mesodorylaimus* Andrássy, 1959		x	x	1961	NCN	2017	Swart et al. (2017)
Dorylaimidae[a]	x	x	x				
Family Ironidae De Man, 1876							
Genus *Ironus* Bastian, 1865 = *Cephalonema* Cobb, 1893, *nec* Stimpson, 1882 = *Nanonema* Cobb in Stiles & Hassal, 1905			x	1962	NCN	2017	Swart et al. (2017)
Family Leptonchidae Thorne, 1935							
Genus *Leptonchus* Cobb, 1920	x	x	x	1961	NCN	2018	Mbatyoti et al. (2018)
Genus *Proleptonchus* Loredello, 1955	x	x		1963	NCN	1990	NCN
Leptonchidae[a]	x		x				
Family Mononchidae Filipjev, 1934							
Genus *Cobbonchus* Andrássy, 1958	x		x	1962	NCN	2018	Habig et al. (2018)
Genus *Granonchulus* Andrássy, 1958		x		1963	NCN	2017	Swart et al. (2017)
Genus *Mononchus* Bastian, 1865 = *Dioncholaimus* Kreis, 1932	x	x	x	1962	NCN	2018	Habig et al. (2018)
Mononchidae[a]	x	x	x				
Family Mydonomidae Thorne, 1964							
Genus *Dorylaimoides* Thorne & Swanger, 1936 = *Leptonema* Jairajpuri, 1961 (preoccupied) = *Tarjania* Brzeski & Szczygiel, 1961 = *Shamimonema* Chawla, Khan & Prasad, 1965 = *Dorylaimoides* (*Arcidorylaimoies)* Jairajpuri & Ahmad, 1992 = *Dorylaimoides* (*Digidorylaimoides*)Jairajpuri & Ahmad, 1992 = *Dorylaimoides* (*Longidorylaimoides)* Jairajpuri & Ahmad, 1992	x			1959	NCN	2015	Du Preez et al. (2015)
Family Mylonchulidae Jairajpuri, 1969							
Genus *Mylonchulus* Cobb, 1916 = *Mononchus* (*Mylonchulus* Cobb, 1916) = *Paramylonchulus* Jairajpuri & Khan, 1982 = *Pakmylonchulus* Khan & Saeed, 1987	x	x		1962	NCN	2018	Habig et al. (2018)
Family Paraxonchiidae Dhanachand & Jairajpuri, 1981							
Genus *Paraxonchium* Krall, 1958 = *Drepanodorus* Altherr, 1954 (*praeocc.)* = *Drepanophorus* Altherr, 1954	x	x	x	1959	NCN	2018	Habig et al. (2018)
Family Primatolaimidae Micoletzky, 1922							
Genus *Prismatolaimus* De Man, 1880 = *Takakia* Yeates, 1967	x	x	x	1962	NCN	2012	Durand et al. (2012)

[a] Indicates specimen that could not be identified due to poor quality of specimens or only juvenile specimens were obtained.
NCN – record information was obtained from the National Collection of Nematodes database (Marais et al. 2017a).

Table 4.2 (continued): Checklist of the terrestrial free–living nematodes found at the Telperion Nature Reserve, Mpumalanga, South Africa

	Habitat			Year first recorded in South Africa	Reference of record	Year of most recent record in South Africa	Reference of record
	Open grassland	Covered rocky grassland	Water related				
Family Qudsianematidae Jairajpuri, 1965							
Genus *Discolaimoides* Heyns, 1963	x	x	x	1963	NCN	2018	Habig et al. (2018)
Genus *Discolaimus* Cobb, 1913 = *Ottolaimus* Kirjanova, 1951	x	x	x	1959	NCN	2018	Habig et al. (2018)
Genus *Eudorylaimus* Andrássy, 1959 = *Witoldinema* Brzeski, 1960 = *Qudsianema* Jarajpuri, 1965		x	x	1959	NCN	2018	Habig et al. (2018)
Genus *Labronema* Thorne, 1939	x	x	x	159	NCN	2018	Swart et al. (2017)
Discolaiminae[a]	x	x					
Family Tobrilidae De Coninck, 1965 Genus *Eutobrilus* Tsalolikhin, 1981 = *Peritobrilus* Gagarin, 1993 = *Setsalia* (Shoshina, 2003) Andrássy, 2007			x	1973	NCN	2017	Swart et al. (2017)
Genus *Tobrilus* Andrássy, 1959 = *Trilobus* Bastian, 1865, *nec* Brünnich, 1781 (*Trilobita*)		x	x	1964	NCN	2018	Habig et al. (2018)
Family Tripylidae De Man, 1876 Genus *Tripyla* Bastian, 1865 = *Promononchus* Micoletzky, 1922 = *Paratripyla* Brzeski, 1964	x	x		1962	NCN	2018	Habig et al. (2018)
Family Tylencholaimellidae Jairajpuri, 1964 Genus *Tylencholaimellus* Cobb in Cobb, 1915 = *Pharetrolaimus* De Man, 1921 = *Tylencholaimellus* (*Coronatylencholaimellus)* Jairajpuri & Ahmad, 1992	x	x	x	1962	NCN	2018	Habig et al. (2018)
Family Tylencholaimidae Filipjev, 1934 Genus *Discomyctus* Thorne, 1939			x	First report			
Genus *Tylencholaimus* De Man, 1876 = *Xenonchium* Siddiqi & Khan, 1964 = *Tylencholaimus* (*Amphitylencholaimus)* Jairajpuri & Ahmad, 1992) = *Tylencholaimus* (*Disctylencholaimus*)Jairajpuri & Ahmad, 1992) = *Tylencholaimus* (*Leptotylencholaimus)* Jairajpuri & Ahmad, 1992) = *Tylencholaimus* (*Opisthotylencholaimus)* Jairajpuri & Ahmad, 1992) = *Tylencholaimus* (*Protylencholaimus)* Jairajpuri & Ahmad, 1992)		x	x	1960	NCN	2018	Mbatyoti et al. (2018)
Tylencholaimidae[a]	x		x				
Unidentified							
Alaimida Siddiqi, 1983[a]	x	x	x				
Aphelenchida Siddiqi, 1980[a]			x				
Chromadorida Chitwood, 1933[a]	x						
Dorylaimida Pearse, 1942[a]	x	x	x				
Rhabditida Chitwood, 1933[a]	x	x	x				
Unknown[a]	x	x					

[a] Indicates specimen that could not be identified due to poor quality of specimens or only juvenile specimens were obtained.
NCN – record information was obtained from the National Collection of Nematodes database (Marais et al. 2017a).

4.6 Discussion

Approximately 149 free–living nematode genera (terrestrial and freshwater) have been reported in South Africa to date (Botha & Heyns 1990; 1993; Durand et al. 2012; Mobara 2014; Du Preez et al. 2015; Swart et al. 2017; Habig et al. 2018; Mbatyoti et al. 2018). Of these 70 have now been found in the Telperion Nature Reserve. These numbers are a clear indication of the high nematode diversity in pristine protected areas in the Grassland Biome of South Africa. The four genera reported for the first time from South Africa were *Discomyctus*, *Drilocephalobus*, *Heterocephalobellus*, and *Seinura*. *Discomyctus* is distributed in Asia, Africa (Cameroon), Europe, North America and South America (Siddiqi 1998; Andrássy 2009). *Drilocephalobus* has been reported from all continents except Antarctica (Andrássy 2005). In Africa species of the genus has been reported from Cameroon, Democratic Republic of Congo, Senegal, Tanzania and Libya (Coomans & Goodey 1965; De Ley & Coomans 1990; Siddiqi 2001). Members of *Heterocephalobellus* have been reported from Africa (Nigeria and Benin), Europe, North America and South America (Andrássy 2005; Holovachov et al. 2009). *Seinura* has a world–wide distribution and in Africa has been reported from the Egypt and Democratic Republic of Congo (Andrássy 2007).

4.7 Conclusion

The survey conducted at the Telperion Nature Reserve resulted in new diversity and distribution records of the terrestrial free-living nematodes in South Africa and particularly the province of Mpumalanga. Seventy terrestrial free-living nematode genera were found at the Telperion Nature Reserve. Nematodes are a diverse and abundant group of organisms and play important roles in ecological systems and soil health. It is therefore important to determine the diversity of these organisms to serve as baseline information for future studies. Future work can be done on the ecology of nematodes in the Grassland Biome and other protected areas for conservation purposes as well as other global issues such as climate change and its effect on natural habitats.

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4.9 Competing interests

The authors declare that they have no financial or personal relationship(s) that may have inappropriately influenced them in writing this article.

4.10 Author contributions

Chantelle Girgan, first author, responsible for the identification and curation of specimens, and writing of the manuscript, participated in field work. Antoinette Swart participated in the identification of specimens, field work, co–supervisor of Ph.D. thesis and editing of manuscript. Mariette Marais co–coordinator of South African Plant–Parasitic Nematode Survey (SAPPNS) and National Collection of Nematodes (NCN) database, responsible for original concept of the project, participated in field work, co–supervisor of Ph.D. thesis and editing of manuscript. Hendrika Fourie participated in editing the manuscript, supervisor of Ph.D. thesis and coordinator at the North West University.

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CHAPTER 5: ARTICLE 4

***Aphelenchoides* spp. (Nematoda: Aphelenchida) and *Panagrolaimus leperisini* (Nematoda: Rhabditida) found associated with grass seeds in the Telperion Nature Reserve, South Africa**

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Aphelenchoides spp. (Nematoda: Aphelenchida) and *Panagrolaimus leperisini* (Nematoda: Rhabditida) found associated with grass seeds in the Telperion Nature Reserve, South Africa

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Abstract

As part of the South African Plant-Parasitic Nematode Survey (SAPPNS), an extensive nematode diversity survey was conducted in the Telperion Nature Reserve, Mpumalanga, South Africa. During this survey, samples of grass seeds were collected. Some of these seeds yielded specimens of the genera *Aphelenchoides, Aphelenchus* and *Panagrolaimus.* Identified species include *Aphelenchoides africanus, A. besseyi, A. lichenicola, A. rutgersi, A. spicomucronatus* and *Panagrolaimus leperisini.* Four of these species are new records for South Africa and the South African populations of these species are described in this paper.

Key words: *Aphelenchoides, Panagrolaimus, Aphelenchoides lichenicola, A. rutgersi, A. spicomucronatus, Panagrolaimus leperisini,* Grassland biome

Introduction

The Nematology Unit of the Agricultural Research Council-Plant Health and Protection (ARC-PHP), Biosystematics Division founded the South African Plant-Parasitic Nematode Survey (SAPPNS) in 1987 with the aim of making a comprehensive assessment of the nematode biodiversity resources of South Africa (Marais 2006). According to Mucina *et al.* (2006) the Rand Highveld Grassland is highly endangered (only 1% protected by conservation) due to cultivation, plantation forestry, urbanisation and dam-building. However, little is known about the diversity of nematodes in this vegetation ecozone.

To address this deficiency, an extensive nematode survey of the Telperion Nature Reserve, Mpumalanga, South Africa, was conducted as part of the SAPPNS. During this survey, various samples, including soil, root, seed (inflorescence) of grass, flowing water and bottom samples of marshland, were collected over a one-year period, (2015-2016) in winter, spring, summer and autumn. As part of this survey, seed samples of various grasses were collected during the summer (2016) season.

In the past, species of *Anguina* Scopoli, 1777, *Subanguina* Paramonov, 1967, *Aphelenchoides* Fischer, 1894 and *Ditylenchus* Filipjev, 1936 have been reported in South African grass seeds (Bekker 2008; Jones & De Waele 1988; Kleynhans *et al.* 1996; SAPPNS; Van den Berg 1985). *Anguina agrostis* (Steinbuch, 1799) Filipjev, 1936 is the only species of the genus *Anguina* reported to date from seeds in South Africa, originally described from *Agrostis tenuis* Sibth, which is now a synonym of *Agrostis capillaris* L. *Anguina agrostis* was recovered from seeds of *Lolium perenne* L. in Western Cape Province (Kleynhans *et al.* 1996) and is a known vector of the bacterium *Rathayibacter rathayi* (Smith 1913) Zgurskaya *et al.* 1993, which causes sheep staggers (Stynes & Bird 1980).

Subanguina wevelli (Van den Berg, 1985) Fortuner & Maggenti, 1987, also known as seed-and -leaf gall nematode, was described from *Eragrostis curvula* (Schrad.) Nees seeds from two localities in Free State and Mpumalanga provinces (Van den Berg 1985). During a survey of *Eragrostis* L. species, *S. wevelli* was also reported from areas of Bethal, Amersfoort and Standerton (Mpumalanga Province), and Potchefstroom (North West Province) (Bekker 2008).

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Aphelenchoides besseyi Christie, 1942 is a migratory ecto- and endoparasite found on leaves, stems and other parts of higher plants, and is also associated with fungi colonising bark (de Jesus *et al.* 2016). This plant-parasitic nematode is widely distributed and has a wide host range, associated with over 35 genera and more than 200 species of higher plants (de Jesus *et al.* 2016, Luc *et al.* 2005). As an agricultural pest, *A. besseyi* is well known on seeds, stems and panicles of rice, causing "white tip" disease (de Jesus *et al.* 2016, Luc *et al.* 2005). In Brazil, this nematode has also frequently been associated with forage grass seeds (de Jesus *et al.* 2016).

According to the SAPPNS, *A. besseyi* has been reported from six provinces in South Africa, associated with crops such as *Allium sativum* L., *Allium cepa* L. and *Fragaria* × *ananassa* Duch; shrubs and ornamentals including *Ixia* L. sp., *Hyacinthoides hispanicus* (Mill.) Chouard *ex* Rothm., *Dahlia* L. sp., *Lilium* L. sp., *Galtonia* Decne. sp. and *Amaryllis* L. sp.; and in the rhizosphere of an unidentified grass in natural vegetation. However, this species has not yet been recorded from seeds in South Africa.

SAPPNS records indicate that *Ditylenchus* species are commonly associated with the rhizosphere of grasses in South Africa. However, only *Ditylenchus africanus* Wendt, Swart, Vrain & Webster, 1995 has been recorded from seed. First reported as *Ditylenchus destructor* Thorne, 1945, *Ditylenchus africanus* was described from groundnuts in the Schweizer-Reneke district in South Africa (Jones & De Waele 1988).

Species of *Panagrolaimus* Fuchs, 1930 are classified as free-living, bacterial-feeding nematodes (Yeates *et al.* 1993), generally occurring in soil and fresh-water bodies. *Panagrolaimus* has also been recorded in various other habitats, including roots, stems, leaves, spikelets, and in rice grains and the seeds of pearl millet (Panchbhai *et al.* 1986). *Panagrolaimus rigidus* (Schneider, 1866) Thorne, 1937 has been associated with dogwood cankers (Self & Bernard 1994) and has also been reported on elderberry branches (*Sambucus* sp.) exhibiting witches' broom symptoms (Lehmann 1963). *Panagrolaimus magnivulvatus* Boström, 1995 was reported in nest material of the snow petrel, *Pagodroma nivea* Forster, 1777, and algae and soils from nunataks in Antarctica, despite the extreme environmental conditions (Swart & Harris 1996). *Panagrolaimus subelongatus* (Cobb, 1914) Thorne, 1937 was recorded in an extensive survey of nematodes associated with the stem malformations of hardwood trees (Santamour & McArdle 1987). There are thirty-eight valid species and six *species inquirendae* or *incertae sedis* classified under *Panagrolaimus* (Abolafia & Peña-Santiago 2006). Although *Panagrolaimus* is frequently found in South African soils (Heyns 1971), very little is known about this nematode in South Africa and only a few records exist where species have been identified. Only two species have previously been reported from South Africa, *P. subelongatus* and *P. magnivulvatus* (Heyns 1971; National Collection of Nematodes (NCN) database). Locality details for these species are given in Table 1.

TABLE 1. *Panagrolaimus* spp. Reported from South Africa (National Collection of Nematodes database).

Species	Locality	Year	Description of sample
Panagrolaimus subelongatus	Rustenburg, North West Province	1959	Garden soil.
	Ventersdorp, North West Province	1961	Grey, sandy soils planted to pumpkin, weeds in field.
Panagrolaimus magnivulvatus	Rietondale, Pretoria, Gauteng Province	1996	Dry growths, caused by psyllids, in the inflorescence of *Searsia lancea*
	Krugersdorp, Gauteng Province	1996	Spongy lesions on the bulbs of garlic infested with a rod-shaped virus.

Due to the lack of information on the diversity of nematodes in natural environments in South Africa, a nematode survey was conducted in the Telperion Nature Reserve of Mpumalanga Province. The objective of this study was to determine whether there are nematodes associated with grass seeds in the reserve and their diversity.

Materials and methods

Twenty seed samples were collected from different grass species as part of a nematode survey of the Telperion Nature Reserve (between 25°39'35.51"S, 29° 0'33.15"E and 25°42'47.58"S, 29°2'1.84"E, North to South; and

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25°40'20.64"S, 29°2'49.30"E and 25°41'42.99"S, 28°58'16.89"E, East to West), Mpumalanga, South Africa during 2015 and 2016. The seed samples were collected as whole plants in February 2016 during the summer season sampling. Telperion Nature Reserve is situated at the extreme north of the grassland biome in South Africa, recognized by Acocks (1988) as Bankenveld, while Bredenkamp & Van Rooyen (1998) classified it as Rocky Highveld Grassland. According to Mucina *et al.* (2006) the area is known as the Rand Highveld Grassland. Telperion Nature Reserve was divided into various management sections from which grass plants were collected. Table 2 lists the sections in which nematodes were found including details about these sites. Grasses were identified by Prof. Sarel Cilliers (Botanist, North West University, Potchefstroom, South Africa) and are listed in Table 2.

TABLE 2. Grass seed collection site data.

	Section	GPS		Altitude (m)	Exposure	Associated Host
		Longitude	Latitude			
MP122	Blaauwpoort 1	25° 40' 34.03"S	29° 2' 7.66"E	1482	Full sun	Aristida canescens
MP123	Blaauwpoort 2	25° 40' 34.03"S	29° 2' 7.66"E	1482	Full sun	Eragrostis gummiflua
MP124	Blaauwpoort 1	25° 39' 32.72"S	29° 0' 56.66"E	1394	Full sun	Bulbostylis burchellii
MP125	Blaauwpoort 2	25° 39' 32.72"S	29° 0' 56.66"E	1394	Full sun	Cyperus longus
MP126	Blaauwpoort 1	25° 40' 41.63"S	29° 2' 13.02"E	1448	Full sun	Perotis patens

Nematode extraction: Grass inflorescence samples were weighed and nematodes were extracted using the adapted Baermann tray method (Kleynhans 1997), fixed in a heated 4% formaldehyde + 1% propionic acid (FPG) solution (Netscher & Seinhorst 1965), dehydrated in a glycerine solution using the method of Seinhorst and mounted in glycerine on permanent glass slides using the wax ring method (Seinhorst 1962).

Light microscopy: Measurements and drawings of the mounted specimens were made with an Olympus BX53F microscope, equipped with a drawing tube, at 1000X magnification. Measurements of curved structures were taken along the median line. Morphometrics according to de Man (1884) were used in descriptions. The specimens were deposited in the National Collection of Nematodes (NCN), ARC-PHP, Biosystematics, Pretoria.

The classification followed in this paper is based on Hunt (1993) for Aphelenchina and Andrássy (2005) for Rhabditida.

Results

Nematodes belonging to the genera *Aphelenchus* Bastian, 1865 (species not determined due to low quality of the one specimen found), *Aphelenchoides* and *Panagrolaimus* were found in five of the 20 samples (Table 3). Identified species include *Aphelenchoides africanus* Dassonville & Heyns, 1984, *Aphelenchoides besseyi, Aphelenchoides lichenicola* Siddiqi & Hawksworth, 1982, *Aphelenchoides rutgersi* Hooper & Myers, 1971, *Aphelenchoides spicomucronatus* Truskova, 1973 and *Panagrolaimus leperisini* Massey, 1974.

One female of *A. africanus* was found associated with seeds of *Cyperus longus* L. during this survey. This is the second report of this species since its description from benthic samples from Skinnerspruit, Pretoria, South Africa and the first report from seeds (Dassonville & Heyns 1984). Specimens were deposited in the NCN with accession number 50542. *Aphelenchoides besseyi* was found associated with seeds of *C. longus* and *Bulbostylis burchellii* (Ficalho & Hiern) C.B. Clarke from two sites. This nematode has previously been reported from six provinces and a number of crops in South Africa (Kleynhans *et al.* 1996; SAPPNS database). This is the first report of this species from seeds. Specimens were deposited in the NCN with accession number 50543. *Panagrolaimus* has been reported from onion seeds submitted to the Nematology Unit for diagnostic services of the ARC-PHP. This is the first report in South Africa of the genus associated with seeds of grasses.

Populations of the species *Aphelenchoides lichenicola, A. rutgersi, A. spicomucronatus* and *Panagrolaimus leperisini*, all reported for the first time from South Africa, are described below. Females and juveniles of *A. rutgersi* was found associated with seeds of *Aristida canescens* Henrard, a female of *A. spicomucronatus* was found

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associated with seeds of *Eragrostis gummiflua* Nees, male and female specimens of *A. lichenicola* was found associated with seeds of *Perotis patens* Gand., and female, male and juvenile specimens of *P. leperisini* were found associated with three different grasses: *B. burchellii, C. longus* and *P. patens*.

TABLE 3. Nematodes found at various sites.

Sample name	Associated host	Sample Weight	Nematode species	Number of individuals found	Feeding type
MP122	*Aristida canescens*	4 g	*Aphelenchoides rutgersi*	3♀ 2j	Fungivore
			Aphelenchoides sp. 1	1♀	Fungivore
MP123	*Eragrostis gummiflua*	< 1 g	*Aphelenchoides spicomucronatus*	1♀	Fungivore
MP124	*Bulbostylis burchellii*	4 g	*Panagrolaimus leperisini*	1♀ 9j	Bacterivore
			Aphelenchoides sp. 2	2 ♀ 2j	Fungivore
			Aphelenchoides besseyi	1♀	Herbivore/fungivore
MP125	*Cyperus longus*	4 g	*Aphelenchus* sp.	1j	Fungivore
			Panagrolaimus leperisini	1♂ 6j	Bacterivore
			Aphelenchoides besseyi	2♀ 2j	Herbivore/fungivore
			Aphelenchoides africanus	1♀	Fungivore
			Aphelenchoides sp. 3	2♀	Fungivore
MP126	*Perotis patens*	1 g	*Aphelenchoides lichenicola*	1♀ 1♂ 2j	Fungivore
			Panagrolaimus leperisini	1♀	Bacterivore

Aphelenchoides lichenicola Siddiqi & Hawksworth, 1982
(Fig. 1&2)

Aphelenchoides lichenicola was first described from galls found on *Cladonia glauca* Flörke from Ireland. It was suggested that this species feeds on the fungal component of the lichen (Siddiqi & Hawksworth, 1982). The original description was the only report found for this species.

Measurements. See Table 4.

Description. *Female* (n = 1) Habitus: curled in on itself ventrally. Cuticle finely annulated, annule width 1 µm at mid-body. Lateral field 2 µm wide, marked with four incisures (Fig. 1D), middle two incisures faint. Head offset (Fig. 1E). Lip region 3 µm high and 6 µm broad. Stylet 10 µm long with basal swellings. Lumen of stylet distinct. Median bulb spherical to slightly rectangular (Fig. 1E). Valve distinct, situated slightly off centre in posterior half. Nerve ring situated 68 µm from anterior end, less than one corresponding body width from base of median bulb. Excretory pore 73 µm from anterior end, opposite posterior end of nerve ring (Fig. 1E). Ovary outstretched with a single row of oocytes (Fig. 1F & 2E). Spermatheca oval in shape containing sperm cells (Fig. 2A). Vulval lips slightly raised with the vagina occupying more than half the corresponding body width, curving slightly anteriorly (Fig. 1G). Post-vulval uterine sac 37 µm in length, about 2.5 times vulval body width and shorter than half the vulva-anus distance (Fig. 1G & 2C). Tail elongate conoid, 34 µm long. Terminus bearing slightly star-shaped mucro with central part more prominent (Fig. 1C & 2B).

Male (n = 1): Habitus: curled in on itself ventrally. Head, lip region and pharynx as in female (Fig. 1A). Stylet 11 µm, longer than in female. Tail 32 µm long with three pairs of subventral papillae (Fig. 1B). Mucro similar to female but less star-shaped. Spicules rose-thorn shaped with well-developed apex (18 µm) and rostrum (11 µm), medially measuring 14 µm. Spicules with an anterior subventral concavity and swelling on dorsal limb (Fig. 1B & 2D).

Discussion. The female and male specimens of *Aphelenchoides* found associated with *Perotis patens* seeds from the Telperion Nature Reserve were identified as *A. lichenicola* based on the characteristic shape of the spicule (having a concavity in the anterior subterminal surface), tail shape, and presence of four lateral field incisures. The specimens have been positively identified based on measurements and morphology similar to those of Siddiqi & Hawksworth (1982). According to the original description of this species the spicules range between 17 and 18 µm; the specimen described here had a slightly smaller spicule (14 µm). Although smaller in size, the spicules are morphologically the same and it was decided that this specimen is conspecific with *A. lichenicola*. This species is

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reported for the first time in South Africa and to our knowledge it is the first report of this species from seeds. Specimens are deposited in the NCN with the accession number 50546.

TABLE 4. Measurements (in µm) of *Aphelenchoides rutgersi* Hooper & Myers, 1971, *Aphelenchoides lichenicola* Siddiqi & Hawksworth, 1982 and *Aphelenchoides spicomucronatus* Truskova, 1973.

	Aphelenchoides rutgersi	*Aphelenchoides lichenicola*		*Aphelenchoides spicomucronatus*
Locality	MP122	MP126		MP123
n	3♀	1♀	1♂	1♀
L	556 ± 22.6 (542–582)	526	461	669
a	34.1 ± 0.8 (33.5–34.9)	36.4	30.2	35.3
b	4.6 ± 0.2 (4.4–4.8)	4.4	3.8	4.9
c	15.8 ± 0.5 (15.5–16.38)	15.4	14.4	16.7
c'	3.6 ± 0.3 (3.3–3.9)	2.9	2.8	3.3
Annule width	1.1 ± 0.15 (1.0–1.3)	1.1	1.1	1.1
Lateral field width	3.3 ± 0.30 (3–4)	2	3	-
Lateral field width / corresponding body width	4.9 ± 0.18 (4.7–5.1)	7	6	-
Lip region width	5.6 ± 0.40 (5–6)	6.3	6.6	6.3
Lip region height	3.2 ± 0.53 (3–4)	2.6	3.2	3.4
Stylet	11 ± 0.4 (11–12)	10	11	11
Excretory pore from anterior end	76 ± 1.9 (75–76)	73	71	85
Median bulb width	10 ± 0.5 (9–10)	10	10	11
Median bulb length	12 ± 0.5 (12–13)	12	12	14
Pharynx length	122 ± 1.5 (121–124)	119	122	138
Pharyngo-intestinal junction distance from base of median bulb	8.9 ± 1.3 (7–10)	-	12	12
Basal bulb length	46 ± 4.1 (43–48)	36	-	49
Basal bulb width	7 ± 0.4 (6–7)	5	-	8
Nerve ring from anterior end	67 ± 0.9 (66–68)	68	63	74
Body diameter : Midbody	16 ± 0.9 (16–17)	15	15	19
Anus / Cloaca	10 ± 0.9 (9–11)	12	11	12
Vulva	16 ± 0.8 (15–17)	15	-	19
Vulva (µm from anterior)	386 ± 13.6 (374–401)	366	-	463
V	69 ± 0.8 (69–70)	70	-	69
Vagina length	7 ± 0.9 (6–8)	7	-	9
Vagina length / Corresponding body width	2.2 ± 0.3 (1.9–2.4)	2.14	-	2.11
Anterior genital branch length	220 ± 5.6 (215–226)	239	-	357
Post-vulval uterine sac length	40 ± 4.1 (37–43)	37	-	57
Egg length	32 ± 4.3 (28–36)	-	-	-
Egg width	11 ± 0.8 (10–12)	-	-	-
Testis length	-	-	252	-
Spicule length	-	-	14	-
Spicule: Dorsal limb	-	-	18	-
Spicule: Ventral limb	-	-	11	-
Tail length	35 ± 2.1 (33–37)	34	32	40

- indicates structures that were not visible or not present.

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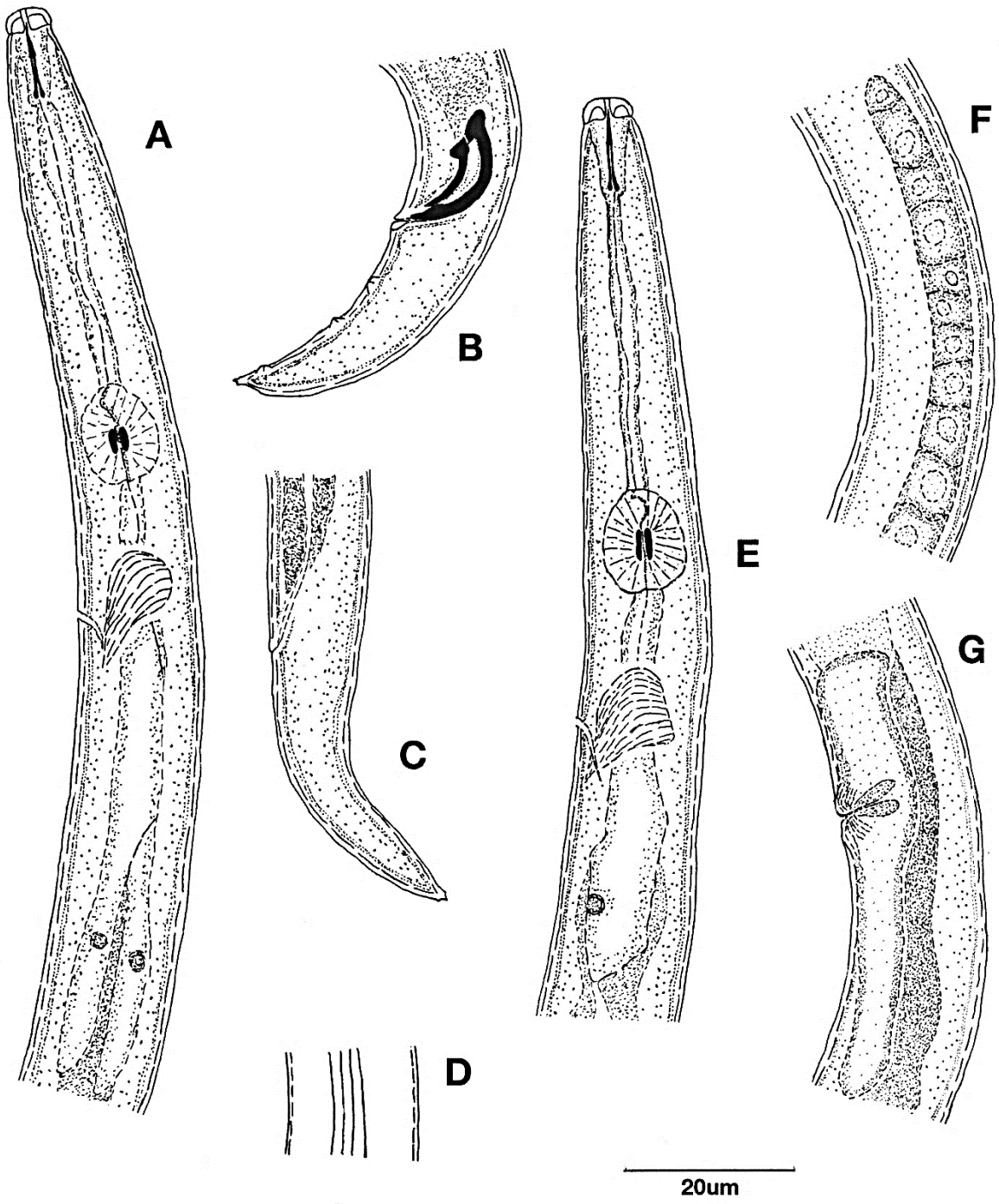


FIGURE 1. *Aphelenchoides lichenicola*. Female (C–G), C: Tail region; D: Lateral field at mid-body; E: Anterior region; F: Anterior end of reproductive system showing arrangement of oocytes; G: Vulval region showing post-vulval uterine sac. Male (A–B), A: Anterior region; B: Tail region showing spicule.

Relationships. The *Aphelenchoides* specimens from Telperion were compared with the following species that are grouped together in the key produced by Shahina (1996). All have a terminal tail outgrowth that is not spine- or star-shaped and four incisures in the lateral field. Females of *A. editocaputis* Shavrov, 1967 and *Aphelenchoides vaughani* Maslen, 1979 differ from our specimen in having shorter body (270–320 µm, and 360–430 µm, respectively, compared to 526 µm in our *A. lichenicola*). The following species are larger in body length and have different tail shapes: *A.hamatus* Thorne & Malek, 1968 (1.00 mm, tail with digitate, centrally located hyaline

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mucro) and *A. kungradensis* Karimova, 1957 (650 µm, double mucro on tail tip) compared to 526 µm in our *A. lichenicola*. The following species have a more posterior vulva (V = 70% in *A. lichenicola*) and, in cases where males were found, larger spicules (spicule = 14 µm in *A. lichenicola*): *A. franklini* Singh, 1969 (V = 79%, spicule = 23 µm), *A. lanceolatus* Tandon & Singh, 1974 (V = 71–73%, spicule = 24 µm) and *A. rarus* Eroshenko, 1968 (V = 72–74%, male unknown).

A. spaskii Eroshenko, 1968 is morphologically the most similar, but differs in tail shape (one long digitate mucro).Other closely related species based on morphology are *A. parietinus* (Bastian, 1865) Steiner, 1932 and *A. saprophilus* Franklin, 1957. *Aphelenchoides parientinus* can be differentiated by a longer stylet (11–13) µm vs. 10 µm in *A. lichenicola*) and different tail shape (digitate mucro in *A. parientinus)*. *Aphelenchoides saprophilus* differs in tail shape (digitate mucro in, *A. saprophilus)* spicule size (23 µm vs. 14 µm in *A. lichenicola*) and *c'* value (3.4 vs. 2.9 in *A. lichenicola*).

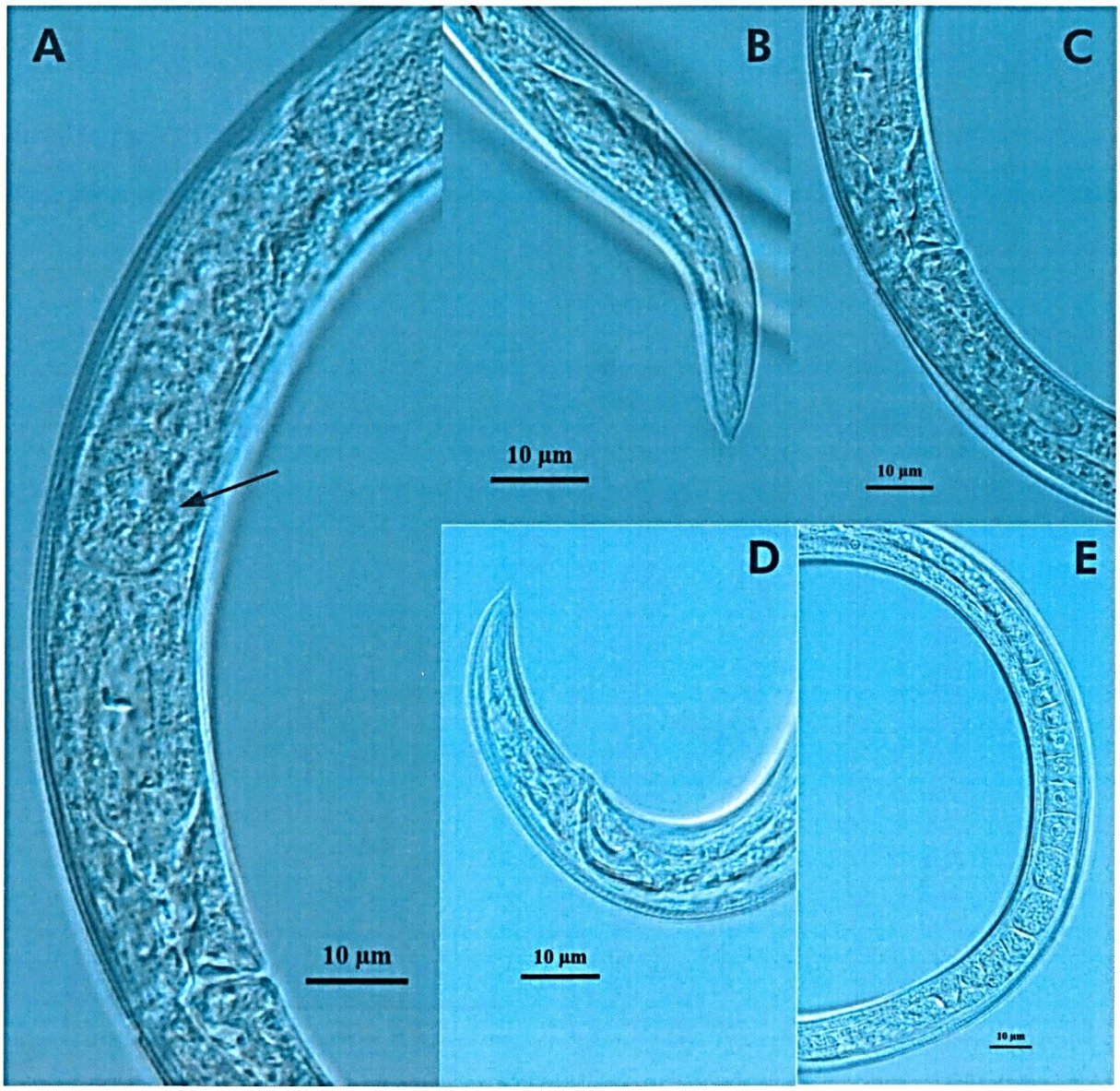


FIGURE 2. *Aphelenchoides lichenicola.* Female (A–C & E), A: Genital tract with arrow indicating spermatheca with sperm cells; B: Tail region; C: Vulval region showing post-vulval uterine sac; E: Anterior end of reproductive system showing arrangement of oocytes; Male, D: Tail region showing spicule.

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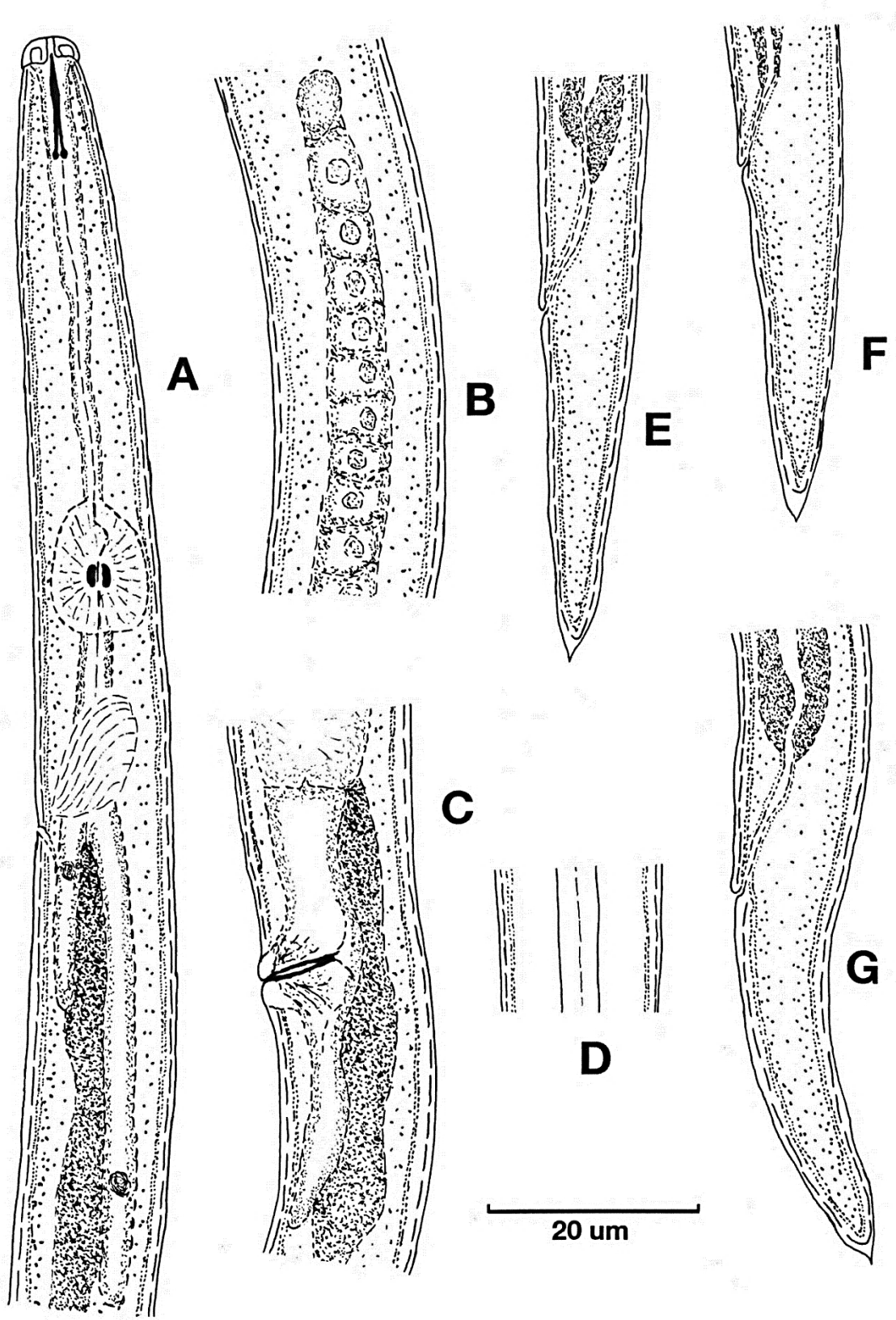


FIGURE 3. *Aphelenchoides rutgersi*, female. A: Anterior region; B: Anterior end of reproductive system showing arrangement of oocytes C: Vulval region showing post-vulval uterine sac D: Lateral field at mid-body; E–G: Tail region.

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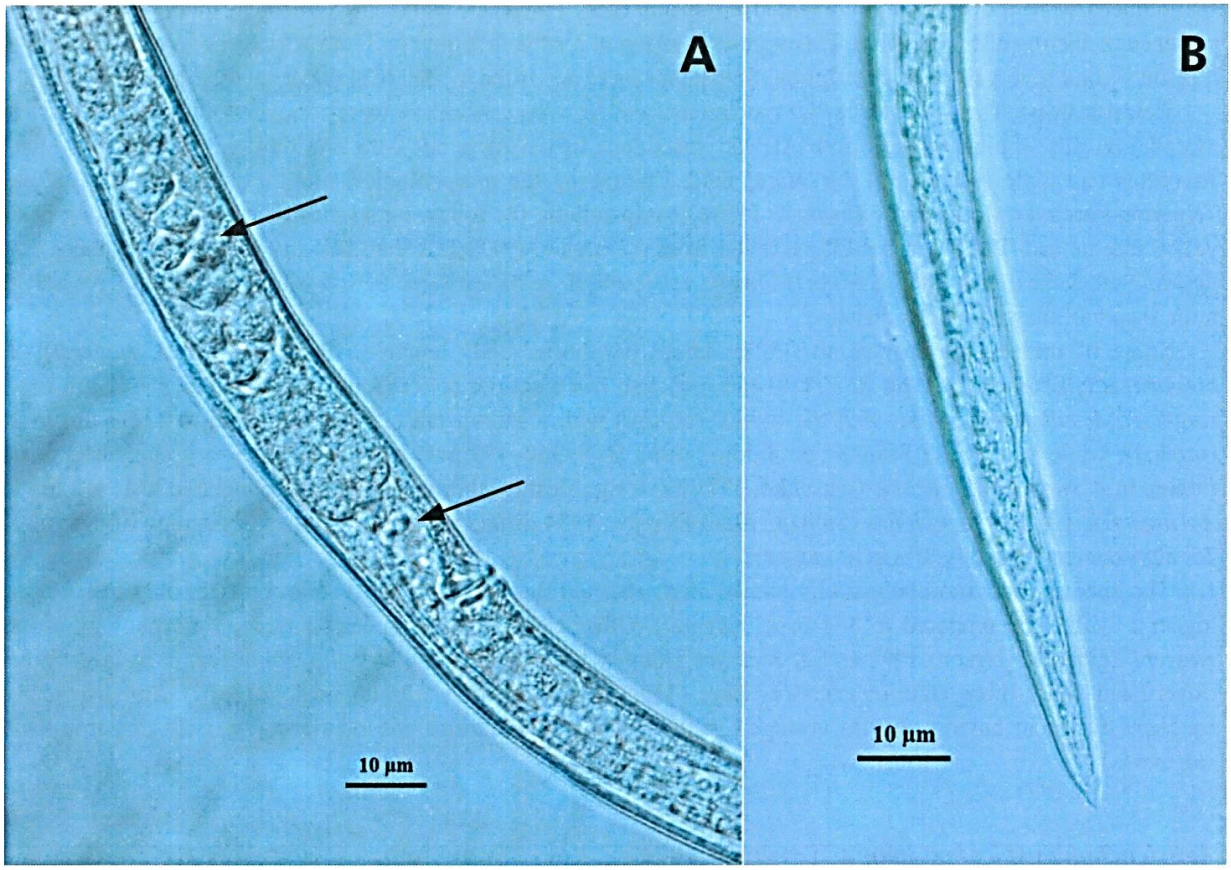


FIGURE 4. *Aphelenchoides rutgersi*, female. A: Vulval region showing spermatheca with arrows indicating sperm cells; and post-vulval uterine sac; B: Tail region.

Aphelenchoides rutgersi Hooper & Myers, 1971
(Fig. 3 & 4)

Aphelenchoides rutgersi was originally collected by W. Feder from around citrus roots Orlando, Florida, U.S.A and described from specimens cultured on *Setophoma terrestris* (H.N. Hansen) Gruyter, Aveskamp & Verkley or *Botrytis cinerea* Pers. (Hooper & Myers 1971). This species was then again found in the rhizosphere of tobacco plants in Bulgaria (Katalan-Gateva & Budurova, 1976).

Measurements. See Table 4.

Description. *Female:* (n = 3) Habitus slightly curved ventrally. Cuticle finely annulated, annule width about 1 µm at mid-body. Lateral field 3–4 µm wide marked with three incisures (Fig. 3D). Head slightly offset (Fig. 3A). Lip region 2–4 µm high and 3–4 µm wide. Stylet 11–12 µm long with slight basal thickenings. Median bulb spherical with centrally located valve (Fig. 3A). Nerve ring situated 66–68 µm from anterior end, less than one corresponding body width from base of median bulb. Excretory pore 75–76 µm from anterior end, opposite nerve ring (Fig. 3A). Ovary outstretched with a single row of oocytes (Fig. 3B). Spermatheca elongate, containing sperm cells (Fig. 4A). Vulval lips slightly protruding with the vagina occupying about half the corresponding body width, curving anteriorly (Fig. 3C). Post-vulval uterine sac 37–43 µm in length, about 2.5 times vulval body width and shorter than half vulva-anus distance (Fig. 3C). Tail elongate conoid, 33–37 µm long. Terminus bearing a short, slightly ventrally-located mucro (Fig. 3E–G & 4B).

Male: Not found.

Discussion. The female specimen of the *Aphelenchoides* sp. found associated with the seeds of *Aristida canescens* collected from the Telperion Nature Reserve were identified as *A. rutgersi* based on tail shape and morphological characteristics matching those of Hooper & Myers (1971). There were only the following

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differences: Spermatheca and sperm cells observed in South African specimen. In the original description, spermatheca were indistinct and contained no sperm cells. However, males are present in this species and the presence of sperm cells is possible. This species is reported for the first time in South Africa and to our knowledge, it is the second report since its description. Specimens were deposited in the NCN under accession number 50541.

Relationships. The *Aphelenchoides* specimens from *Aristida cansescens* were compared with species that are morphologically similar to the South African specimens in having a tail with one or two terminal mucronate structures and three incisures in the lateral field. Comparing the morphological and morphometric data of our Telperion specimens to those in Shahina's (1996) compendium, the following species with a relatively long post-vulval uterine sac (PUS) can be differentiated from *A. rutgersi* (PUS less than half the vulva-anus distance): *A. agarici* Seth & Sharma, 1986, *A. minoris* Ebsary, 1991, and *A. myceliophagus* Seth & Sharma, 1986 all have a PUS more than half the vulva-anus distance.

Some of the smaller species also differ based on shorter body length (542–582 µm in *A. rutgersi*): *A. breviuterallis* Eroshenko, 1968 (L = 410–460 µm) also has a shorter stylet (8 µm compared to 11–12 µm in *A. rutgersi*); *A. cibolensis* Riffle, 1970 (L = 360–460 µm) with a shorter tail (27 µm compared to 33-37 µm in *A. rutgersi*); while *A. trivialis* Franklin & Siddiqi, 1963 (L = 360–490 µm) has a reflexed ovary compared to no flexure in *A. rutgersi*. *A. curiolis* Gritsenko, 1971 has a significantly shorter stylet (6 µm compared to 11–12 µm in *A. rutgersi*). *A. chamelocephalus* (Steiner, 1926) Filipjev, 1934 differs in the more posterior position of the vulva: 75–80% compared to 69–70% in *A. rutgersi*.

The species most morphologically similar is *A. swarupi* Seth & Sharma, 1986 and differs only in having a higher *c'* value (5 compared to 3–4 in *A. rutgersi*). Other closely related species based on morphology are *A. eltayebi* Zeidan & Geraert, 1991 and *A. sacchari* Hooper, 1958. *Aphelenchoides eltayebi* differs in having a more offset head and a more anterior excretory pore (44µm from anterior vs. 76µm in *A. rutgersi*). *Aphelenchoides sacchari* differs in having oocytes arranged in two rows at the anterior end of the ovary (only one row in *A. rutgersi*).

Aphelenchoides spicomucronatus Truskova, 1973

(Fig. 5 & 6)

Aphelenchoides spicomucronatus was first described from the roots of *Glycine hispida* (Moench) Maxim. (Truskova, 1973). However, only three females were examined in describing the species. It was reported for the second time by Rashid *et al.* (1986) from soil samples collected in Brazil. To date these are the only reports of this species.

Measurements. See table 4.

Description. *Female* (n = 1): Body slender, habitus slightly curved ventrally. Cuticle finely annulated, annule width approximately 1 µm at mid-body. Lateral field marked with four incisures (Fig. 5B). Head slightly offset. Lip region flattened anteriorly (Fig. 5A) 3 µm high and 6 µm broad. Stylet 11 µm long with distinct basal swellings. Median bulb spherical in shape with prominent centrally located valves (Fig. 5A). Nerve ring situated 74 µm from anterior end, less than one corresponding body width from base of median bulb. Excretory pore 85 µm from anterior end, opposite posterior end of nerve ring (Fig. 5A). Ovary outstretched with oocytes arranged in a single row (Fig. 5D & 6A). Spermatheca containing spherical sperm cells (Fig. 6B). Sperm cells also observed in uterus (Fig. 6B). Vulval lips slightly protruding with the vagina occupying less than half the corresponding body width, curving anteriorly. Post-vulval uterine sac 57 µm in length, about three times vulval body width and shorter than half vulva-anus distance (Fig. 5E & 6D). Tail conical, 40 µm long with a centrally located mucro (Fig. 5C & 6C). Anus distinct, with the anterior lip protruding slightly.

Male: Not found.

Discussion. The female *Aphelenchoides* specimen recovered from *Eragrostis gummiflua* samples was identified as *A. spicomucronatus* based on the characteristic tail mucro shape and presence of four lateral field incisures. Measurements and morphological details match those of Truskova (1973) and Rashid et al. (1986). This specimen differs only in having the excretory pore situated opposite the posterior end of the nerve ring. In both published accounts of *A. spicomucronatus*, the excretory pore is situated between the median bulb and the nerve ring. However, Rashid *et al.* (1986) did state that the excretory pore position varied from opposite the median bulb to one median bulb length posterior to it.

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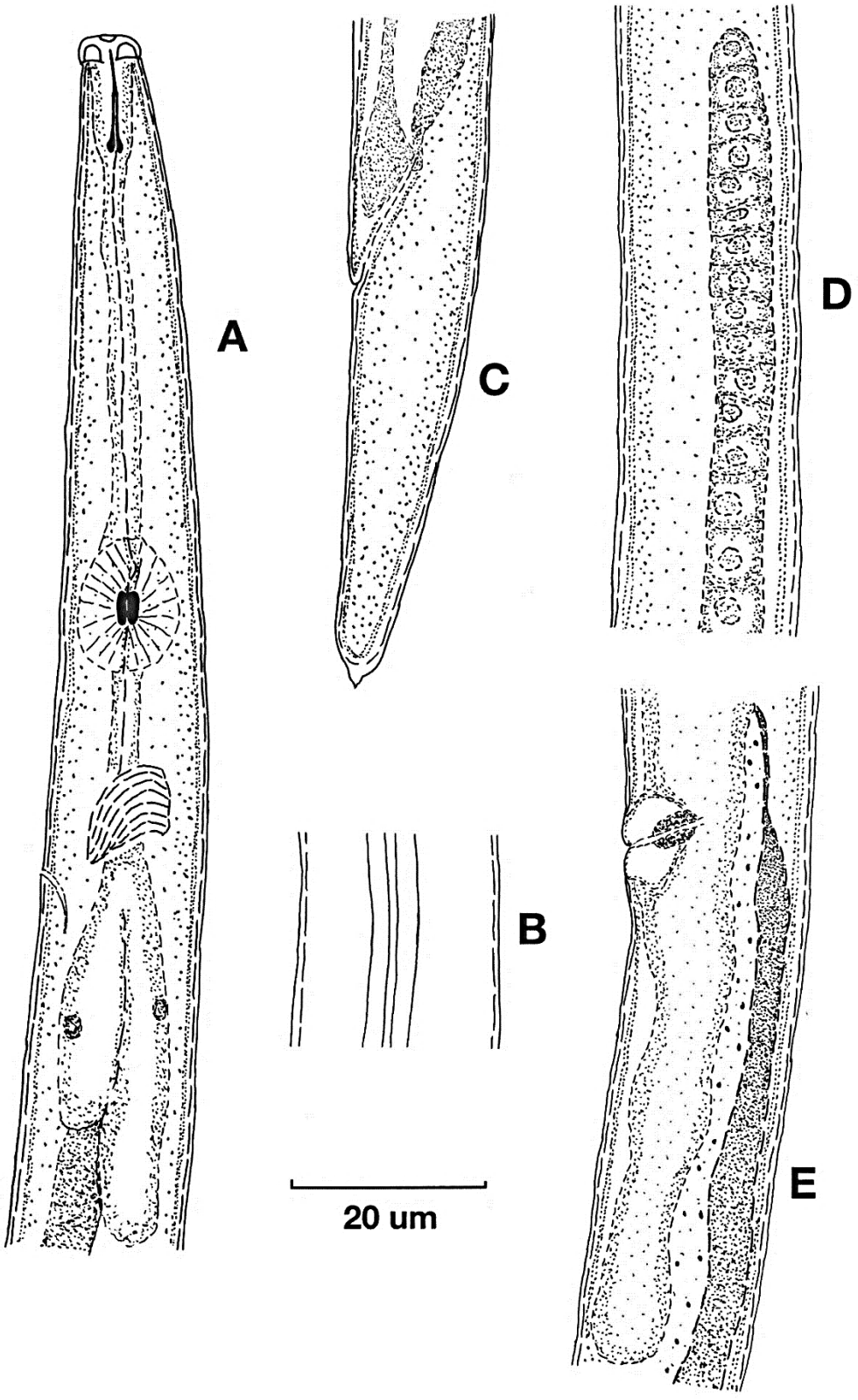


FIGURE 5. *Aphelenchoides spicomucronatus* female. A: Anterior region; B: Lateral field at mid-body; C: Tail region; D: Anterior end of reproductive system showing arrangement of oocytes; E: Vulval region showing post-vulval uterine sac.

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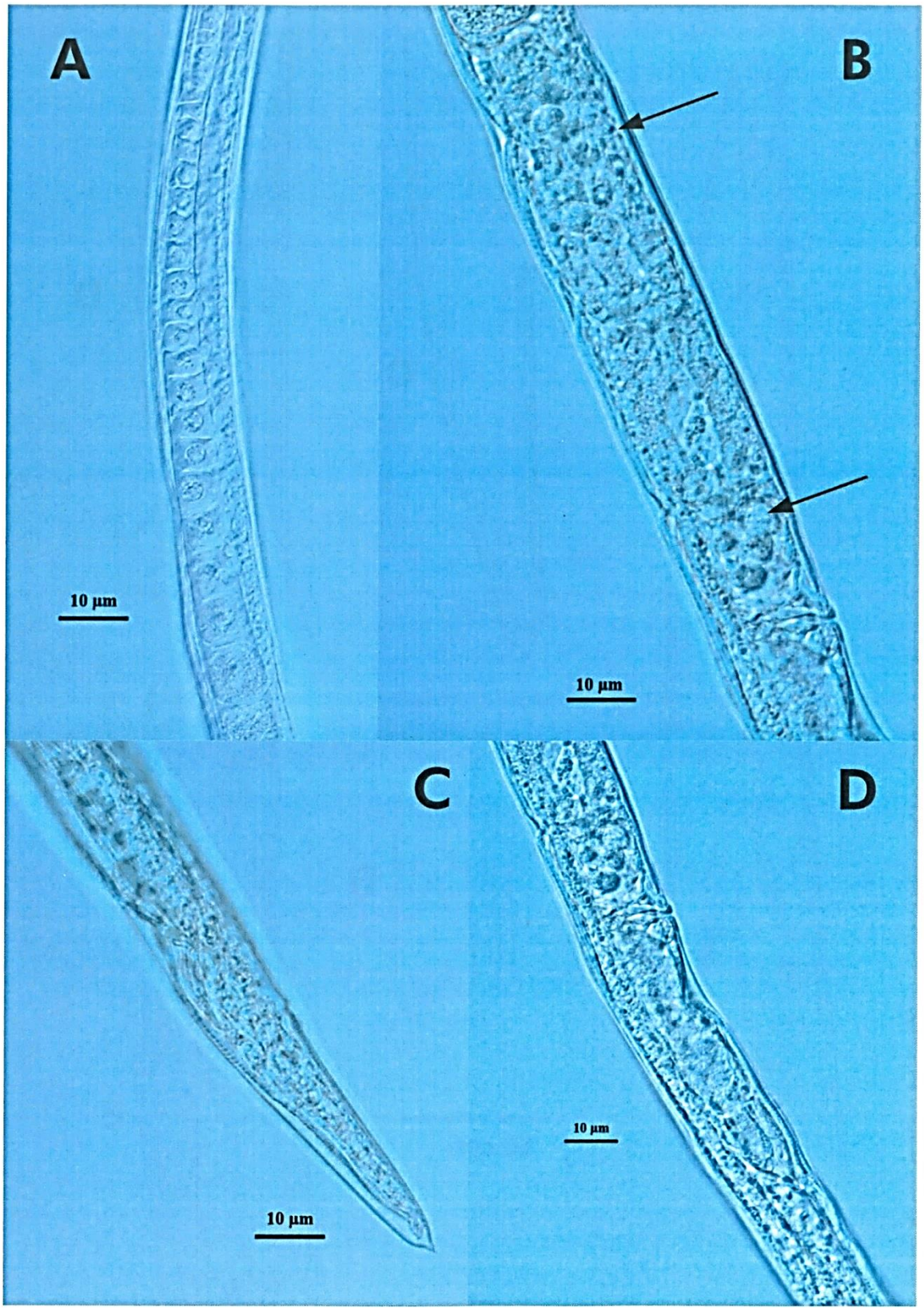


FIGURE 6. *Aphelenchoides spicomucronatus* female. A: Anterior end of reproductive system showing arrangement of oocytes; B: Genital tract with arrow indicating spermatheca with sperm cells; C: Tail region; D: Vulval region showing post-vulval uterine sac.

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Other discrepancies were noted in the above-mentioned descriptions. In Rashid et al. (1986) the spermathecae of 19 females were reported to be empty. However, the original description by Truskova (1973) indicated that sperm cells were observed in the spermatheca and PUS, in all three specimens studied. Sperm cells were seen in the spermatheca of our Telperion specimen. In the original description, the lateral field was described as not visible, but Rashid et al. (1986) observed four incisures in the lateral field in cross sections. Our South African specimen has a lateral field with four incisures. This species is reported for the first time from South Africa. The specimen (accession number 50544) was deposited in the NCN.

Relationships. The *Aphelenchoides* specimen from Telperion was compared to those species with a tail with one (or sometimes two) terminal mucronate structures and four incisures in the lateral field (Shahina 1996). *Aphelenchoides haguei* Maslen, 1979 and *A. tuzeti* B'Chir, 1979 both have longer stylets - 13 µm and 14 µm, respectively - compared to 11 µm in *A. spicomucronatus*. The following species have a slightly more posterior vulva position (vs. 69% in the South African species): *A. absari* Husain & Khan, 1967 (69–77%), *A. brassicae* Edward & Misra, 1969 (68–74%), *A. cyrtus* Paesler, 1959 (70–73%), and *A. echinocaudatus* Haque, 1968 (72%). *A. parasaprophilus* Sanwal, 1965 (680–750 µm), and *A. subtenius* (Cobb, 1926) Steiner & Buhrer, 1932 (870–1150 µm) are larger (L = 669 µm for *A. spicomucronatus)* and can also be differentiated by tail shape. *A. eradicitus* Eroshenko, 1968 (300–310 µm), *A. parabicaudatus* Shavrov, 1967 (310-350 µm), and *A. platycephalus* Eroshenko, 1968 (240-270 µm) are much smaller species. *A. eradicitus* also has a shorter stylet (10 µm compared to 11 µm in *A. spicomucronatus*) and more anterior vulva (V = 64% vs. 69%). *A. parabicaudatus* can be differentiated by its shorter stylet (8 µm vs. 11 µm) and bifurcate tail tip. *Aphelenchoides platycephalus* has a more anterior nerve ring and a longer mucro.

Aphelenchoides richardsoni Grewal, Siddiqi & Atkey, 1992 is the most morphologically similar species in having more developed stylet knobs than the South African specimens. Other closely related species based on morphology are *A. daubichaensis* Eroshenko, 1968, and *A. tsalolikhini* Ryss, 1993. *A. daubichaensis* differs in tail shape, with a more centrally located terminal mucro and a shorter tail length (29–31 µm compared to 40 µm in *A. spicomucronatus*). *Aphelenchoides tsalolikhini* also has a longer tail (*c'* = 4.6–6.2 compared to 3.3 in *A. spicomucronatus*), and different tail shape (long, thorn-like, ventrally situated mucro).

Panagrolaimus leperisini **Massey, 1974**
(Fig. 7)

Panagrolaimus. Leperisini was first described in the U.S.A. as an associate of the bark beetle *Leperisinus aculeatus* (Say) in green ash (*Fraxinus pennsylvanica* Marshall) (Massey 1974). This is the only known report of this species.

Measurements. See table 5.

Description. *Female* (n = 2): Habitus slightly curved ventrally. Cuticle moderately annulated, annule width 2 µm at midbody. Fine longitudinal striations. Lateral field with three incisures, about one-seventh of corresponding body width. Lips rounded, each bearing one small seta (Fig. 7A–B). Six labial and four cephalic setae. Stoma 11–12 µm in length. Cheilostom distinct, 3 µm in length, gymnostom 4–5 µm and stegostom 3 µm long. Meso-, meta- and telorhabdions fused (Fig. 7A–B). Metarhabdions bearing prominent dorsal tooth (Fig. 7A–B). Corpus twice the isthmus length (Fig. 7A–B). Corpus-isthmus junction distinct, isthmus narrower than corpus (Fig. 7A–B). Nerve ring situated at mid-isthmus, 124–130 µm from anterior end. Basal bulb ovoid with well-developed valves (Fig. 7A–B). Excretory pore slightly posterior to nerve ring, passing through hemizonid (Fig. 7A–B). Cardia distinct, 6 µm in length. Vulva at 56%, vulval lips protruding (Fig. 7C). Vagina one-third of corresponding body width, directed anteriorly. Reproductive system monodelphic-prodelphic (Fig. 7C). Ovary reflexed. Post-vulval uterine sac short, less than half the corresponding body width (Fig. 7C). Anus and rectum distinct with prominent anal glands (Fig. 7D–E). Phasmid situated at 65–73 % of the tail length (Fig. 7D–E). Tail conoid to acute terminus, 47–55 µm long.

Male: (n = 1) Pharyngeal morphology as in female. Body more ventrally curved at posterior end. Testis single, reflexed dorsally anteriorly. Spicules 29 µm in length, manubrium spheroid and lamina curved ventrally (Fig. 7F). Gubernaculum less than half the length of spicules, curved ventrally. Five pairs of caudal papillae: two preanal ventrosubmedian, 1 subdorsal and 2 postanal ventrosubmedian. Tail conoid to acute terminus (Fig. 7F).

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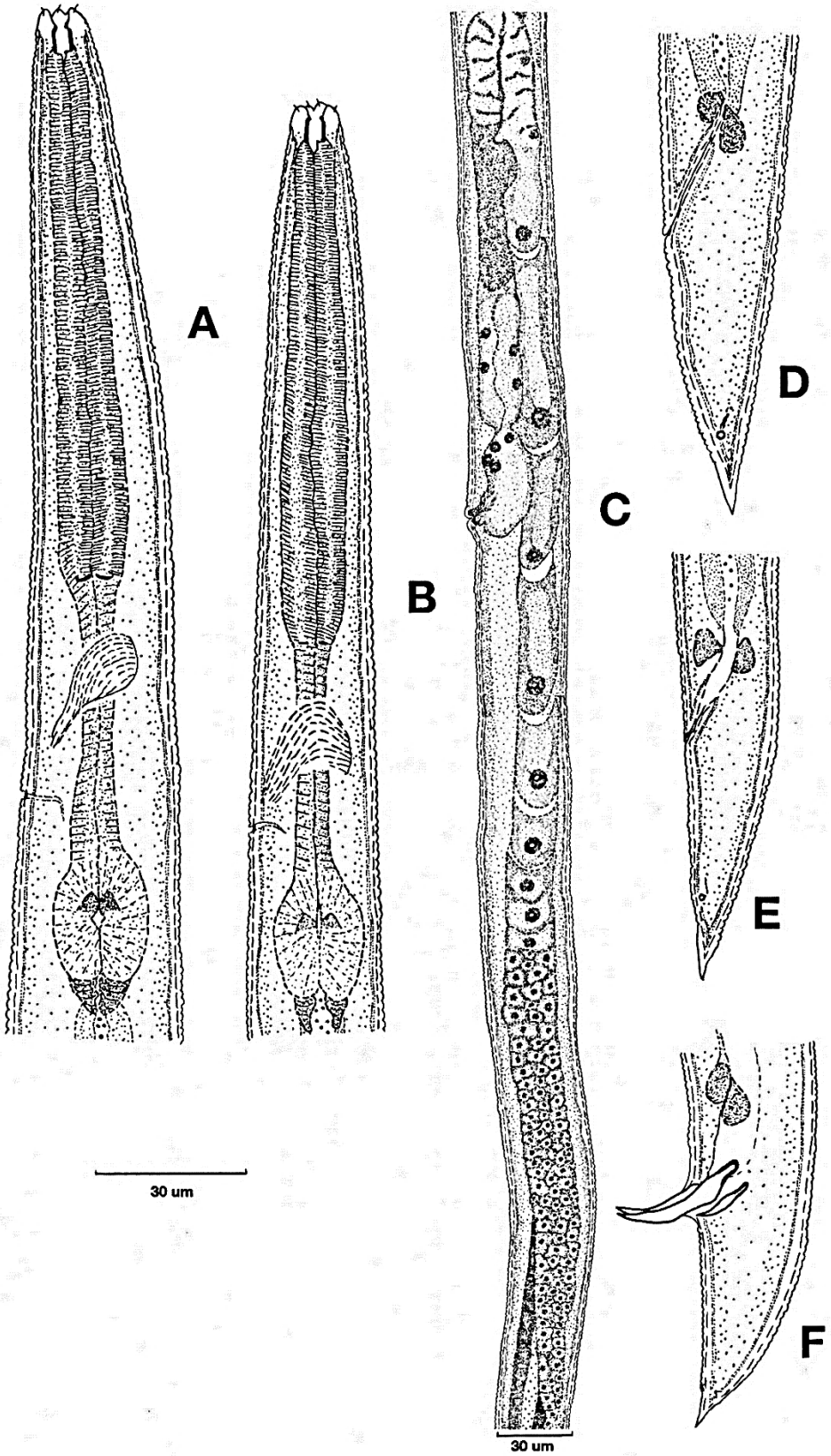


FIGURE 7. *Panagrolaimus leperisini*, female (A–E). A–B: Anterior region; C: Reproductive system; D-E: Tail region. Male, F: Tail region showing spicules and gubernaculum.

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TABLE 5. Measurements (in µm) of *Panagrolaimus leperisini* Massey, 1974. - indicates structures that were not visible or not present in the specimen.

	Panagrolaimus leperisini		
Locality	MP126	MP124	MP125
n	1♀	1♀	1♂
L	1049	892	963
a	31.7	29.2	25.4
b	5.4	5.0	5.7
c	19.2	19.1	18.7
c'	2.6	2.5	1.7
Annule width	2	2	1
Lateral field width	5	4	-
Lateral field width / corresponding Body width	7.0	7.3	-
Stoma length	12	11	9
Cheilostom	3	3	3
Gymnostom	5	4	4
Stegostom	3	3	3
Nerve ring from anterior	130	124	132
Nerve ring length	9	8	-
Pharyngeal corpus Length	114	107	102
Isthmus length	56	49	44
Corpus/isthmus	2.0	2.2	2.3
Pharynx length	194	179	168
Excretory pore from anterior	158	144	-
Basal bulb width	17	17	21
Basal bulb length	23	23	23
Cardia	6	6	7
Body diameter : Midbody	33	31	38
Anus / Cloaca	21	18	31
Vulva	35	34	-
Vagina length	10	-	-
Vagina length / corresponding body width	3.53	-	-
V-A/T	7.4	7.4	-
V (µm from anterior)	590	498	-
V %	56	56	-
Post-vulval uterine sac (PUS)	11	-	-
PUS/midbody diameter	0.3	-	-
Anterior genital branch length	745	611	-
G1%	71	68	-
Spicule length	-	-	29
Gubernaculum Length	-	-	11
Testis length	-	-	540
Tail length	55	47	52
Rectum	26	24	-
Rectum / Width at anus	1.2	1.3	-
Phasmid, % of tail length	73	65	-

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Discussion. *Panagrolaimus* specimens recovered from *B. burchellii, C. longus* and *P. patens* samples collected in the Telperion Nature Reserve were identified as *P. leperisini* based on the characteristic shape of the lips and presence of a dorsal tooth on each metarhabdion (Fig. 7A–B). Identification was aided by the key provided by Abolafia & Peña-Santiago (2006) and measurements and morphological features were compared with those in Massey (1974). This species is reported for the first time in South Africa. Only two species have been previously reported from South Africa: *P. magnivulvatus* and *P. subelongatus*. Specimens were deposited in the NCN with accession numbers 50542, 50544, and 50545.

Relationships. Closely related species, based on morphology, are *P. goodeyi* Rühm, 1956 and *P. subelongatus* (Cobb, 1914) Thorne, 1937. *Panagrolaimus subelongatus* differs in the absence of a dorsal tooth on the metarabdions, head with three lips and six pairs of caudal papillae in males. *P. goodeyi* differs in having seven pairs of caudal papillae in males; longer tails (c = 16.2–18.4, in both males and females vs. c>19 in *P. leperisini*) and a more anterior phasmid (54 % of tail length vs. 65–73 % in *P. leperisini*).

Conclusion

This survey produced four new species records for South Africa, as well as six new species records associated with grass inflorescences. Knowledge of the nematodes associated with natural vegetation in South Africa has essentially been limited to those found in soil and root samples. Our study indicates that surveys need to include other types of samples, including grass inflorescences, to give a more complete estimate of nematode diversity within a given region. This survey adds to our knowledge of the Grassland biome in South Africa. Given the importance of natural habitats and diversity in these ecosystems, as well as the lack of information on nematodes found in natural grass inflorescences, there is a definite need for more surveys of nematode diversity in grassland habitats throughout South Africa.

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CHAPTER 6: ARTICLE 5

Free-living freshwater nematodes from the Telperion Nature Reserve (Mpumalanga, South Africa) with the description of three new and five known species (Nematoda)

Prepared for submission to **Zootaxa**

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6.1 Abstract

A nematode survey conducted in the Telperion Nature Reserve (Mpumalanga, South Africa) resulted in three new species (belonging to the genera *Aphanolaimus, Ironus* and *Makatinus*) being described; two species reported for the first time from South Africa (*Chronogaster aspinata* and *Paraphanolaimus behningi* and); and three others (*Chronogaster africana, Eutobrilus annetteae* and *Neotobrilus ampiei*) known to occur in South Africa but for the first time described with scanning electron microscope graphs. *Aphanolaimus strilliae* n. sp. is characterised by a body length of 1240-1613 µm, more than 800 body annules, lateral field originating between the first and second lateral body pore at the 34th-46th annule, first lateral body pore located at the 25th-35th annule, vagina V-shaped and bent anteriorly, 142-195 µm long uterus and 165-207 µm long tail with offste spinneret. This species is ovoviviparous and no males were found. *Ironus telperionensis* n. sp. is characterised by a long (1456-1864 µm) and slender body (50 times longer than width at mid-body), long stoma (87-129 µm), and a 212-257 µm long tail with a ventral curl at the tip. *Makatinus africanus* n. sp. is characterised by a large, thick body (3228-4128 µm long, a = 30-39), slightly set off lip region with amalgamated lips; 31-34 µm long odontostyle; long tongue-shaped cardia, and stout, short tail (30-43 µm long) with a small peg / digitate extension and males absent. A total of 48 genera belonging to 38 families were found during the survey and are listed in the present paper.

Keywords: Aphanolaimus, Chronogaster africana, Chronogaster aspinata, Eutobrilus annetteae, Ironus, Makatinus, Neotobrilus ampiei, new records, new species, *Paraphanolaimus behningi*, SEM.

6.2 Introduction

Approximately 71 % of the earth's surface is covered in water, of which only 3 % is freshwater (Danovaro *et al.* 2009). Nematodes are the most abundant and possibly the most diverse Metazoa in these freshwater systems and present in all freshwater environments (Eyualem-Abebe *et al.* 2001; Eyualem-Abebe *et al.* 2008). However, the taxonomy and ecology of this group of nematodes have received the least amount of attention globally compared to those of marine and terrestrial nematodes (Traunspurger 2000; Eyualem-Abebe *et al.* 2008). Studies on nematodes in freshwater systems in South Africa dates back to work done by Victoria Coetzee between 1965 and 1968 (Coetzee 1965; 1966; 1967a; 1967b; 1968a; 1968b) while Prof Juan Heyns and collaborators proceeded with such studies between 1972 and 2002. In 1984 a review of the free-living freshwater nematodes of Africa was published (Jacobs 1984). According to Jacobs (1984) 91 nematode species were reported from South Africa In 2002, Annermarié Avenant-Oldewage published posthumously a checklist of free-living nematodes in Southern Africa compiled by Heyns (Heyns 2002a) that served as a supplement to a key published by Heyns in 2002 (Heyns 2002b). More recent work in South Africa on freshwater nematodes includes that of Van den Berg (2007; 2009), Durand *et al.* (2012), Mobara (2014) and Du Preez *et al.* (2015). During a recent nematode survey (2015-2016) conducted in the Telperion Nature Reserve (Mpumalanga Province), novel and updated information was generated for South African free-living species occurring in freshwater sources. This includes three new species belonging to the genera *Aphanolaimus* De Man, 1880, *Ironus* Bastian, 1865 and *Makatinus* Heyns, 1965. Moreover, *Paraphanolaimus behningi* Micoletzky, 1923 and *Chronogaster aspinata* Raski & Maggenti, 1984 are first reports for South Africa and are described. *Chronogaster africana* Heyns & Coomans, 1980, *Eutobrilus annetteae* (Joubert & Heyns, 1979) Tsalolikhin, 1981 and *Neotobrilus ampiei* (Joubert & Heyns, 1979) Tsalolikhin, 1981 were also found and have been studied for the first time with the scanning electron microscope (SEM).

Members of the genus *Aphanolaimus* are mainly found in freshwater environments. However, some species have been reported from brackish water bodies, moist soils (Andrássy 2005) moss and litter (Holovachov & De Ley 2006). This genus is found on all continents except Antarctica (Holovachov & De Ley 2006) and consists of 34 nominal species of which, according to Andrássy (2005), 25 are regarded as valid. Three species are ovoviviparous, namely *Aphanolaimus aymarae* De Waele & Coomans, 1993, *Aphanolaimus spiriferus* Cobb, 1914 and *Aphanolaimus viviparus* Plotnikov, 1899. Although specimens of *Aphanolaimus* have been reported from the Free State, KwaZulu-Natal and Western Cape provinces of South Africa [Mobara 2014; National Collection of Nematodes (NCN)], none have been identified to species level

The genus *Paraphanolaimus* Micoletzky, 1923 has a global distribution and contains eight valid species (Holovachov & De Ley 2006). Members of this genus have mostly been reported from freshwater, but also from moist soil and marine environments and have been reported from all continents excluding Antarctica (Andrássy 2005; Holovachov & De Ley 2006). According to Holovachov & Sturhan (2003), the two most widely distributed species are *P. anisitsi* (Daday, 1905) Andrássy, 1968 and *P. behningi* Micoletzky, 1923. This is the first report of the genus in South Africa. *Paraphanolaimus behningi* is the type species of the genus and occurs in various freshwater environments, which include both stagnant and running waters. This species has been reported from Europe, Asia, Africa and South America (Andrássy 2005). In Africa, this species has only been reported from Ethiopia and is now reported for the first time in South Africa.

Members of the genus *Chronogaster* Cobb, 1913 are aquatic nematodes and have been reported from freshwater environments, thermal springs and brackish water. However, several species have also been found in terrestrial environments (Andrássy 2005). The genus contains 48 valid species of which four have been reported in South Africa, namely *C. africana* Heyns & Coomans, 1980 from the Eastern Cape, Gauteng, Mpumalanga and North West provinces*; C. glandifera* Heyns & Coomans, 1980 and

C. longicauda Heyns & Coomans, 1980 from the Gauteng Province, and *C. multispinata* Heyns & Coomans, 1980 from Limpopo Province (Heyns & Coomans 1980; Dassonville 1981; Botha & Heyns 1992; Heyns 2002a). Diagnostic keys and a compendium to the species were provided by Heyns & Coomans (1980; 1983) and Raski & Maggenti (1984).

The genus *Makatinus* is a group of large, predacious nematodes with a worldwide distribution. Despite having a cosmopolitan distribution, species of this genus are rare with only two of the eight species reported from outside their type localities, namely *M. aquaticus* Jiménez-Guirado, 1994 and *M. crassiformis* (Kreis, 1924) Andrássy, 1986 (Peña-Santiago & Varela 2017). The genus was proposed by Heyns in 1965 to accommodate two South African species, *M. capensis* Heyns, 1965 and *M. punctatus* Heyns, 1965, that were described from soils from Bathurst (Eastern Cape Province) and the Makatini flats (KwaZulu-Natal Province), respectively (Heyns 1965).

Members of the genus *Ironus* are considered freshwater species with only a few species reported from other environments such as brackish water, soil and mosses (Smol & Coomans 2006). *Ironus ignavus* Bastian, 1865 is the most widely reported species, from both from freshwater and brackish water systems (Eyualem-Abebe & Coomans 1997; Smol & Coomans 2006). According to Heyns (1971), *Ironus* is commonly found in South Africa in various habitats and seven species have been reported or described from this country i.e. *I. crassatus* Argo & Heyns, 1972, *I. dentifurcatus* Argo & Heyns, 1972, *I. ernsti* Argo & Heyns, 1972 and *I. lautus* Argo & Heyns, 1972. *Ironus ignavus*, *I. longicaudatus* De Man, 1884 were reported by Botha & Heyns (1992; 1993) and *I. tenuicaudatus* De Man, 1876, was reported by Andrássy (1970). A diagnostic key and compendium of the genus was provided by Andrássy (2011).

The family Tobrilidae De Coninck, 1965 is classified as a true freshwater family as most of the species are aquatic (Andrássy 2007). In South Africa, the following species of this family have been reported from various aquatic habitats: *Epitobrilus stefanskii* (Micoletzky, 1925) Andrássy, 2007 from the Free State, Gauteng and Mpumalanga provinces*; Eutobrilus annetteae* (Joubert & Heyns, 1979) Tsalolikhin, 1981 from the Western Cape and Gauteng provinces; *E. heptapapillatus* (Joubert & Heyns, 1979) Tsalolikhin, 1981 from the Eastern Cape, Gauteng, KwaZulu-Natal and Western Cape provinces; *Macrotobrilus elephas* (Andrássy, 1964) Tsalolikhin, 1981 from the Eastern Cape Province*; Neotobrilus ampiei* (Joubert & Heyns, 1979) Tsalolikhin, 1981 from the Eastern Cape and North West provinces*; N. breviductus* (Loof & Riemann, 1976) Tsalolikhin, 1981 from the Free-State and Gauteng provinces*; N. diversipapillatus* (Daday, 1905) Tsalolikhin, 1981 from the Free State, Gauteng, North West and Mpumalanga provinces*; N. floridensis* (Joubert & Heyns, 1979) Tsalolikhin, 1981 from the Gauteng and Mpumalanga provinces; and *N. longus* (Leidy, 1852) Tsalolikhin, 1981 from the Gauteng and Western Cape provinces (Andrássy 1970; Argo & Heyns 1973; Joubert & Heyns 1979; Dassonville 1981; Swart & Heyns 1988; Botha & Heyns 1992; Heyns 2002a). However, *N. floridensis* is considered a *species inquirenda* (Tsalolikhin & Shoshin 2009).

Due to limited and fragmented knowledge of free-living freshwater nematodes in South Africa, a recent nematode survey (2015-2016) was conducted in the Telperion Nature Reserve (Mpumalanga Province) to determine the diversity of freshwater nematode in the reserve and to update existing information.

6.3 Materials and methods

6.3.1 Site description and collection of samples.

The study was undertaken in the Telperion Nature Reserve, Mpumalanga, South Africa during 2015 - 2016. Telperion Nature Reserve is situated in the extreme north of the Grassland Biome in South Africa, classified as the Rand Highveld Grassland in the Mesic Highveld Grassland Bioregion (Mucina

et al. 2006). The Telperion Nature Reserve forms part of the farm Elandsfontein 493 JR, (-25.659860, 29.009210 and -25.713220, 29.033840, north to south; and -25.67240, 29.047030 and -25.695270, 28.971360, east to west) (Figure 6.1). The area lies on Ecca and Dwyka formations of the Waterberg and Karoo groups, which were formed during the Mokolian and Palaeozoic eras, respectively. Telperion Nature Reserve is bordered by the perennial Wilge River and contains three streams that originate from higher lying wetlands and sponge areas (Grobler 1999). Four water-sediment samples (2 l/sample) were collected from four water sites during four consecutive seasons, totalling 12 samples (Figure 6.2 and Table 6.1). Each sampling date was allocated a separate locality number. Sample MP00127 was, however, obtained after seasonal sampling. Samples were collected by scraping sediment at least 10 cm deep with a sediment collection cup and consisted of both water and sediment.

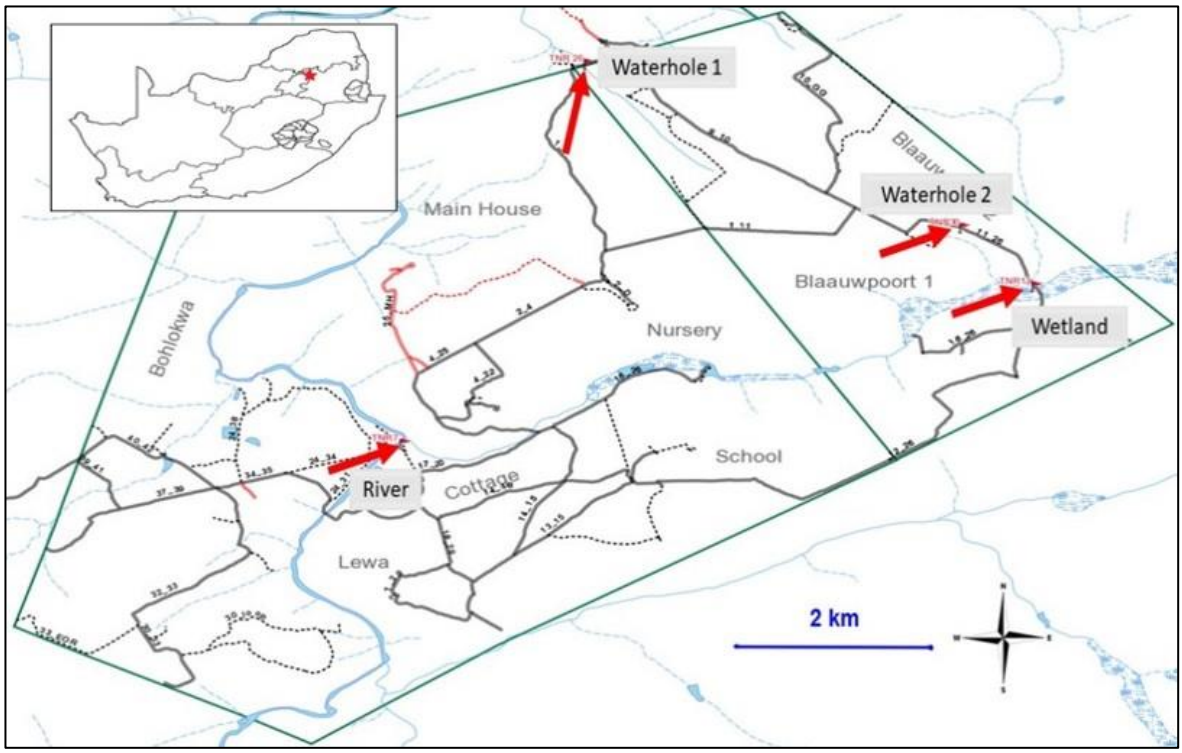


Figure 6.1: Telperion Nature Reserve, Mpumalanga, indicating the four study sites from which water and sediment samples were taken. Blue colour indicates water. The red star on the map of South Africa (top left) indicates the location of the Telperion Nature Reserve, Mpumalanga. (Shorthouse 2010).

6.3.2 Nematode extraction and preservation.

Nematodes were extracted from the 2l samples by using an adapted decanting and sieving method, followed by a sugar centrifugal flotation method as soon as possible after collection. The nematodes were then fixed in a heated 4 % formaldehyde + 1 % propionic acid (FPG) solution, dehydrated in a glycerine solution and mounted in glycerine on permanent glass slides using a wax ring method (Marais *et al.* 2017).

6.3.3 Classification and identification of species.

Measurements and drawings of the mounted specimens were made with an Olympus BX53F microscope and with a Nikon LABOPHOT-2 microscope equipped with dedicated drawing tubes. All measurements and identifications were made at 1000× magnification. Curved structures were measured along the median line. Morphometrics according to De Man (1884) were used in descriptions; standard morphometric calculations and terms were used throughout this paper. All measurements in the descriptions are given in micrometers (µm) except when stated otherwise, and in the form: mean ± standard deviation (range). All specimens (new species and specimens from known species) are deposited in the National Collection of Nematodes (NCN), Biosystematics Division, Agricultural Research Council (ARC) - Plant Health and Protection (PHP), Pretoria. Nematode classification was based on a synthesis of Maggenti *et al.* (1988) and Geraert (2011) for the Tylenchina; Kleynhans *et al.* (1996) for the Heteroderidae; Hunt (1993) for the Aphelenchida; Decraemer (1995) and Duarte *et al.* (2010) for the Trichodoridae; Andrássy (2009) for the Dorylaimida; and Andrássy (2005; 2007) for other free-living nematodes. Descriptions as given in Abolafia (2006), Bert & Borgonie (2006); Coomans & Eyualem-Abebe (2006), Decraemer & Smol (2006), Holovachov & De Ley (2006), Peña-Santiago (2006), Smol & Coomans (2006); Vinciquerra (2006), Zullini (2006), and Zullini & Peneva (2006) were also used to identify specimens.

6.3.4 Scanning Electron Microscopy (SEM) and Light Microscopy (LM) studies.

For SEM study, specimens were fixed, dehydrated in an ethanol series, critical point-dried, mounted on microscope stubs and coated with gold-palladium (Marais *et al.* 2017). Light micrographs of mounted specimens were taken with a Zeiss Axio Imager2 with an Axiocam MRC camera.

Table 6.1: Detailed description of the sites selected for the water-sediment samples taken from the Telperion Nature Reserve, Mpumalaga, South Africa.

Sample site	GPS coordinates		Description
	Longitude	Latitude	
River	-25.706140	28.990590	Bank of the Wilge River upstream from the point where a stream flowing from a wetland joins the river. Samples deposited in the NCN[1] with locality numbers: MP00008; MP00038; MP00068; MP00098.
Wetland	-25.687030	29.055690	Water from a wetland flows (in the summer rainfall season) through the reserve and joins the Wilge River at the border that separates Telperion Nature Reserve from the Ezemvelo Nature Reserve. Samples deposited in the NCN with locality numbers: MP00013; MP00043; MP00073; MP00103.
Waterhole 1	-25.660000	29.009570	A waterhole regularly used by animals, the source of the waterhole is not clear however, it might be from fountains in the koppie (referring to a small hill in an area with a generally flat topography) adjacent to the site. In summer rainfall seasons the water from this site also flows to join the Wilge River. Samples deposited in the NCN with locality numbers: MP00027; MP00057; MP00087; MP00117.
Waterhole 2	-25.679900	29.048240	The source of the water collected is probably from fountains in the koppie adjacent to the site. The water flows (in summer rainfall seasons), towards the wetland and collects in a hole at the site from which samples were collected. Samples deposited in the NCN with locality numbers: MP00031; MP00061; MP00091; MP00121; MP00127.

[1]NCN = National Collection of Nematodes, Nematology Unit, Biosystematics, Agricultural Research Council-Plant Health and Protection, Pretoria, South Africa

6.4 Results

A total of 48 genera belonging to 38 families were identified during the survey (Table 6.2). The most well represented class was Secernentia Von Linstow, 1905 (22 genera) followed by Penetrantia Andrássy, 1974 (14 genera) and finally Torquentia Andrássy, 1974 (12 genera). The order Tylenchida (Filipjev, 1934) Thorne, 1949 was the most prominent order with 12 representative genera. Seventeen species were identified (Table 6.2). The species of a number of specimens could not be determined because either not enough specimens of a species were found, or the quality of the fixed specimens were inadequate for species identification. Seven of the known species are new reports for freshwater in South Africa and two species, *C. aspinata* and *P. behningi*, are reported for the first time in the country (Table 6.2).

Table 6.2: Checklist of nematodes found at different water sites from Telperion Nature Reserve

Class Torquentia Andrássy, 1974
Family Aphanolaimidae Chitwood, 1936
Genus *Aphanolaimus* De Man, 1880 [a]
Aphanolaimus strilliae n. sp.
Genus *Paraphanolaimus* Micoletzky, 1923
Paraphanolaimus behningi Micoletzky, 1923 [ab]
Family Chromadoridae Filipjev, 1917
Genus *Punctodora* Filipjev, 1930 [bc]
Family Chronogasteridae Gagarin, 1975
Genus *Chronogaster* Cobb, 1913
Chronogaster africana Heyns & Coomans, 1980
Chronogaster aspinata Raski & Maggenti, 1984 [abc]
Chronogaster longicauda Heyns & Coomans, 1980
Family Cyatholaimidae Filipjev, 1918
Genus *Achromadora* Cobb, 1913
Family Leptolimidae Örley, 1880
Genus *Leptolaimus* De Man, 1876
Family Metateratocephalidae Ersenko, 1973
Genus *Eutertocephalus* Andrássy, 1958
Family Microlaimidae Micoletzky, 1922
Genus unidentified

[a] Species described in this chapter.
[b] New report for South Africa.
[c] New report for freshwater in South Africa.

Table 6.2 (continued): Checklist of nematodes found at different water sites from the Telperion Nature Reserve

Class Torquentia Andrássy, 1974
Family Monhysteridae De Man, 1876
Genus *Eumonhystera* Andrássy, 1981
Genus *Monhystera* Bastian, 1865
Genus *Monhystrella* Cobb, 1918
Genus *Tridentulus* Eyualem-Abebe & Coomans, 1995 [bc]
Family Plectidae Örley, 1880
Genus *Plectus* Bastian, 1865
Class Secernentia Von Linstow, 1905
Family Anguinidae Nicoll, 1935 (1926)
Genus *Ditylenchus* Filipjev, 1936
Family Aphelenchidae Fuchs, 1937 (Steiner, 1949)
Genus unidentified
Family Aphelenchoididae (Skarbilovich, 1947) Paramonov, 1953
Genus *Aphelenchoides* Fisher, 1894
Family Cephalobidae (Filipjev, 1934) Chitwood & Chitwood, 1934
*Genus Acrobeloides (*Cobb, 1924) Thorne, 1937
Genus *Cephalobus* Bastian, 1865
Genus *Eucephalobus* Steiner, 1936
Genus *Seleborca* Andrássy, 1985 [c]
Family Criconematidae (Taylor, 1936) Thorne, 1949
Genus *Criconema* Hofmänner & Menzel, 1914
Genus *Hemicycliophora* De Man, 1921
Hemicycliophora typica De Man, 1921 [c]
Hemicycliophora poranga Monteiro & Lordello, 1978 [b]
Family Diplogastridae (Micoletzky, 1922) Steiner, 1929
Genus unidentified
Family Dolichodoridae Chitwood, 1950
Genus *Tylenchorhynchus* Cobb, 1913
Family Heteroderidae Filipjev & Schuurmans Stekhoven, 1941
Genus *Meloidogyne* Göldi, 1892
Family Hoplolaimidae (Filipjev, 1934) Wieser, 1953
Genus *Helicotylenchus* Steiner, 1945
Helicotylenchus dihystera (Cobb, 1893) Sher, 1961 [c]
Genus *Rotylenchus* Filipjev, 1936
Rotylenchus gracilidens (Sauer, 1958) Sauer, 1958 [c]
Genus *Rotylenchulus* Linford & Oliveira, 1940
Genus *Scutellonema* Andrássy, 1958
Scutellonema brachyurus (Steiner, 1938) Andrássy, 1958 [c]
Family Mesorhabditidae Andrássy, 1976
Genus *Mesorhabditis* (Osche, 1952) Dougherty, 1953
Family Neodiplogastridae (Paramonov, 1952) Andrássy, 1984
Genus *Koerneria* Meyl, 1960 [c]

[a] Species described in this chapter.
[b] New report for South Africa.
[c] New report for freshwater in South Africa.

Table 6.2 (continued): Checklist of nematodes found at different water sites from the Telperion Nature Reserve

Class Secernentia Von Linstow, 1905
Family Neotylenchidae (Thorne, 1941) Thorne, 1949
Genus unidentified
Family Panagrolaimidae (Thorne, 1937) Paramonov, 1956
Genus *Panagrolaimus* Fuchs, 1930
Family Pratylenchidae Thorne, 1949
Genus *Pratylenchus* Filipjev, 1936
Pratylenchus pseudopratensis Seinhorst, 1968
Family Rhabditidae Örley, 1880
Genus *Rhabditis* Dujardin, 1845
Family Teratocephalidae Andrássy, 1958
Genus *Teratocephalus* De Man, 1876 [c]
Family Tylenchidae Örley, 1880
Genus *Coslenchus* Siddiqi, 1978 [c]
Genus *Tylenchus* Bastian, 1865
Genus unidentified
Family Tylenchulidae (Skarbilovich, 1947) Kirjanova, 1955
Genus unidentified
Class Penetrantia Andrássy, 1974
Family Actinolaimidae Thorne, 1939
Genus *Actinolaimus* Cobb, 1913
Family Aporcelaimidae Heyns, 1965
Genus *Aporcelaimellus* Heyns, 1965
Aporcelaimellus obtusicaudatus (Bastian, 1865) Altherr, 1968
Genus *Makatinus* Heyns, 1965 [a,c]
Makatinus africanus n.sp.
Family Belondiridae Thorne, 1939
Genus *Axonchium* Cobb, 1920 [c]
Family Dorylaimidae De Man, 1876
Genus *Mesodorylaimus* Andrássy, 1959
Genus *Afrodorylaimus* Andrássy, 1964
Genus unidentified
Family Ironidae De Man, 1876
Genus *Ironus* Bastian, 1865
Ironus telperionensis n. sp.[a]
Family Mononchidae Filipjev, 1934
Genus unidentified
Family Primatolaimidae Micoletzky, 1922
Genus *Prismatolaimus* De Man, 1880
Family Qudsianematidae Jairajpuri, 1965
Genus unidentified

[a] Species described in this chapter.
[b] New report for South Africa.
[c] New report for freshwater in South Africa.

Table 6.2 (continued): Checklist of nematodes found at different water sites from the Telperion Nature Reserve

Class Penetrantia Andrássy, 1974
Family Tobrilidae De Coninck, 1965
Genus *Tobrilus* Andrássy, 1959
Genus *Eutobrilus* Tsalolikhin, 1981
Eutobrilus annetteae (Joubert & Heyns, 1979) Tsalolikhin, 1981 [a]
Genus *Neotobrilus* Tsalolikhin, 1981
Neotobrilus ampiei (Joubert & Heyns, 1979) Tsalolikhin, 1981 [a]
Genus *Tobriloides* Loof, 1973
Tobriloides loofi Swart & Heyns, 1990
Family Trichodoridae (Thorne, 1935) Clark, 1961
Genus *Nanidorus* Siddiqi, 1974 [c]
Family Tripylidae De Man, 1876
Genus *Tripyla* Bastian, 1865
Order Mermithida Hyman, 1951
Unidentified

[a] Species described in this chapter.
[b] New report for South Africa.
[c] New report for freshwater in South Africa.

6.4.1 Family Aphanolaimidae Chitwood, 1936

6.4.1.1 *Aphanolaimus strilliae* n. sp.

Figures 6.2-6.6

Measurements: Table 6.3

Description:

Female (n=26): Body 1240-1613 µm long, tapering towards both extremities, habitus mostly ventral curvature of whole body, some specimens curved dorsally. Body with 808-887 well defined smooth annules, mean width 2 µm. Cuticle 2-4 µm thick at mid-body, lateral field a single ridge with two lines, originating on 34th to 46th annule (56-70 µm from the anterior end), mean width of 3 µm at mid body. Lateral field ends 15-55 µm anterior to tail tip. Lateral body pores paired, 21-26 pairs on each side, arranged randomly on both ventral and dorsal side of lateral field; five pairs in region of oesophagus, one pair and one single pore on tail. First lateral body pore situated on 25th to 35th annule

(30-55 µm from anterior end); second pore situated on 56th to 71st annule (89-108 µm from anterior end). Each pore contains a small, hollow projection (observed with SEM) that varies in structure (Figure 6.5). Each pore connected to an epidermal gland. Cephalic region not annulated, 7-10 µm high and 7-10 µm wide with cephalic setae measuring 7 µm long, situated at the anterior end of the amphids. Amphid located at base of cephalic region, 4-7 µm high and 4-6 µm wide, circular with no central elevation. Stoma a narrow tube, not sclerotized, 6-10 µm long. Oesophagus a narrow tube, 217-251 µm long, 4-7 µm wide at widest point, ending in a 6-16 µm long cardia. Ventral gland 48-67 µm long, 6-9 µm wide, situated at base of oesophagus, gland opening / excretory pore not observed (excretory pore described as opening in stoma in this genus). Nerve ring situated at 52-59% of oesophagus length. Reproductive system didelphic-amphidelphic, genital tracts almost equal in length, often posterior tract (mean length 222 µm) slightly longer than anterior tract (mean length 190 µm). Simple sphincter muscles separate ovijector from both uteri. Anterior uterine branch 70-96 µm long, posterior branch 72-99 µm in length (measured in females without eggs or developing embryos in uterus). Eggs and fully-grown juveniles observed in more than half of specimens, indicating the species is ovoviviparous. Vulva a transverse slit occupying about half corresponding body width, situated at 47-52 % of total body length. Vagina bent anteriorly in V-shape, 16-22 µm long and occupies less than half of corresponding body width. No sperm cells observed in reproductive system of females, indicating that species might be parthenogenetic. Rectum 1.2-2.0 times anal body width. Tail 165-207 µm long ending in an offset 2-4 µm spinneret. Three distinct caudal glands present in anterior half of tail.

Male: Unknown.

Table 6.3: Morphometrics of *Aphanolaimus strilliae* n. sp. females found in freshwater sediment in the Telperion Nature Reserve, South Africa. All measurements in the form: mean ± standard deviation (range).

Morphological feature	Holotype	25 Paratypes
L	1394	1377 ± 86.8 (1240–1613)
a	48.8	40.9 ± 4.1 (36.3–49.5)
b	5.7	5.7 ± 0.3 (5.1–6.6)
c	6.9	7.2 ± 0.3 (7.0–8.0)
c'	10.5	9.5 ± 0.8 (7.7–10.9)
V	49	49 ± 1.1 (47–52)
G1	12	14 ± 2.5 (11–19)
G2	13	16 ± 3.3 (12–22)
Body width at mid–body	29	33 ± 3.9 (28–41)
Body width at vulva	32	34 ± 3.9 (28–43)
Body width at anus	19	20 ± 1.9 (17–26)
Cuticle width at mid–body	3	3 ± 0.5 (2–4)
Annule width at mid–body	2	2 ± 0.3 (1–2)
Number of annuli	833	837 ± 24.4 (808–887)
Number of annuli from anterior to end of oesophagus	167	156 ± 9.5 (139–179)
Number of annuli from anterior to vulva	412	412 ± 18.5 (388–452)
Number of annuli between vulva and anus	280	287 ± 11.6 (253–313)
Number annuli from anterior to anus	692	699 ± 21.7 (674–746)
Number of annuli from anus to tail terminus	141	138 ± 6.5 (120–152)
Start of lateral field (annule)	41	41 ± 3.3 (34–46)
Start of lateral field (µm)	65	64 ± 3.4 (56–70)
End of lateral field from tail tip (µm)	26	29 ± 9.0 (15–55)
Lateral field width at mid–body	2	3 ± 0.7 (2–5)
Lateral body pores (in pairs)	22	23 ± 1.4 (21–26)
Lateral body pores (single)	1	1[a]
Lateral body pores (total)	45	48 ± 2.9 (43–53)
Lateral body pore pairs (oesophageal region)	5	5[a]
Lateral body pore pairs (anterior to vulva)	7	10 ± 2.8 (7–14)
Lateral body pore pairs vulva to anus)	9	8 ± 0.8 (7–9)
Lateral body pore pairs (tail)	1	1[a]
Lateral body pore single (tail)	1	1[a]
First body pore from anterior (annuli)	35	30 ± 2.4 (25–35)
First body pore from anterior (µm)	51	49 ± 4.9 (30–55)
Second body pore from anterior (annuli)	71	64 ± 4.2 (56–71)
Second body pore (µm)	101	98 ± 4.5 (89–108)
Labial region length	9	9 ± 0.8 (7–10)
Labial region width	8	8 ± 0.5 (7–10)

[a] Value same for all specimens.

Table 6.3 (continued): Morphometrics of *Aphanolaimus strilliae* n. sp. females found in freshwater sediment in the Telperion Nature Reserve, South Africa. All measurements in the form: mean ± standard deviation (range).

Morphological feature	Holotype	25 Paratypes
Cephalic setae length	6	7 ± 1.4 (6–9)
Amphid length	6	6 ± 0.6 (4–7)
Amphid width	4	5 ± 0.5 (4–6)
Stoma length	7	7 ± 0.8 (6–10)
Oesophagus length	243	237 ± 7.7 (217–251)
Oesophagus width at widest point	6	6 ± 1.0 (4–7)
Ventral gland length	56	57 ± 5.9 (48–67)
Ventral gland width at nucleus	7	7 ± 1.0 (6–9)
Nerve ring from anterior	131	131 ± 5.2 (122–140)
Nerve ring length	9	9 ± 0.9 (8–11)
Cardia length	13	10 ± 2.6 (6–16)
Vulva from anterior	689	664 ± 50.0 (560–759)
Vagina length	21	20 ± 2.1 (16–22)
Anterior genital tract length	160	190 ± 42.9 (130–274)
Posterior genital tract length	185	222 ± 54.5 (150–337)
Anterior uterus length	70	79 ± 9.6 (70–96)
Posterior uterus length	92	88 ± 8.5 (72–99)
Rectum length	36	35 ± 4.1 (25–43)
Rectum / anal body width	1.9	1.7 ± 0.2 (1.3–2.1)
Nerve ring as percentage of oesophagus	54	55 ± 1.8 (52–59)
Vagina as percentage of corresponding body width	32	36 ± 5.3 (29–48)
Tail length	201	188 ± 11.7 (165–207)
Spinneret length	2	3 ± 0.4 (2–4)

[a] Value same for all specimens.

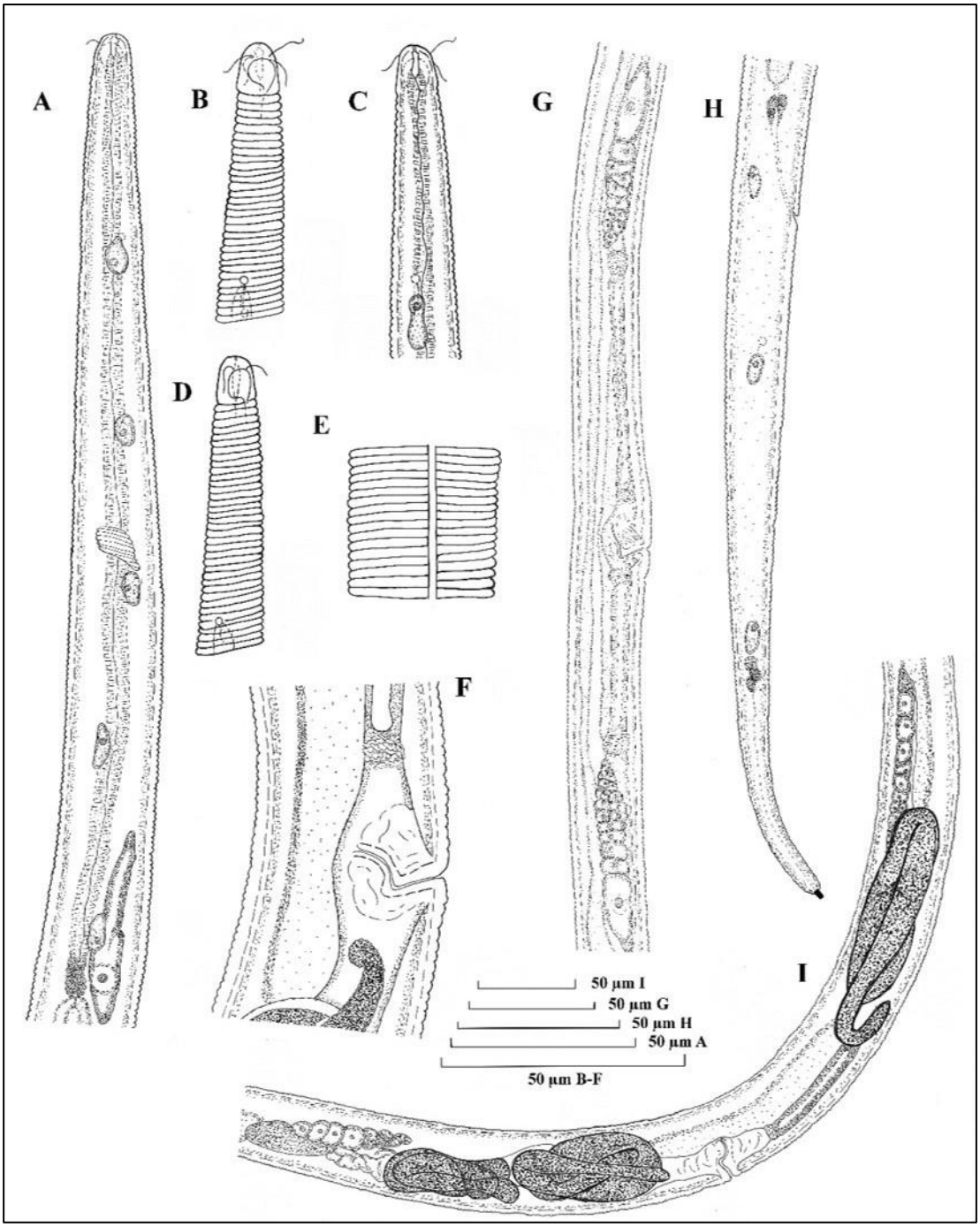


Figure 6.2: *Aphanolaimus strilliae* n. sp. female A: Oesophageal region with ventral gland; B-D: Anterior region with oesophagus, stoma, position of amphid and position of first lateral body pore; E: Lateral field at mid-body; F: Vulval region; G: Reproductive system; H: Tail region; I: Genital tract with developing juveniles.

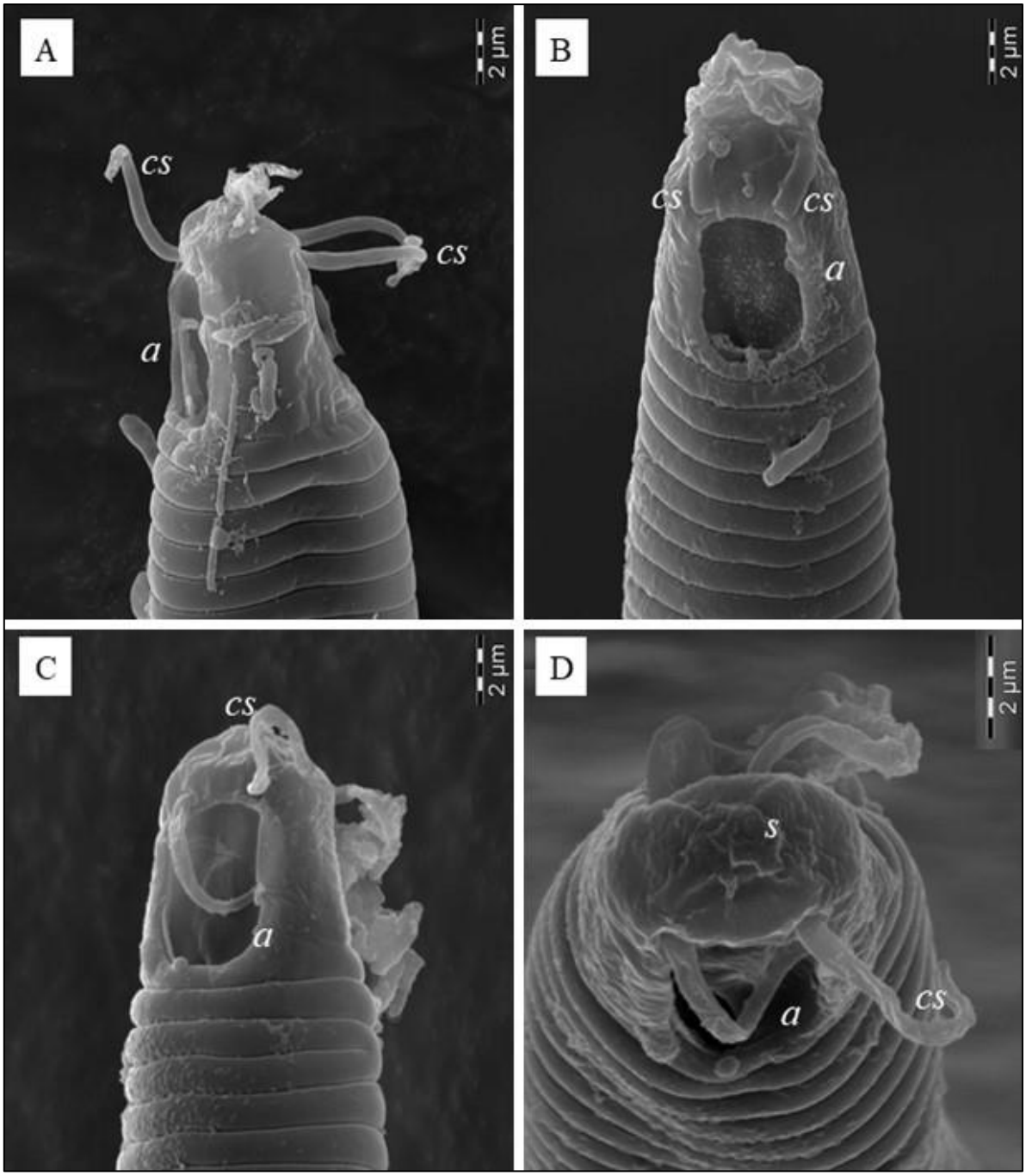


Figure 6.3: Scanning electron micrographs of *Aphanolaimus strilliae* n. sp. female A: Labial region in ventral view with cephalic setae (*cs*) and amphid (*a*); B + C: Labial region in lateral view with cephalic setae (*cs*) and amphid (*a*); D: Lip region in e*n face* view of lip region with amphid opening (*a*) and stoma opening (*s*).

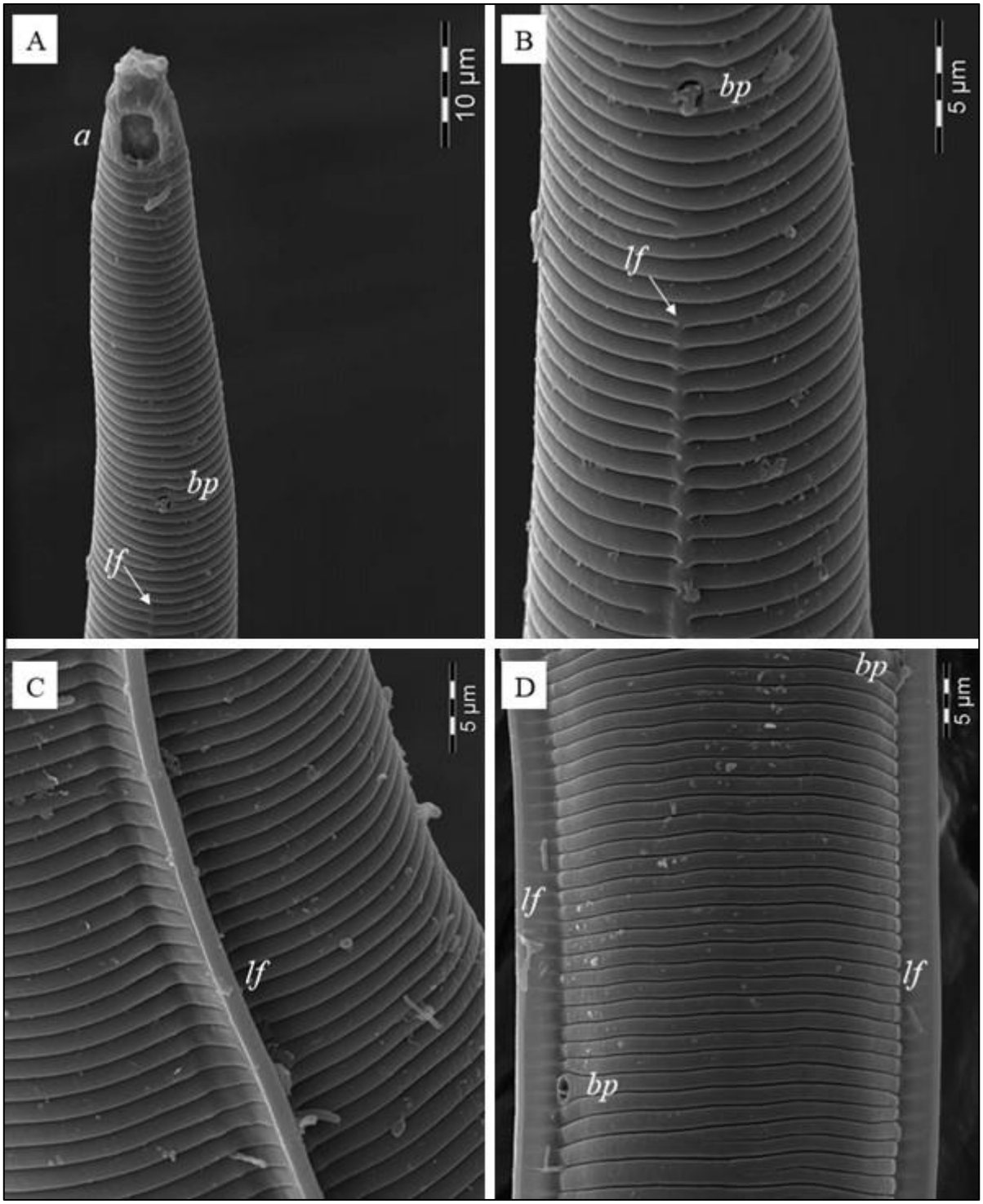


Figure 6.4: Scanning electron micrographs of *Aphanolaimus strilliae* n. sp. female A: Lateral view of anterior region with amphid (*a*), first body pore (*bp*) and the beginning of the lateral field (*lf*); B: First body pore (*bp*) and lateral field (*lf*); C: Lateral field (*lf*) at mid-body; D: Ventral view mid-body with lateral field (*lf*) and two body pores (*bp*).

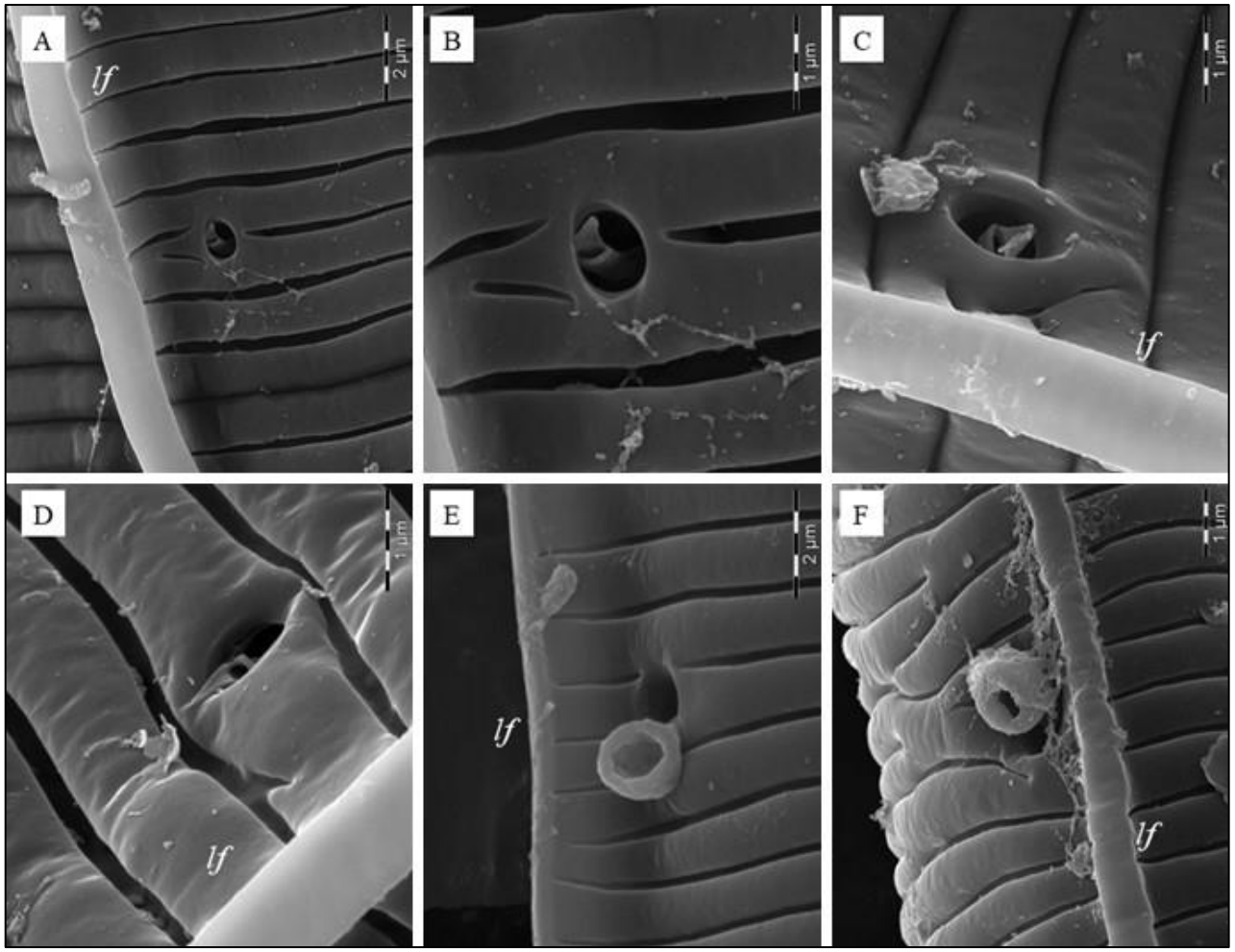


Figure 6.5: Scanning electron micrographs of *Aphanolaimus strilliae* n. sp. female A-D: Body pores detail all located in anterior half of body; E-F: Body pores in posterior half of body (*lf* = lateral field).

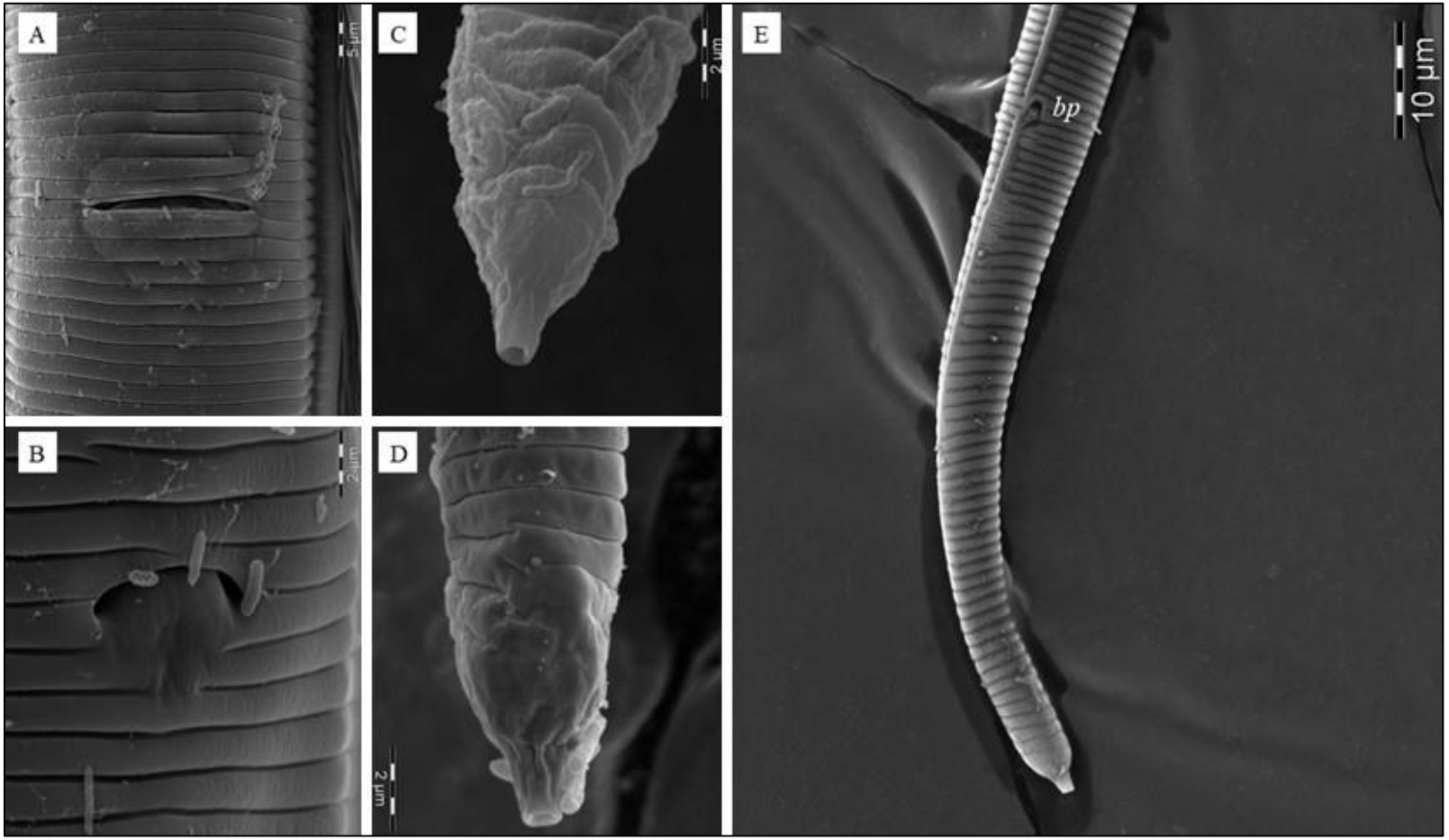


Figure 6.6: Scanning electron micrographs of *Aphanolaimus strilliae* n. sp. female A: Ventral view of vulva; B: Ventral view of anus; C-D: Tail tip with opening of spinneret; E: Tail region with lateral field and last body pore (*bp*).

Diagnosis and relationships:

Aphanolaimus strilliae n. sp. is characterised by body length of 1240-1613 µm, more than 800 annuli, 7 µm long cephalic setae, lateral field originating between the first and second lateral body pore at the 34th-46th annule, first body pore located at the 25th-35th annule, vulva equatorial, vagina V-shaped and bent anteriorly, 142-195 µm long uterus and 165-207 µm long tail with one paired and one single lateral body pore, spinneret offset. Species is ovoviviparous. Males absent.

Aphanolaimus strilliae n. sp. belongs to the ovoviviparous group of larger species with more than 700 body annuli. This group includes *Aphanolaimus aymarae* De Waele & Coomans, 1993, *Aphanolaimus spiriferus* Cobb, 1914 and *Aphanolaimus viviparus* Plotnikov, 1899, which are all ovoviviparous species. *Aphanolaimus aquaticus* Daday, 1894 and *Aphanolaimus tudoranceai* Zullini, 1988 belong to the group of large species, but are not ovoviviparous. *Aphanolaimus strilliae* n. sp. is morphologically closest to *A. spiriferus*. The new species was compared to the type that was described from the Potomac River, United States of America (USA) and other descriptions (Lake Tahoe and Mendocino, USA) of *A. spiriferus* (Cobb 1914; Raski & Coomans 1990). *Aphanolaimus strilliae* n. sp. compares well to *A. spiriferus* in body length (1240-1613 µm *vs* 1200-1700 µm), total number of annuli (more than 800) and ovoviviparous development of offspring. It differs from the type population in the shape of the amphid (circular with no elevation *vs* spiral), morphology of the vagina (V-shaped *vs* inverted V-shape) and the presence of males (absent in *Aphanolaimus strilliae* n. sp.). *Aphanolaimus strilliae* n. sp. is however separated from the *A. spiriferus* from Lake Tahoe and Mendocino based on: lower number of lateral body pores (21-26 pairs *vs* 26-30 pairs); lateral body pores on the tail (one pair and one single pore vs two pairs); and presence of males (absent in *Aphanolaimus strilliae* n. sp.). Based on SEM observations of both *A. strilliae* n. sp. and *A. spiriferus* the following differences were observed: different morphology of annuli (rounded, smooth annuli *vs* plate-like annuli folding over each other) and different morphology of setae in lateral body pores (various shapes found in all lateral body pores *vs* simple setae with no setae in first three pores) (Raski & Coomans 1990). *Aphanolaimus strilliae* n. sp. was also compared to various descriptions available of *A. aquaticus* namely the type description

by Daday (1894) and other descriptions by Jägerskiöld (1909), Höfmanner & Menzel (1914), Micoletzky (1922), Coomans & De Waele (1983), Traunspurger (1989) and Andrássy (2005). The new species differs from the type population of *A. aquaticus* described from Lake Balaton, Hungary, in the presence of males. Unfortunately, the original description of *A. aquaticus* is vague and does not include characteristics currently used for species identification. Morphologically *Aphanolaimus strilliae* n.sp. was therefore compared to reported populations of *A. aquaticus* and not the original one. The new species differs from all other populations of *A. aquaticus* in higher number of body annuli {808-887 *vs* 665 (calculated from Traunspurger [1989]) which only stated that "more than 600 annuli" are present in *A. aquaticus*}, vaginal morphology (bent vagina *vs* straight), structure of amphid (no central elevation *vs* central elevation), longer stoma (6-10 µm *vs* 2 µm) (Coomans & De Waele 1983), longer oesophagus (217-251 µm *vs* 190-200 µm) (Coomans & De Waele 1983), the absence of males (present in *A. aquaticus*) (except population of (Coomans & De Waele [1983] where males were absent) and the development of offspring within the female (oviparous in *A. aquaticus*). *Aphanolaimus strilliae* n.sp. differs from type specimens of *A. aymarae* described from mud in Bolivia in: lower body width (28-41 µm *vs* to 57-75 µm), number of annuli (600-700 in *A. aymarae*), number of lateral body pores (26-37 pairs in *A. aymarae*), higher labial region (7-10 µm *vs* 10-12 µm), longer stoma (2-3 µm in *A. aymarae*); morphology of the vagina (inverted V-shape in *A. aymarae*) and spinneret morphology (offset *vs* not offset) (De Waele & Coomans 1993). *Aphanolaimus aymarae* was not found after its description. *Aphanolaimus strilliae* n. sp. is morphologically close to the type description of *A. tudoranceai* reported from Lake Zway, Ethiopia, as both are of the larger species with over 700 annuli (Zullini 1988). The new species differs in: vaginal morphology (straight vagina in *A. tudoranceai*), amphid shape (circular *vs* kidney shaped), longer tail (165-207 µm *vs* 145-165 µm) and spinneret morphology (spinneret not offset in *A. tudoranceai*). *Aphanolaimus tudoranceai* was never found after its original description (Zullini 1988). *Aphanolaimus strilliae* n. sp. is closely related to *A. viviparus* however, the validity of this species is in question. According to Goodey (1963), *A. viviparus* may be a synonym of *A. aquaticus* but unfortunately, he did not stipulate the reasons for this. In the literature available on *A. viviparus*, no distinct differences between *A. viviparus* and *A. aquaticus* can be determined apart from one described male of *A. viviparus* having 13 pre-cloacal supplements compared to the 7-12 supplements reported for

A. aquaticus (Schneider 1916; 1922). The description of the type female by Plotnikov (1899) could not be located in the present study and apart from the species name being mentioned in literature, no original morphological data could be found for this species. As the validity of *A. viviparus* is in question, *Aphanolaimus* strilliae n.sp. therefore is not compared to it.

Type material: Holotype female on slide 50587 (first female from the left side) and paratypes on slides 50577-50578, 50580, 50582-50584, 50586-50590 (18 females) deposited in the NCN (ARC-PHP, Biosystematics Division, Pretoria).

Type locality: Specimens were found in sediment and water samples collected at the wetland site. This wetland is situated along the 11_26 road on the border of the Blaauwpoort 1 and Blaauwpoort 2 sections of the reserve. The site is described in Table 6.4.1. Locality number MP00013 in the NCN (ARC- PHP, Biosystematics, Pretoria).

Etymology: *Aphanolaimus strilliae* n. sp. is named after Mrs. Strilli Oppenheimer, owner of Telperion Nature Reserve and well known for her work in conservation.

6.4.1.2 *Paraphanolaimus behningi* Micoletzky, 1923

Figures 6.7-6.10

Measurements: Table 6.4

Description:

Female (n = 2): Body 924-1078 µm long. Habitus: slightly curved ventrally in one specimens and fully curved ventrally in posterior region in the other. Cuticle coarsely annulated. Annuli 2 µm thick

at mid-body. Lateral field with two lines, originating between second and third lateral body pore (88 - 92 μm from anterior end) and terminating on tail. Twenty-four pairs of lateral body pores arranged alternatively ventral and dorsal to lateral field. Four to five pairs in oesophageal region; 9-10 pairs between cardia and vulva; seven to eight pairs between vulva and anus; and one to two pairs on tail. Each body pore connected internally to an epidermal gland cell. First body pore situated 42-51 μm from anterior end. Lip region truncate, 4-5 μm long, 6-7 μm wide. Cephalic setae 2-4 μm long. Amphids large, unispiral, situated on unstriated lip region. Stoma barrel shaped, 7-8 μm long, wide with sclerotized walls in anterior half. Oesophagus cylindrical, 171-183 μm long. Nerve ring situated at mid oesophagus (approximately 57 % of oesophagus length from anterior). Cardia 17-20 μm long. Reproductive system didelphic, amphidelphic. Vagina anteriorly directed (30-45 μm long, 1.0-1.1 times the corresponding body width). Vulva not protruding, situated at 51-53 % of body length. Tail 139- 160 μm long, narrowing gradually and terminating in swollen, unstriated tip, ending in a short spinneret about 2.5 μm long. Rectum 39- 42 μm long. Three caudal glands in tail region.

Male (n = 2); Morphology similar to that of female except the following. Ventral curvature of body more accentuated, completely coiled at posterior end in one specimen. Lateral body pores approximately 25 pairs; five pairs in oesophageal region; 17-18 pairs in cardia to cloaca region and two pairs on tail. First and fourth body pore contains a setum, absent in female. Reproductive system monorchic. Spicules 106-110 μm long, slender with faint transverse striations for most of its length. Gubernaculum prominent (21-26 μm). Thirteen to fourteen pre-cloacal supplements, each supplement approximately 11 μm long. One pair of pre-cloacal setae and four pairs on the tail (2 subdorsal and 2 subventral). Last pair of setae on the tail the shortest.

Table 6.4: Morphometrics of *Paraphanolaimus behningi* Micoletzky, 1923 females and males found in freshwater sediment in the Telperion Nature Reserve, South Africa. All measurements in μm and in the form: mean ± std. (range).

	1♀	1♀	1♂	1♂
L	1078	924	915	1039
a	26.6	27.9	32.8	39.5
b	5.9	5.4	6.0	5.5
c	6.7	6.7	7.0	7.1
c'	8.0	7.5	6.2	6.5
V	51	53	–	–
G1	21	18	–	–
G2	7	8	–	–
Body width at mid body	41	33	28	26
Body width at anus / cloaca	20	18	21	23
Body width at vulva	42	31	–	–
Lateral field start from anterior	92	88	–	145
Labial region length	5	4	–	–
Labial region width	7	6	6	–
Cephalic setae length	–	–	3	–
Stoma length	7	8	5	6
Annule width at mid body	–	2	–	–
Number of lateral body pores	47	48	49	50
First body pore from anterior	51	42	39	49
Second body pore from anterior	86	77	385	86
Oesophagus length	183	171	154	189
Nerve ring from anterior	106	102	-	110
Cardia length	21	22	–	19
Vulva from anterior	549	486	–	–
Vagina length	45	30	–	–
Vagina / corresponding body width	1.1	1.0	–	–
Anterior genital tract length	222	166	–	–
Posterior genital tract length	76	74	–	–
Tail length	160	139	130	147
Rectum length	42	39	–	–
Rectum / Anal body width	2	2	–	–
Spinneret length	2	3	2	3
Spicule length	–	–	106	110
Gubernaculum length	–	–	26	21
Number of supplements	–	–	13	14
Supplements in spicule range	–	–	–	5
Pre–cloacal setae	–	–	2	–
Tail setae	–	–	4	–
First supplement from anterior	–	–	536	608
First supplement to cloaca	–	–	249	298
Last supplement to cloaca	–	–	35	35
Nerve ring as percentage of oesophagus	58	59	–	58

– Indicates structures that were not visible or not present.

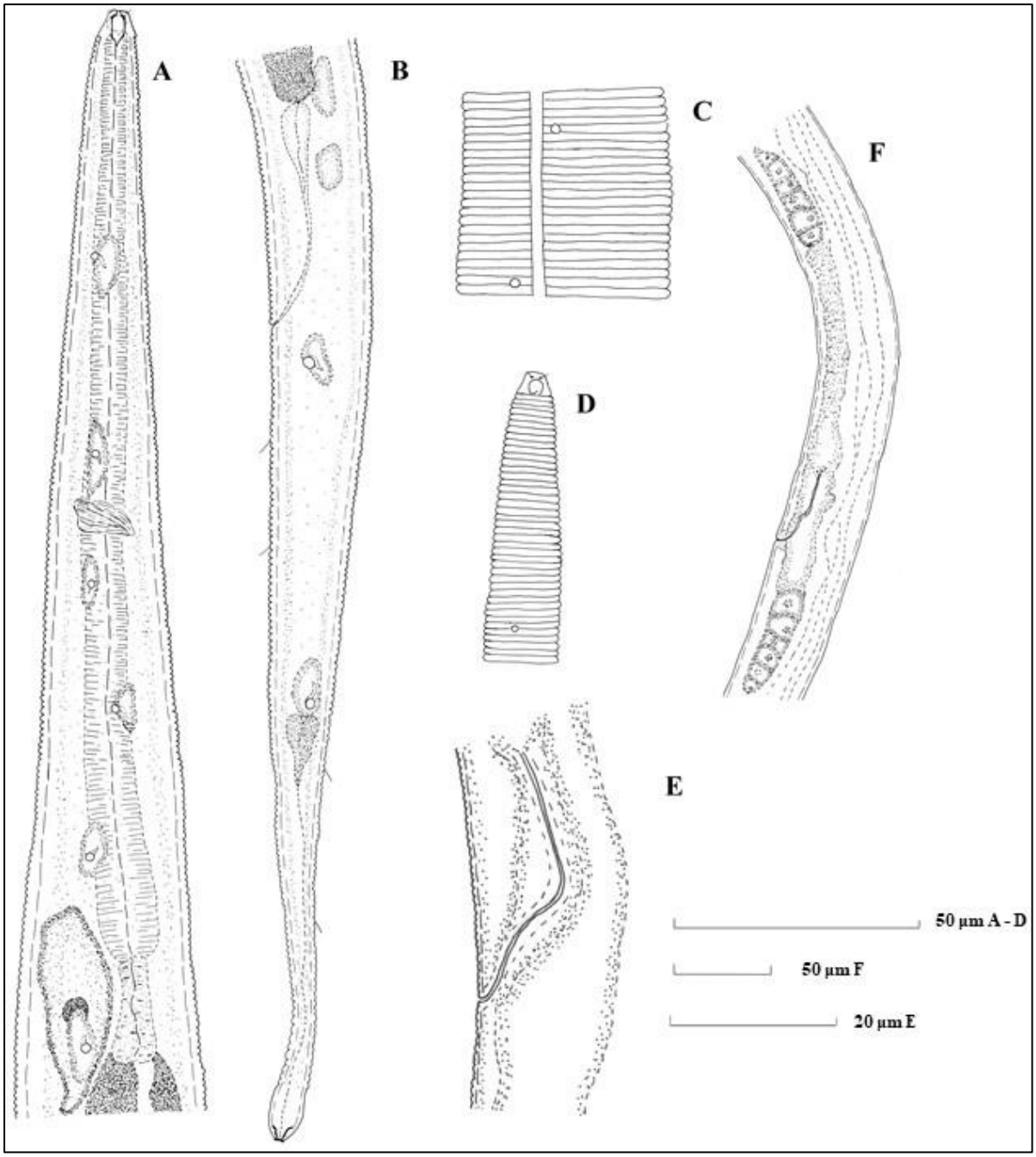


Figure 6.7: *Paraphanolaimus behningi* Micoletzky, 1923 female A: Anterior region with oesophagus and ventral gland; B: Tail with spinneret; C: Lateral field at mid-body with two lateral body pores; D: Anterior region with amphid opening and first body pore; E: Vulval region; F: Genital tract.

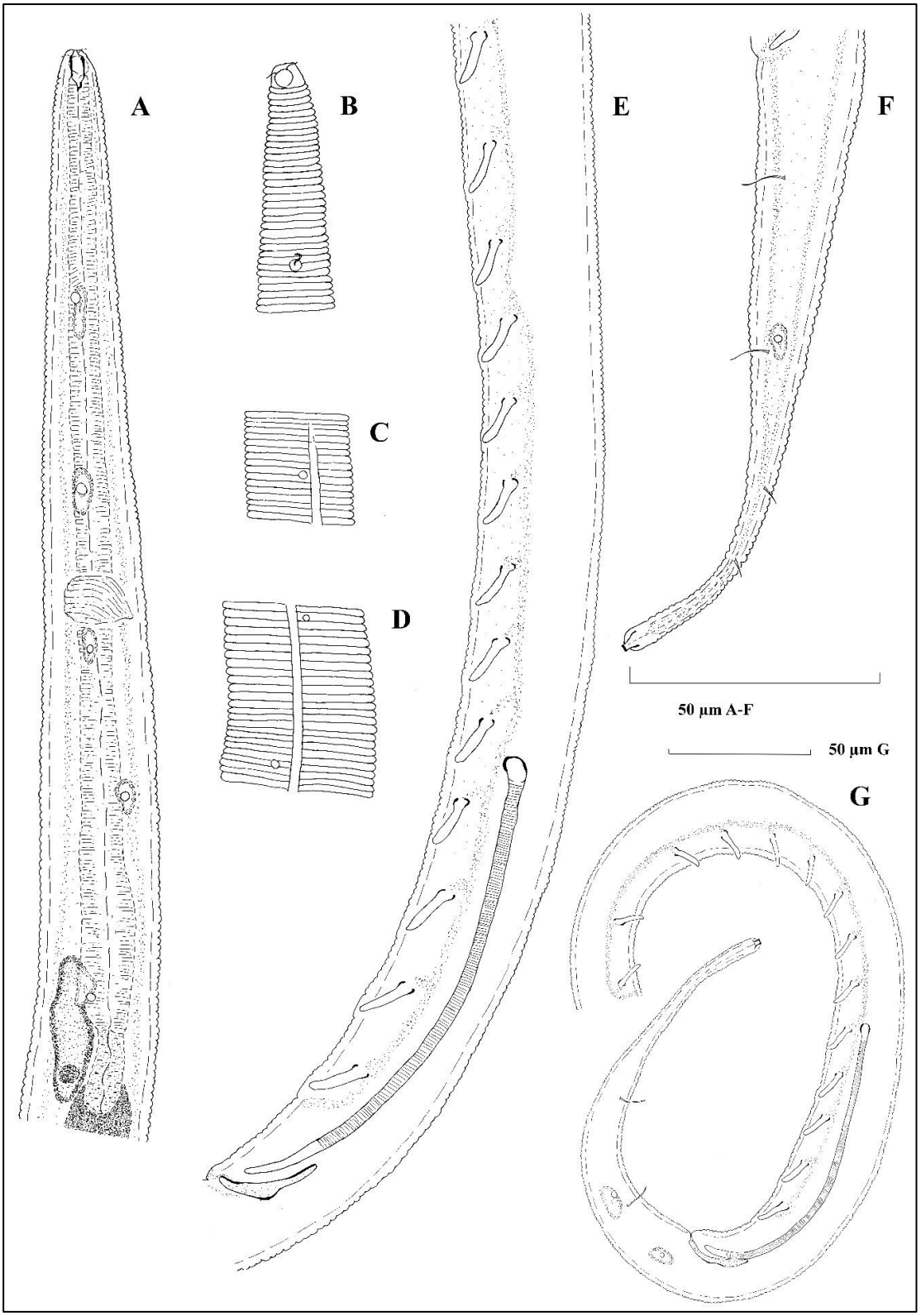


Figure 6.8: *Paraphanolaimus behningi* Micoletzky, 1923 male A: Anterior region with oesophagus and ventral gland; B: Anterior region, with amphid opening and first body pore with setae; C: Start of lateral field after second body pore; D: Lateral field and body pores at mid-body; E: Posterior region with supplementary row, spicule and gubernaculum; F: Tail; G: Tail form of second male.

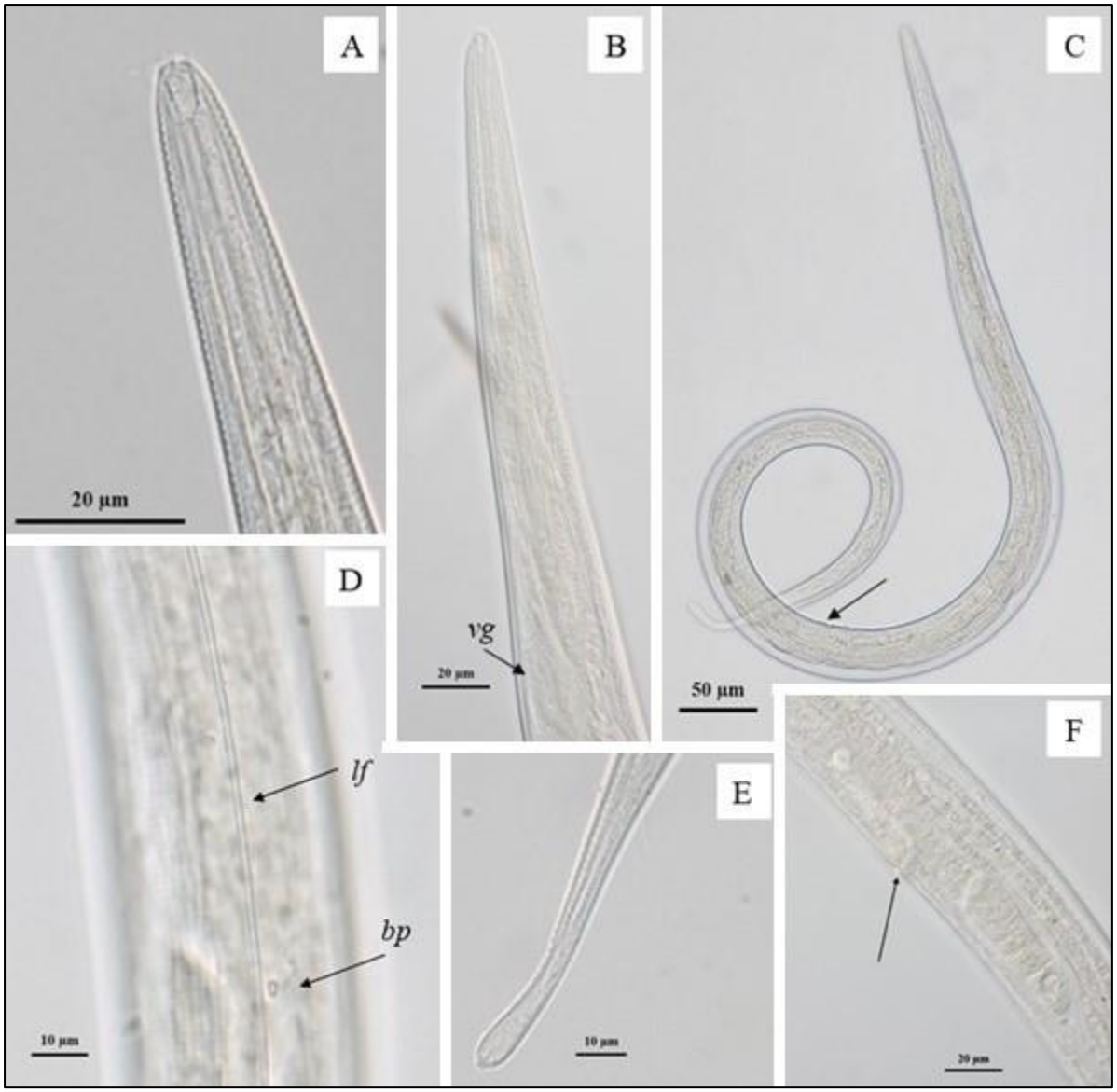


Figure 6.9: Light microscope pictures of *Paraphanolaimus behningi* Micoletzky, 1923 female A: Anterior region with stoma; B: Anterior region with oesophagus and ventral gland (*vg*); C: Habitus of female arrow indicating vulva; D: Mid-body showing lateral field (lf) and body pore (bp); E: Tail with spinneret; F: Vulval region, arrow indicating vulva and vagina.

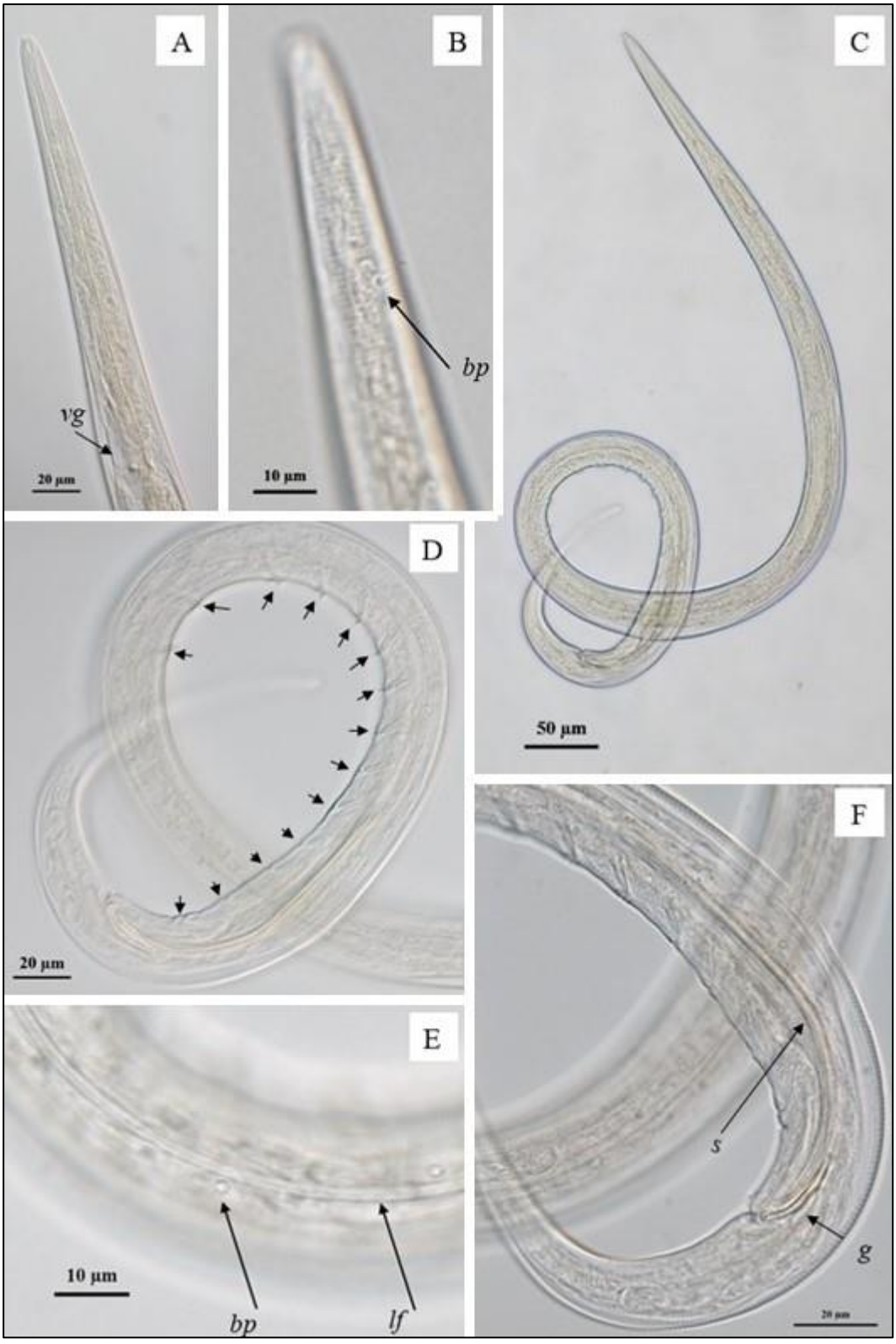


Figure 6.10: Light microscopic pictures of *Paraphanolaimus behningi* Micoletzky, 1923 male A: Anterior region with oesophagus and ventral gland (*vg*); B: Anterior region, arrow indicating first body pore with setae; C: Habitus of male; D: Posterior region arrows indicating supplements; E: Mid-body with lateral field and body pore; F: Cloacal region, with spicule (*s*) and gubernaculum (*g*).

Discussion:

The *Paraphanolaimus* specimens from Telperion were identified as *P. behningi* based on the following morphological characteristics: in female; anteriorly bent vagina, in male; spicule length (106 and 110 µm), number of supplements (13 and 14). Females collected from Telperion matches those of the type description of *P. behningi* from the River Volga (Russia) by Micoletzky (1923), except for the following: development of juveniles (oviparous *vs* ovoviviparous), position of start of the lateral field (posterior to second body pore *vs* anterior to the first body pore), morphology of the vagina (anteriorly bent *vs* straight). The Telperion males fit the description of the first males found in Lake Balaton (Biró, 1968) except in: slightly higher a-value (32.8-39.5 *vs* 29-35); higher b-value (5.5-6.0 *vs* 4-4.5) and longer spicule (106-110 µm *vs* 80-95 µm). The morphology of the vagina differs among populations. In the type populations as well as according to keys of the species by Raski & Coomans (1991) and Tsalolikhin (1989) the vagina is straight. However, in the populations described by Alekseev (1981), Eyualem-Abebe (1996), Holovachov & Sturhan (2003) and Andrássy (2005) all the females are described with an anteriorly bent vagina which matches that of the Telperion populations.

Males and females of *P. behningi* were compared to various populations of *P. behningi* described (Micoletzky 1925; Biró 1968; Gagarin 1971; Alekseev 1981; Zullini 1982; Gagarin 1993; Eyualem-Abebe 1996; Holovachov & Sturhan 2003; Andrássy 2005). The females of the Telperion population of *P. behningi* matches all other populations of this species except in the following. The Telperion population differs from the Russian population reported by Alekseev (1981) in higher a-value (26.6-27.9 *vs* 17.0-18.0); higher b-value (4.8-5.0 in *P. behningi* by Alekseev [1981]); higher mean V (52 % *vs* 46 %); longer stoma (7-8 µm *vs* 5-6 µm); shorter oesophagus (171-183 µm *vs* 201-226 µm); shorter rectum (mean value 41 µm *vs* 80 µm). From the Italian population reported by Zullini (1982) the females of Telperion differed in lower c'-value (7.5-8.0 *vs* 8.7-10.0) and total number of lateral body pores (47-48 *vs* 40). The females of the Telperion population was also compared to a Russian population described by Gagarin (1993) and the following differences were observed: higher a-value (21.2-24.7 in *P. behningi* of Gagarin [1993]); wider annuli at mid body (2 µm in both specimens *vs* 1.3-1.5 µm); and

longer stoma (5-6 µm in *P. behningi* of Gagarin [1993]). The females from the Telperion population differs from the Ethiopian population of Eyualem-Abebe (1996) in: wider labial region (4-5 µm in *P. behningi* of Eyualem-Abebe [1996]) and longer stoma (4-6 µm). The Telperion females differs from the German population of *P. behningi* by Holovachov & Sturhan (2003) in the following: higher b-value (4.4-4.7 in German population); wider annuli at mid body (1.3-1.4 µm in German population); position of second body pore (77-86 µm from anterior *vs* 85-122 µm) and shorter oesophagus (207-339 µm German population). The females from Telperion differs from the Hungarian population (Andrássy, 2005) in only the slightly lower c'-value (8.0-11.0 in the Hungarian population) and longer stoma (5-6 µm in the Hungarian population). Based on all the morphometric and morphological data available, the female specimens are most similar to the Italian, Ethiopian and Hungarian populations.

The males of *P. behningi* from Telperion were compared to previously reported males of this species. The Telperion population differ from the first report of males from Hungary (Biró 1968) in higher b-value (4.5) and longer spicules (106-110 µm *vs* 91.4 µm). The males from Telperion differ from the Russian population described by Gagarin (1971) by the following: lower a-value (32.8-39.5 *vs* 26.4-31.4); longer stoma (5-6 µm *vs* 7-8 µm) and longer tail 130-147 µm *vs* 100-125 µm). The Telperion males differs from the Italian males (Zullini 1982) in the following: lower c'-value (6.2-6.5 *vs* 8.7-10.0); more lateral body pores (49-50 *vs* 40) and more supplements in the range of the spicules (4-5 *vs* 3). The males of the Telperion population were compared to that of the Ethiopian population described by Eyualem-Abebe (1996) and the following differences were observed: longer body (915-1039 µm *vs* 612-877 µm); higher a-value (20.6-32.5 in the Ethiopian population); higher b-value (6.0 in both specimens *vs* 3.7-5.0); wider labial region width (6-7 µm *vs* 3.5-4 µm); longer stoma (4-6 µm in the Ethiopian population); longer tail (80-90 µm in the Ethiopian population) and longer gubernaculum (21-26 µm *vs* 18-21 µm). The males from Telperion differs from the Hungarian population in Andrássy (2005) in longer tail (80-90 µm in Hungarian population) and longer gubernaculum (18-20 µm in Hungarian population). Based on all the morphological and morphometric

data on the males of *P. behningi*, the Telperion population is most similar to both populations reported from Hungary.

Locality and material examined: The *P. behningi* specimens were found in sediment and water samples taken at the river site (Table 6.1) on the bank of the Wilge River with the locality number MP00098 and are on slides 50569 (two females) and 50568 (two males) deposited in the National Collection of Nematodes (ARC- Plant Health and Protection, Biosystematics, Pretoria).

6.4.2 Family Chronogasteridae Gagarin, 1975

6.4.2.1 *Chronogaster africana* Heyns & Coomans, 1980

Figures 6.11 & 6.12

Measurements: Table 6.5

Description:

The female specimen was identified as *Chronogaster africana* Heyns & Coomans, 1980 based on morphometrics and morphology, especially the morphology of the tail terminus displaying a mucro with six short spines, and the absence of crystalloids in the body cavity. The female specimen found in Telperion Nature Reserve (no males found) matches that of the original description by Heyns & Coomans (1980) with only difference being the length of the post-uterine branch (12.5 µm *vs* 6-9.5 µm). First time SEM observations of this species revealed the following: Lip region three annuli long with four cephalic setae and amphid openings on second annule with their posterior margins protruding slightly. Amphid aperture with two protrusions towards inside. Cuticle shows distinct annuli, no lateral field. Excretory pore located 140 µm from anterior end its opening one body annule wide. Lateral caudal pore on tail 30 µm posterior from anus on lateral side of body. This pore indicates two openings, one in the anterior and one in the posterior margin (Figure 6.12 B). Tail terminus consists of six small

projections on the base of one larger mucro. In some instances, an extra projection noticed on larger mucro (Figure 6.12 C-F). Annuli at the base of the tail tip showed some anastomoses / breaks in striations on lateral sides (Figure 6.12 F).

Discussion:

Chronogaster africana was first described in South Africa in 1980 from water sediment samples in Boskop Dam and The Kloof (North West Province), Natalspruit (Gauteng Province), and Krom River (Western Cape Province). It was again reported in South Africa in 1992 from sand near the weir in the Sabie River near Lower Sabie (Mpumalanga Province) (Botha & Heyns 1992). As this species has been reported in this country only, it is considered endemic to South Africa.

Locality and material examined: In the Telperion Nature Reserve a female specimen of *C. africana* was collected at the Wetland site (Table 6.1) with locality number MP00043 and deposited in the NCN (ARC- PHP, Biosystematics, Pretoria) on slide number 50567.

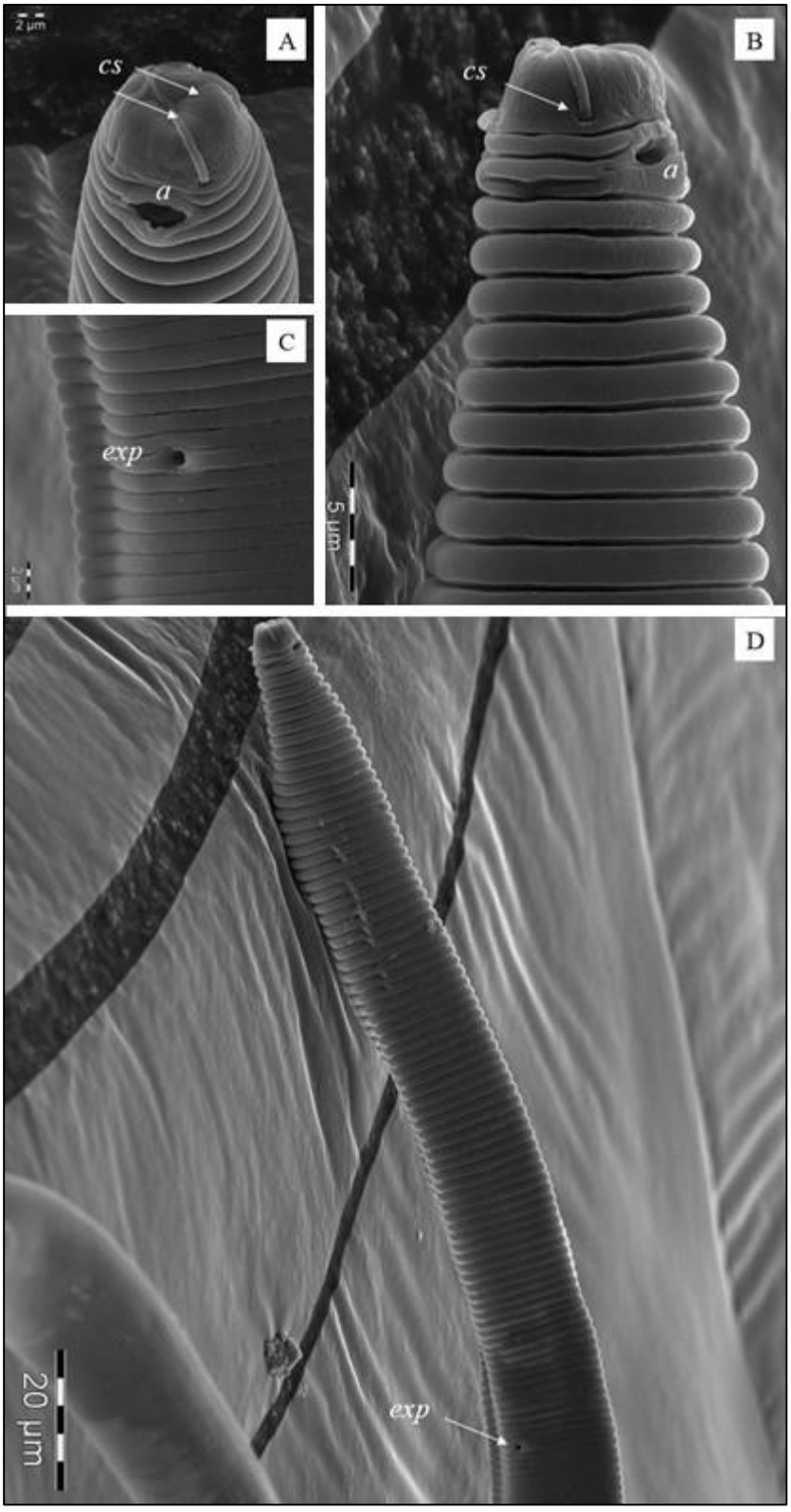


Figure 6.11: Scanning electron micrograph of *Chronogaster africana* Heyns & Coomans, 1980 female, A: Anterior end showing cephalic setae (*cs*) and amphid opening (*a*), B: Ventral side of body showing cephalic region with cephalic setae (*cs*) and amphid (*a*); C: Close up of excretory pore (*exp*); D: Anterior region showing the position of the excretory pore (*exp*).

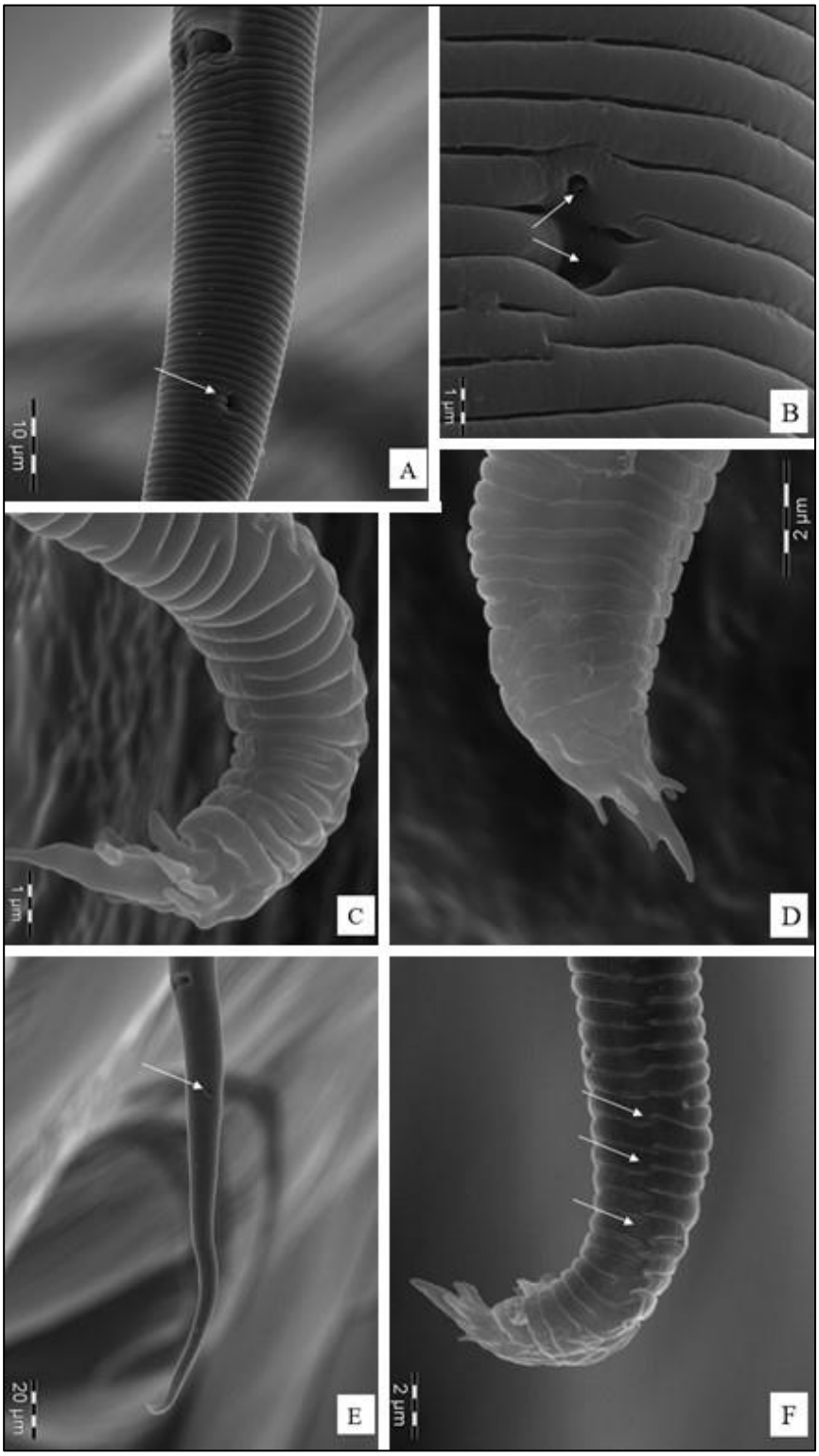


Figure 6.12: Scanning electron micrograph of *Chronogaster africana* Heyns & Coomans, 1980 female, A: Posterior end showing anus and arrow indicating caudal pore; B: Close up of caudal pore arrows indicating two possible openings; C, D, F: Tail tip showing projections, arrows indicate anastomosis; E: Posterior end indicating anus and caudal pores (arrow).

6.4.2.2 *Chronogaster aspinata* Raski & Maggenti 1984

Figure 6.13

Measurements: Table 6.5

Description:

Female (n=2): Body 1203-1127 µm long, ventrally curved after fixation with tail hook-like appearance. Cuticle annulated, annuli 1-2 µm wide in neck region, 2 µm at mid-body, 1 µm on dorsal side of the tail. Lip region 7 µm wide bearing 7 µm long cephalic setae. Stoma 7 µm long and 3 µm wide. Amphids oval with two protrusions on posterior side, about 3 annuli from anterior end. Radial tubules 18-22 µm from base of stoma. Excretory pore not observed. Oesophagus 235-243 µm long, ending in a 22 µm long and 10-14 µm wide basal bulb. Basal bulb contains nine fine denticles in anterior half. Post-bulbar extension 15-22 µm long overlapping a 5 µm long cardia. Vulva situated at 48-51 % of the body, genital tract monodelphic. Anterior tract 9-11 % of total body length; post-uterine branch is 7 µm long. Vulva indistinct from surrounding body annuli, vagina 7 µm long. Rectum 21-26 µm long, twice anal body width. Tail 135-165 µm long ending in finely rounded tip.

Males: Not observed

Discussion:

The *Chronogaster* specimens from Telperion were identified as *C. aspinata* based on the following: body length (1203-1127 µm); cephalic setae length (6.5 µm); finely rounded tail terminus without a mucro or additional spines and no longitudinal ridges. Two female specimens of *C. aspinata* was found in Telperion, one of which matches the type description from Raski & Maggenti (1984) exactly. The second female differs in the following: higher a-value (67 *vs* 55-72); lower c-value (7.3*vs* 8.5-9.1) and slightly anterior position of vulva (V = 48 % *vs* V = 50-52 %). *Chronogaster aspinata* was

described in soil from a rice field in India. Tail shape in the genus *Chronogaster* is a useful diagnostic characteristic and *C. aspinata* is one of three species that have a tail terminus that is finely rounded without a mucro or additional spines. The other species with the same tail characteristics are *C. alata* Gerlach, 1954 and *Chronogaster lissa* Loof, 1973.

Locality and material examined: This is the first time the species has been reported in South Africa and the second report for this species since its description. It was found in sediment from the Wilge River as well as a wetland on the Telperion Nature Reserve (Table 6.1) with locality numbers MP00038 and MP00013. Specimens were deposited in the NCN (ARC- PHP, Biosystematics, Pretoria) on slide numbers 50564-50565.

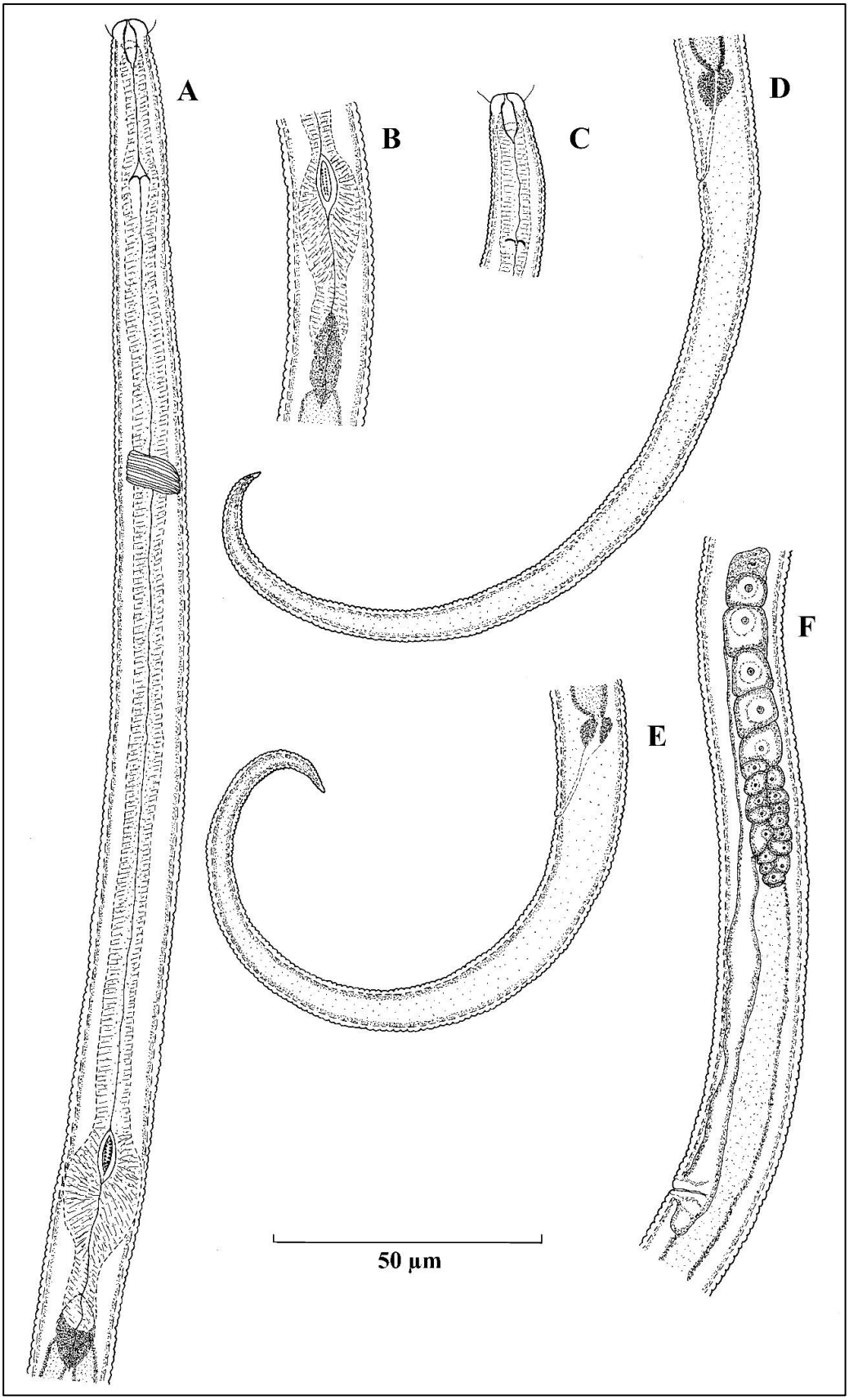


Figure 6.13: *Chronogaster aspinata* Raski & Maggenti 1984 A: Anterior region with oesophagus; B: Basal bulb with denticles and postbulbar extension; C: Labial region; D-E: Tail region; F: Genital tract.

Table 6.5: Morphometrics of female specimens of *Chronogaster africana* Heyns & Coomans, 1980 and *Chronogaster aspinata* Raski & Maggenti, 1984 found in freshwater sediment in the Telperion Nature Reserve.

Species	***Chronogaster africana***	***Chronogaster aspinata***	
Morphometric feature	**n = 1**	**n = 2**	
L	1661	1203	1127
L'	1465	1036	990
a	68.7	75	66.7
a'	60.6	64.4	58.6
b	6.2	5	4.8
c	8.2	7.3	8.4
c'	10.4	15	11.2
V	50	48	51
G1	9	9	11
Body width at anus	19	11	12
Body width at mid body	24	16	17
Body width at vulva	26	17	17
Annule width at neck region	1	1	2
Annule width at base of oesophagus	2	2	2
Annule width at mid body	2	2	2
Annule width opposite anus	2	2	2
Annule width on tail	1	1	1
Lip region height	5	3	3
Lip region width	9	7	7
Cephalic setae length	7	–	7
Stoma length	11	7	7
Stoma width	3	2	3
Radial tubules from base of stoma	23	22	18
Excretory pore from anterior	153	–	–
Basal bulb length	27	22	22
Basal bulb width	17	14	10
Oesophagus length	269	243	235
Postbulbar extension	16	15	22
Cardia length	–	5	5
Vagina length	9	–	7
Length of anterior genital tract	153	107	118
Length of post-uterine branch	12	–	7
Rectum length	–	21	26
Tail length	202	165	135
Rectum / width at anus	–	1.9	2.12

– Indicates structure not present or not measured.

6.4.3 Family Aporcelaimidae Heyns, 1965

6.4.3.1 *Makatinus africanus* n. sp.

Figures 6.14 & 6.15

Measurements: Table 6.6

Description:

Female (n = 6): Habitus strongly curved ventrally. Body 3228-4128 µm long and moderately thick (a = 30-39). Cuticle 6-7 µm thick in oesophageal region, 7-11 µm at mid-body and 9-11 µm on dorsal side of tail. Cuticle consisting of two distinct layers, a thin outer layer and a thicker inner layer with prominent radial lines that end as fine punctations on the outer surface. Lateral chord 15-20 µm wide, occupying about 15-20% of the corresponding body width at mid-body. Three dorsal and three ventral pores always present at level of odontostylet. The dorsal pores (6-8 pores) are always restricted to the oesophageal region, whereas the ventral pores (24-33 pores) are distributed over the entire body, with 12-16 in oesophageal region. Lateral pores abundant and distributed over entire body, four at level of spear and 12-16 in oesophageal region. Lateral pores over rest of body difficult to distinguish, less distinct due to musculature of body and presence of lateral chord. Lips amalgamated, lip region set off by slight constriction, 6-10 µm high and 27-29 µm wide. Amphid stirrup shaped, aperture 11-14 µm wide, occupying less than half corresponding body width. Odontostylet strong, 31-34 µm long (measured medially from apex to base of dorsal side) and odontophore 52-65 µm long. Odontostyle aperture occupies 60-70% of the total odontostyle length. Guide ring simple, situated 12-18 µm from anterior end. Oesophagus muscular, 747-882 µm long, gradually expanding in posterior half, basal expansion more muscular, 51-61 µm wide at its widest point. Oesophageal gland nuclei obscure due to muscular basal expansion. Nerve ring not observed. Cardia 32-37 µm long, tongue-like expansion at base of oesophagus. Genital tract didelphic-amphidelpic with both branches equal in length. Vulva situated at 47-52 % of the total body length. Vagina more than half the corresponding body width, consisting of 4-6 µm long *pars distalis vaginae,* 12-18 µm *pars refringens vaginae* and 37-57 µm *pars*

proximalis vaginae. Pars refringens vaginae consists of two large sclerotized pieces. Uterine eggs observed in most females, measuring 118-124 µm long and 62-65 µm wide. Ovijector muscular and distinct. Uterus long and complex in structure consisting of two oval structures containing sclerotized structures / spines. First oval structure located at junction of ovijector and uterus, second structure smaller, located just before *pars dilatata uteri*. *Pars dilatata oviductus* distinct with few sperm cells. Oviduct distinct, ovaries reflexed, typical of dorylaimids. Oocytes arranged in single row except in growth zone. Length of pre-rectum 113-167 µm, that of rectum 63-76 µm. Tail short, 30-43 µm long, with digitate extension, 4-7 µm long. Two caudal pores present.

Male: Unknown.

Table 6.6: Morphometrics of *Makatinus africanus* n. sp. females found in freshwater sediment in the Telperion Nature Reserve, South Africa. All measurements in the form: mean ± standard deviation (range).

Morphometric feature	Holotype	5 Paratypes
L	3961	3775 ± 325.4 (3228–4128)
a	39.3	33.9 ± 3.1 (29.6–39.3)
b	4.7	4.6 ± 0.3 (4.2–4.9)
c	112.7	107.3 ± 15.5 (91.8–131.0)
c'	0.6	0.6 ± 0.1 (0.5–0.7)
V	51	50 ± 1.7 (47–52)
G1	17	16 ± 1.4 (14–17)
G2	16	16 ± 0.7 (16–17)
Lateral chord as percentage of corresponding body width	17	15 ± 2.5 (12–18)
Aperture as percentage of odontostyle	63.6	63 ± 3.7 (60–70)
Body width at vulva	104	113 ± 11.5 (97–130)
Body width at mid–body	101	112 ± 12.0 (94–126)
Body width at anus	56	59 ± 3.1 (56–63)
Cuticle width at neck	6	6 ± 0.5 (6–7)
Cuticle width at mid–body	7	8 ± 1.3 (7–11)
Cuticle width at dorsal side of tail	11	11 ± 0.8 (9–11)
Lateral chord width	17	17 ± 2.3 (15–20)
Ventral pores (total)	28	28 ± 3.5 (24–33)
Ventral pores (from anterior to end of spear)	3	3[a]
Ventral pores (from anterior to end of oesophagus)	14	14 ± 1.5 (12–16)

[a] Only measured in one specimen.

[b] Value same for all specimens.

Table 6.6 (continued): Morphometrics of *Makatinus africanus* n. sp. females found in freshwater sediment in the Telperion Nature Reserve, South Africa. All measurements in the form: mean ± standard deviation (range).

Morphometric feature	Holotype	5 Paratypes
Dorsal pores (total)	6	7 ± 0.8 (6–8)
Dorsal pores (spear)	3	3 ± 0.0 (3)
Dorsal pores (oesophagus)	6	7 ± 0.8 (6–8)
Lateral pores (spear)	4	4 [a]
Lateral pores (oesophagus)	12	15 ± 1.6 (12–16)
Lip region height	9	8 ± 1.4 (6–10)
Lip region width	28	28 ± 0.7 (27–29)
Amphid aperture width	11	12 ± 1.1 (11–14)
Amphid aperture / corresponding width	38.8	43 ± 5.5 (38–50)
Odontostyle length	31	32 ± 1.1 (31–34)
Odontostyle length (ventral)	27	28 ± 0.5 (27–29)
Odontostyle length (dorsal)	31	32 ± 1.1 (31–34)
Odontophore length	59	60 ± 4.3 (52–65)
Odontostyle aperture length	20	20 ± 1.0 (19–21)
Guide ring from anterior	18	15 ± 2.5 (12–18)
Oesophagus length	851	816 ± 53.4 (747–882)
Basal bulb length	444	444 [b]
Basal bulb width	61	56 ± 7.2 (51–61)
Cardia length	35	35 ± 1.9 (32–37)
Vulva from anterior	2006	1871 ± 210.5 (1512–2067)
Anterior genital tract length	672	614 ± 100.2 (436–720)
Posterior genital tract length	633	605 ± 53.3 (543–666)
Egg length	118	121 ± 2.8 (118–124)
Egg width	62	63 ± 2.1 (62–65)
***Pars distalis vaginae (pdv)* length**	6	5 ± 0.8 (4–6)
***Pars refringens vaginae (prv)* length**	12	15 ± 2.4 (12–18)
***Pars proximalis vaginae (ppv)* length**	37	43 ± 9.4 (37–57)
Vagina length	55	63 ± 10.1 (55–78)
Vagina as percentage of corresponding body width	52.7	59 ± 7.1 (53–68)
Pre–rectum length	167	143 ± 18.1 (113–167)
Rectum length	65	67 ± 4.6 (63–76)
Tail length	35	36 ± 4.6 (30–43)
Number of caudal pores	2	2 [a]
Digitate extension length of tail	7	5 ± 1.4 (4–7)

[a] Only measured in one specimen.

[b] Value same for all specimens.

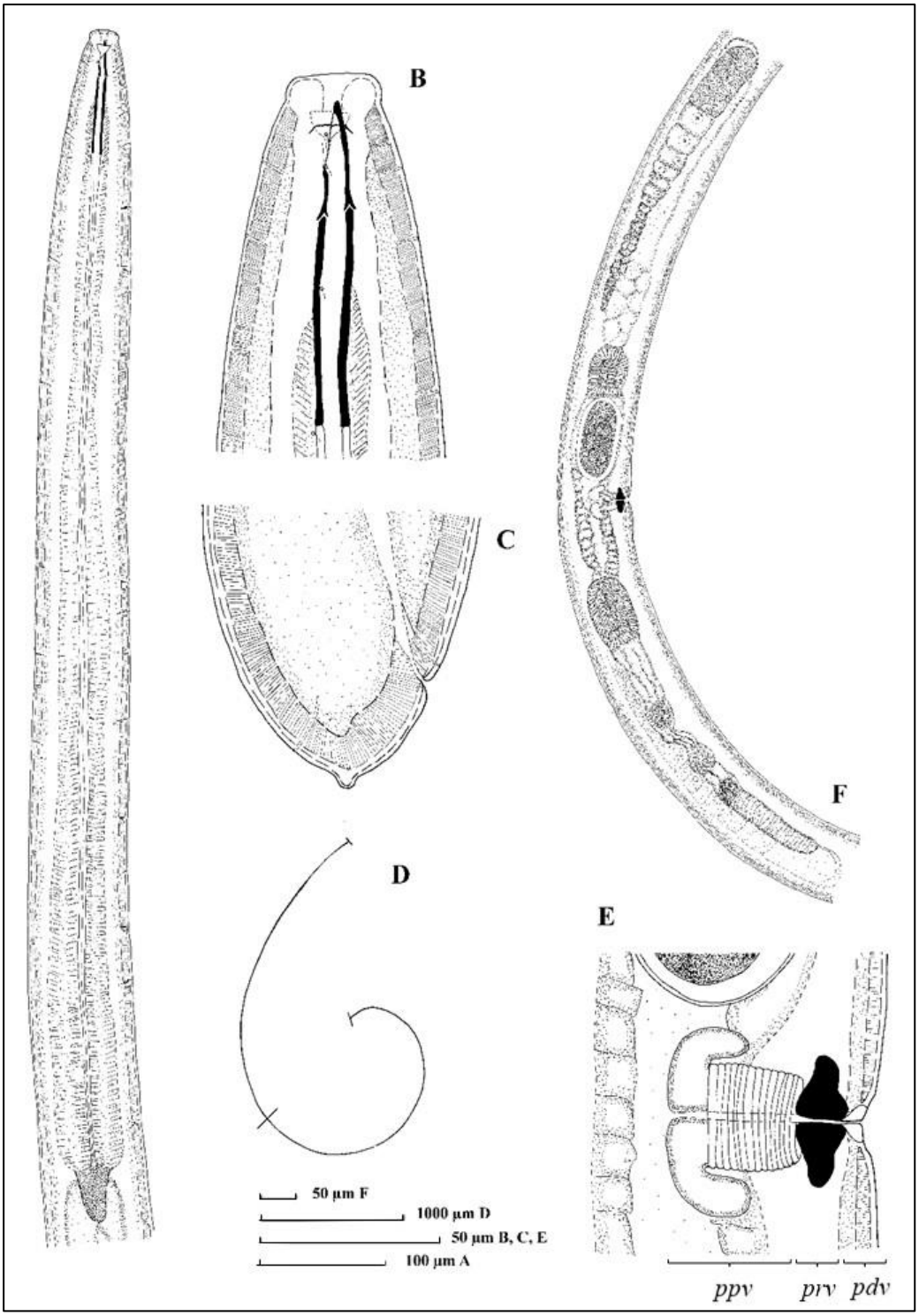


Figure 6.14: *Makatinus africanus* n. sp. female A: Anterior region with oesophagus; B: Anterior region with spear and position of amphid; C: Tail region; D: Habitus; E: Vulval region with *pars distalis vaginae* (*pdv*), *pars refringens vaginae* (*prv*) and *pars proximalis vaginae* (*ppv*); F: Reproductive system.

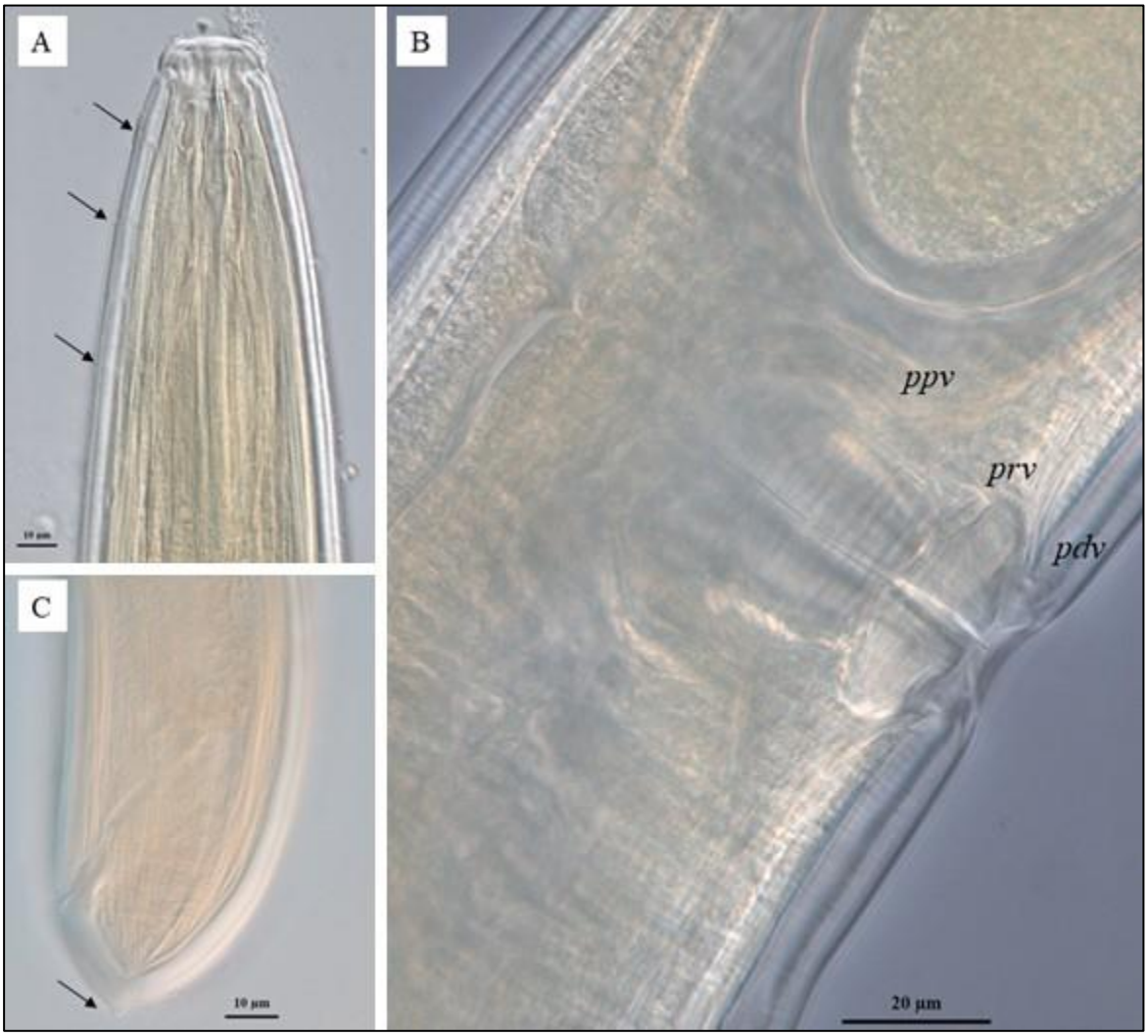


Figure 6.15: Light microscope photos of *Makatinus africanus* n. sp. female A: Anterior region arrows indicate dorsal median pores; B: Vulval region with *pars distalis vaginae* (*pdv*), *pars refringens vaginae* (*prv*) and *pars proximalis vaginae* (*ppv*); C: Tail region with arrow indicating peg.

Diagnosis and relationships:

Makatinus africanus n. sp. is characterised by a large body (3228-4128 µm long, a = 30-39 wide), slightly set off lip region with amalgamated lips; 31-34 µm long odontostyle; long tongue-shaped cardia, and stout, short tail (30-43 µm long) with a small peg / digitate extension. Male absent.

According to the latest compendium of the genus *Makatinus* there are eight valid species (Peña-Santiago & Varela 2017). The type females of these species were compared to *Makatinus africanus* n. sp. and the following differences were observed. Based on the presence of a digitate structure on the tail, the closest related species to *Makatinus africanus* n. sp. is *M. punctatus* Heyns, 1965. *Makatinus africanus* n. sp. differs from *M. punctatus* in the longer body (3228-4128 µm *vs* 2400-3200 µm); longer odontostyle (31-34 µm vs 25-27 µm) and medial position of vulva (V = 47-52 % *vs* 56-59 %) (Heyns 1965). In large body length *Makatinus africanus* n. sp. is also close to *M. capensis* Heyns, 1965, and *M. crassiformis* (Kreis, 1924) Andrássy, 1986. The new species can however be distinguished from *M. capensis* in the longer odontostyle (31-34 µm *vs* 24-28 µm), less ventral pores (24-33 *vs* 48-50) and digitate structure on the tail tip (absent in *M. capensis*) (Heyns 1965). The new species can be distinguished from the type females of *M. crassiformis* in the higher a-value or thinner body (29.6-39.3 *vs* 21-27); higher b-value (4.2-4.9 *vs* 3.6-4.3) and long tail with digitate extension (30-43 µm *vs* 26 µm without digitate extension) (Andrássy 1986; Peña-Santiago & Varela 2017). The following, remaining species differ from *Makatinus africanus* n. sp. in having a body shorter than 2700 µm ; an odontostyle shorter than 28 µm and the absence of a digitate structure on the tail: *M. aquaticus* Jiménez-Guirado, 1994 (L=1920-2260 µm, odontostyle 22-26 µm long and absence of digitate structure on tail) (Peña-Santiago & Varela 2017); *M. heynsi* Ahmad & Ahmad, 1992 (L=1960-2420 µm in length, 21-25 µm long odontostyle and digitate structure on tail absent) (Ahmad & Ahmad 1992); *M. minor* (Loos, 1945) Ahmad, 1997 (L=1900-2200 µm, 21-28 µm long odontostyle and digitate structure on tail absent)(Ahmad 1997, Peña-Santiago & Varela 2017); *M. silvaticus* Ahmad, Sturhan & Wouts, 2003 (L=2350-2720 µm, 25-28 µm long odontostyle and digitate structure on tail absent) (Ahmad *et al.* 2003). *Makatinus africanus* n. sp. could not be compared to *M. goodeyi* Jiménez-Guirado, 1994 as the latter

species is described from males only. However, based on the male body length (2200 µm in *M. goodeyi*) and the length of its odontostyle (22 µm in *M. goodeyi*) it is unlikely that the females found in the Telperion Nature Reserve belongs to the males of *M. goodeyi* (Peña-Santiago & Varela 2017).

Type material: Holotype female on slide 50559 and paratypes (5 females) on slides 50558-50559 deposited in the NCN (ARC- PHP, Biosystematics Division, Pretoria).

Type locality: Specimens were found in sediment and water samples collected at the Waterhole 2 site (Table 6.4.1). It is located next to road 11_26 in the Blaauwpoort 1 section of the reserve and in high rainfall seasons water flows downstream from the Wilge River and collects in this site. Locality number MP00127 in the NCN (ARC- PHP, Biosystematics Division, Pretoria).

Etymology: *Makatinus africanus* n. sp. is named after the continent of origin.

6.4.4 Family Ironidae De Man, 1876

6.4.4.1 *Ironus telperionensis* n. sp.

Figures 6.16-6.18

Measurements: Table 6.7

Description:

Female (n = 11): Habitus mostly straight, sometimes slightly curved ventrally with a strong ventral curl of tail tip, 1456-1864 µm long. Body moderately slender, 33-39 µm wide at mid body (a = 37-53). Cuticle smooth, measuring 0.7 -1.5 µm at anterior level of stoma, 1.5-1.8 µm at level of mid body and 0.7-1.8 µm on dorsal side of tail. Labial region 5-7 µm high and 11-14 µm wide. Labial region

(*en face*) with six cephalic papillae, four cephalic setae (hollow tubes measuring 4-6 µm) at same plane. Six labial papillae encircles stoma opening. Body at base of oesophagus three times as wide as labial region. Amphid situated 5-8 µm from the anterior end, stirrup shaped, aperture less than half the corresponding body width. Stoma a long, sclerotized tube, 87-129 µm, from anterior end, 20-31 % of the oesophagus length and 5-8 % of the total body length. Three hook-like teeth (3-6 µm) at anterior end of stoma. Oesophagus mostly cylindrical, widening to posterior base, 373-446 µm long (excluding 8-18 µm long cardia) and 16-24 µm wide at widest point. Nerve ring prominent, 140-153 µm from anterior; excretory pore not observed. Lateral body pores, 48 in total (24 pairs), observed with SEM. First five pores in line with rest arranged irregularly along rest of the body: 16 pairs from anterior to vulva, five between vulva and anus, and three on tail. First lateral body pore situated just posterior to amphid aperture (2 µm). Reproductive system didelphic-amphidelpic, ovaries reflexed. Anterior branch 11-19 % of body length and posterior branch 12-21 %. Vulva equatorial, transversal slit, one third of the corresponding body width with slightly protruding lips. Vagina 8-15 µm long and only 8-15 % of the corresponding body width. Uterine egg seen in only one female, large, 161 µm long and 31 µm wide. Ovijector distinct and slightly muscular, uterus, oviduct and sphincter muscles not well developed. Ovaries reflexed and arranged in single oocytes. Anus a deep transversal slit, about a third of corresponding body width. Tail 212-257 µm long, straight for most of length, narrowing to a strong ventral curl of the tip. Conical for more than half its length, narrowing to filiform tip. Tail 10-15 times anal body width.

Male: Unknown.

Table 6.7: Morphometrics of *Ironus telperionensis* n. sp. females found in freshwater sediment in the Telperion Nature Reserve, South Africa. All measurements in the form: mean ± standard deviation. (range). – indicates structures that were not visible or not present

	Holotype	10 Paratypes
L	1714	1724 ± 107.6 (1456–1864)
L'	1482	1489 ± 102.7 (1244–1612)
a	51.8	49.2 ± 4.3 (37.4–53.0)
a'	44.8	42.5 ± 3.9 (31.9–46.1)
b	3.9	4.0 ± 0.1 (3.9–4.2)
b'	3.3	3.5 ± 0.1 (3.3–3.6)
c	7.4	7.3 ± 0.5 (6.5–8.0)
c'	12.4	12.6 ± 1.6 (9.5–15.2)
V	50.7	7.74 ± 1.2 (49.1–53.7)
V'	58.6	58.8 ± 1.6 (56.5–62.9)
G1	11.9	14.5 ± 2.4 (11.2–19.3)
G2	15.0	15.3 ± 2.9 (12.0–21.5)
Stoma as percentage of body length	7.3	6.8 ± 1.0 (4.8–8.1)
Stoma as percentage of body length (L')	8.5	7.8 ± 1.2 (5.5–9.4)
Stoma as percentage oesophagus length	28.1	27.0 ± 3.8 (19.7–31.4)
Body width at base of oesophagus	32	35 ± 2.2 (32–39)
Body width at anus	19	19 ± 1.5 (17–22)
Body width at mid–body	33	35 ± 1.9 (33–39)
Body width at vulva	33	35 ± 1.4 (33–37)
Cuticle width at neck	1	1.1 ± 0.3 (0.7–1.5)
Cuticle width at mid–body	1	1.5 ± 0.2 (1.5–1.8)
Cuticle width at dorsal side of tail	1	1.2 ± 0.3 (0.7–1.8)
Lip region length	5	6 ± 0.7 (5–7)
Lip region width	13	12 ± 0.9 (11–14)
Amphid aperture width	5	5 ± 0.6 (4–6)
Cephalic setae length	4	5 ± 0.6 (4–6)
Amphid from anterior end	7	7 ± 0.9 (5–8)
Tooth length	4	4 ± 0.8 (3–6)
Stoma length	125	116 ± 14.6 (87–129)
Stoma width	4	4 ± 0.4 (4–5)
Oesophagus length	445	432 ± 20.5 (373–446)
Basal bulb width	20	20 ± 2.6 (16–24)
Nerve ring from anterior	150	147 ± 4.1 (140–153)
Cardia length	15	13 ± 3.1 (8–18)
Vulva from anterior	869	874 ± 43.1 (782–948)
Vagina length	11	12 ± 2.2 (8–15)
Vagina/ corresponding body width	32.6	34.4 ± 5.5 (22.8–41.2)
Egg length	–	161[a]

[a] Only measured in one specimen.

– Indicates structure not present or not measured.

Table 6.7 (continued): Morphometrics of *Ironus telperionensis* n. sp. females found in freshwater sediment in the Telperion Nature Reserve, South Africa. All measurements in the form: mean ± standard deviation. (range). – indicates structures that were not visible or not present

	Holotype	10 Paratypes
Egg width	–	31[a]
Anterior genital tract length	205	255 ± 47.2 (204–359)
Posterior genital tract length	257	267 ± 49.8 (215–368)
Rectum length	25	26 ± 1.4 (24–27)
Tail length	232	235 ± 14.5 (212–257)
Tail/body width at anus	12.4	12.6 ± 1.6 (9.5–15.2)

[a] Only measured in one specimen.

– Indicates structure not present or not measured.

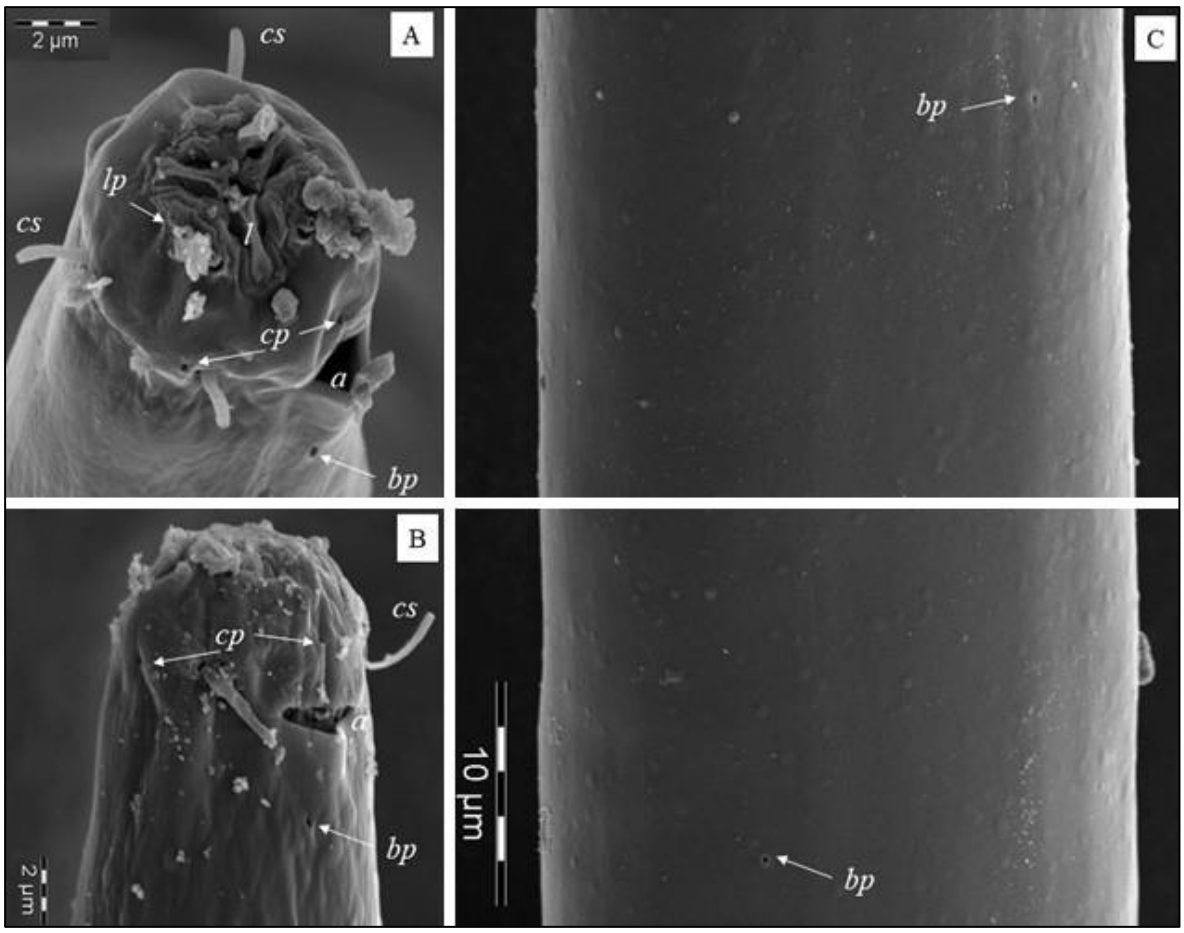


Figure 6.16: Scanning electron micrograph of *Ironus telperionensis* n. sp. female A: *En face* view of labial region with cephalic setae (cs), cephalic papillae (cp), labial papillae (lp), liplets (l), amphid (a) and fist body pore (bp); B: Lateral view of labial region with cephalic setae (cs), cephalic papillae (cp), amphid (a) and fist body pore (bp); C: Lateral view of mid-body region with two body pores (bp).

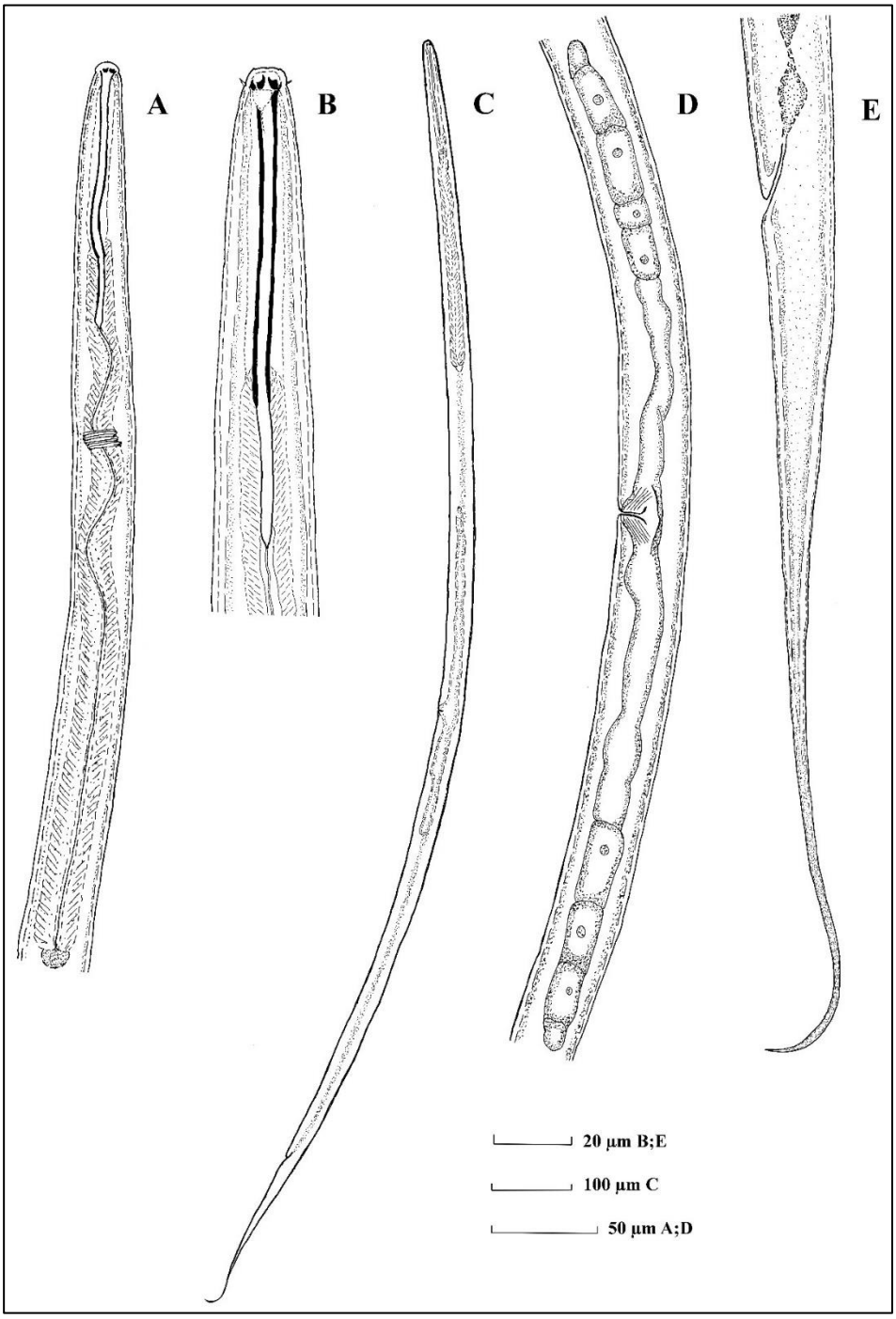


Figure 6.17: *Ironus telperionensis* n. sp. female A: Anterior region with oesophagus; B: Anterior region with position of teeth, stoma and position of amphid; C: Whole female body indicating habitus; D: Genital tract; E: Tail.

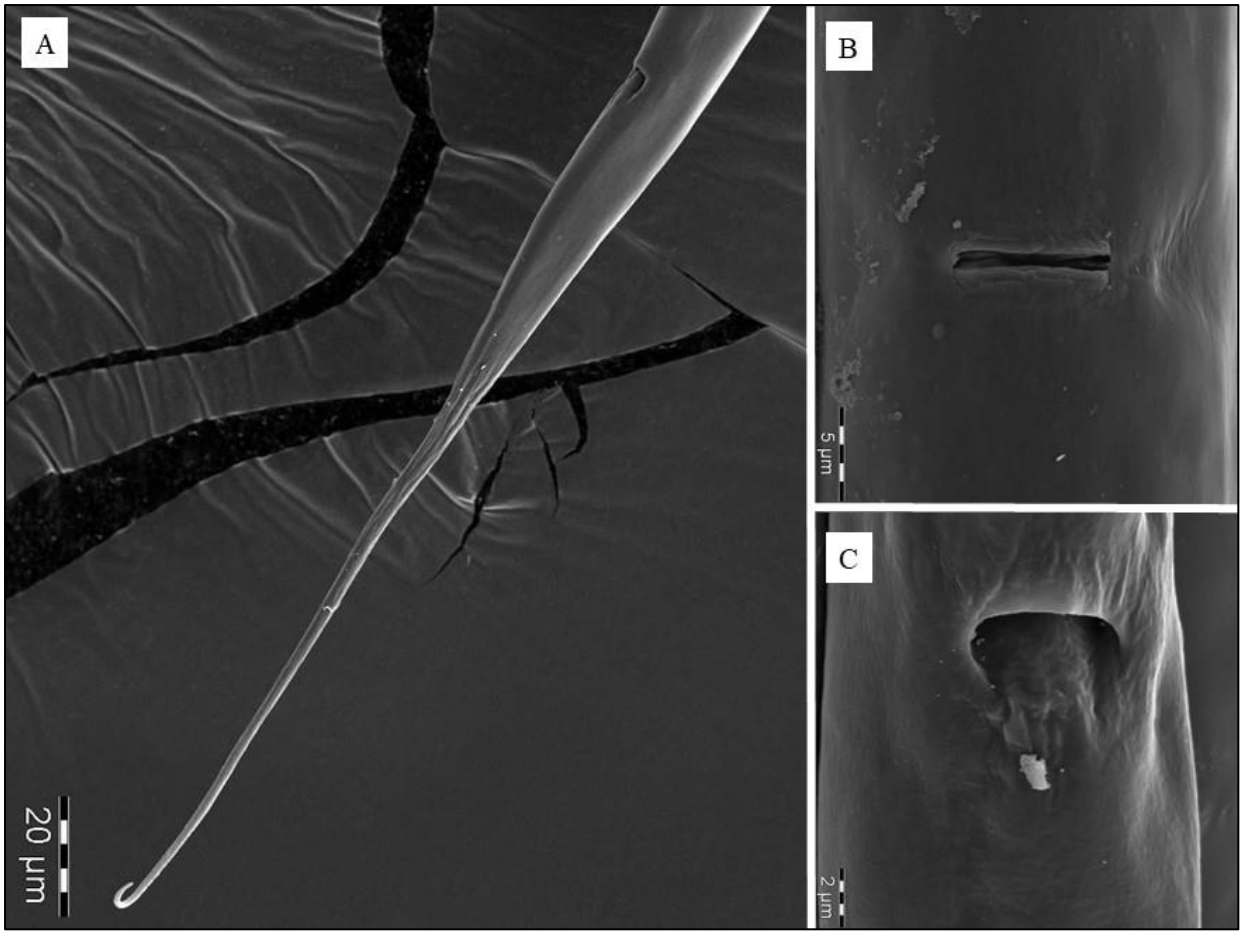


Figure 6.18: Scanning electron micrograph of *Ironus telperionensis* n. sp. female A: Tail region; B: Vulva ventral view; C: Anus ventral view.

Diagnosis and relationships:

Ironus telperionensis n. sp. is characterised by a long (1456-1864 µm), slender body (50 times longer than wide, a = 37-53), long stoma (87-129 µm), long oesophagus (373-446 µm), absence of body crystals, equatorial vulva, paired reproductive system, branches equal in length, tail length (212-257 µm) or 12 anal body widths long, curled ventrally at the tip.

Ironus telperionensis n. sp. is similar to seven other species. These are *Ironus americanus* Cobb, 1914; *I. crassatus*; *I. dentifurcatus*; *I. gagarini* Tsalolikhin, 1987; *I. ignavus*; *I. leoborkini* Tsalolikhin, 1987; and *I. paludicola* Schneider, 1937 in having a body less than 2500 µm long, paired ovaries, and an elongate-conoid tail shorter than 20 anal body widths. *Ironus telperionensis* n. sp. is morphologically the most closely related to *I. ignavus* and *I. paludicola*. However, the new species can be separated from *I. ignavus* based on lower c-value (6.5-8.0 *vs* 8.0-11.0); longer stoma (88-129 µm *vs* 76-90 µm); morphology of the tail (212-257 µm long, conoid for more than half its length, then filiform *vs* 180-225 µm long and conoid for less than half its length, then filiform) and presence of males (present in *I. ignavus*). *Ironus telperionensis* n. sp. can be distinguished from *I. paludicola* by a lower a-value (37.4-53.0 *vs* 60.0-80.0); lower c-value (8.0-13.0 in *I. paludicola*) and stoma length (73-100 µm in *I. paludicola*). Although there is an overlap in these measurements, it must be noted that only two of the specimens found at Telperion exhibited a stoma length shorter than 100 µm. *Ironus telperionensis* n. sp. can be distinguished from *I. americanus* by longer body (1456-1864 µm *vs* 2300-2500 µm), shorter stoma (mean length 116 µm *vs* 85 µm) and longer tail (212-257 µm *vs* 160-170 µm). *Ironus telperionensis* n. sp. differs from the South African species *I. crassatus* in tail length ([186]-670 µm in *I. crassatus*) and lip region width (11-14 µm *vs* 14-21 µm). The new species can be distinguished from a second South African (*I. dentifurcatus*) species by a slightly shorter tail ([207]-520 µm in *I. dentifurcatus*) and lip region width (13-19 µm in *I. dentifurcatus*). Measurements in blocked brackets indicate specimens with obvious broken tails according to Andrássy (2011). *Ironus telperionensis* n. sp. differs from *I. gagarini* in longer stoma (68-76 µm in *I. gagarini*) and longer tail (125-190 µm in *I.*

gagarini). *Ironus telperionensis* n. sp. is separated from *I. leoborkini* by the longer stoma l (77-83 µm in *I. leoborkini*) and shorter tail (275-310 µm in *I. leoborkini*).

Type material: Holotype female on slide 50571 and paratypes (10 females) on slides 50571-50575 deposited in the NCN (ARC- PHP, Biosystematics, Pretoria).

Type locality: Specimens were found in sediment and water samples collected at the waterhole 2 site (during winter, summer and autumn) (Table 6.4.1). It is located next to road 11_26 in the Blaauwpoort 1 section of the reserve and in high rainfall seasons water flows downstream from the Wilge River and collects in this site. Locality numbers MP00031, MP00091 and MP00121 in the NCN (ARC- PHP, Biosystematics, Pretoria).

Etymology: *Ironus telperionensis* n. sp. named after the Telperion Nature Reserve where specimens were found.

Discussion (Remarks and notes on *I. ignavus* in South Africa):

Ironus ignavus has been previously reported from South Africa by Botha & Heyns (1993) and these specimens were identified based on descriptions by Andrássy (1964;1968). Andrássy (1968) stated that the specimen found had an unusually long stoma for *I. ignavus* but matched the description of Andrássy (1964) of specimens from mountains in Kenya, Uganda and the Democratic Republic of the Congo. However, according to Van der Heiden (1974) the specimen of Andrássy (1964) was conspecific with *I. tenuicaudatus*. Therefore, *I. ignavus* described by Botha & Heyns (1993) is conspecific with the *I. tenuicaudatus* described by Andrássy (1964). The presence of *Ironus ignavus* in South Africa is therefore questionable.

6.4.5 Family Tobrilidae De Coninck, 1965

6.4.5.1 *Eutobrilus annetteae* (Joubert & Heyns, 1979) Tsalolikhin, 1981

Figures 6.19-6.21

Measurements: Table 6.8

Description:

Female (n = 18): Body 1049-1547 µm long, habitus C-shape, bent ventrally or dorsally. Cuticle 1-4 µm thick at mid-body, annulated. Body with several somatic setae, the first 14-63 µm from anterior end. Lip region 17-23 µm wide, cephalic setae 4-6 µm long. *En face* view of labial region shows first circlet of six labial papillae, second circlet of 10 cephalic setae consisting of six long cephalic setae and four slightly shorter cephalic setae. Amphid stirrup shaped with slit-like aperture, 14 µm from anterior. Stoma 22-27 µm long, with wide barrel shaped buccal cavity, 6-11 µm wide followed by two overlapping tooth pockets. The first tooth 10-19 µm from anterior end, second tooth 2-9 µm posterior from first. Oesophagus cylindrical, muscular, 225-300 µm long. Nerve ring situated 102 µm from anterior. Cardia 12-20 µm long with three prominent cells situated at junction of cardia and oesophagus. Excretory pore not observed. Reproductive system didelphic-amphidelphic, anterior and posterior tract equal in length. Vulva a transversal slit, equatorial at 41-50 % of the body. Vagina less than half corresponding body width. Vaginal musculature simple, weakly developed. Uterus simple, sphincter muscles indistinct. Ovaries reflexed with oocytes in a single row except on growth point. Rectum 23-33 µm long, slightly longer than anal body width. Tail 210-253 µm long ending in a 3-5 µm spinneret, subterminal setae absent. Three prominent caudal glands present in tail region.

Male: Not found.

Table 6.8: Morphometrics of three populations of *Eutobrilus annetteae* (Joubert & Heyns, 1979) Tsalolikhin, 1981 females found in freshwater sediment in the Telperion Nature Reserve, South Africa. All measurements in the form: mean ± standard deviation (range).

Locality	**MP00038**	**MP00013**	**MP00091**
Morphometric feature	**n = 1**	**n = 14**	**n = 3**
L	1343	1396 ± 118.9 (1049–1547)	1411 ± 56.1 (1353–1456)
a	31.3	33.1 ± 3.0 (26.2–37.3)	32.8 ± 3.0 (30.6–36.2)
a'	25.9	27.5 ± 2.8 (20.8–31.8)	27.8 ± 2.5 (26.0–30.6)
b	5.13	5.2 ± 0.3 (4.7–5.5)	4.8 ± 0.1 (4.7–4.9)
c	5.73	5.9 ± 0.5 (4.9–6.7)	6.5 ± 0.1(6.4–6. 7)
c'	9.10	9.6 ± 0.4 (8.9–10.4)	9.6 ± 0.4 (9.1–9.8)
V	44	44 ± 2.3 (41–60)	46 ± 0.9 (45–47)
G1	7	8 ± 3.1 (5–18)	11 ± 0.2 (11–12)
G2	7	8 ± 1.2 (6–10)	9 ± 0.9 (8–10)
Body width at mid–body	43	42 ± 2.5 (39–48)	43 ± 5.1 (37–46)
Body width at vulva	43	42 ± 2.5 (39–48)	43 ± 5.1 (37–46)
Body width at anus	26	25 ± 1.5 (21–27)	23 ± 1.8 (22–25)
Cuticle width at neck	2	2 ± 0.3 (1–2)	2 ± 0.5 (1–2)
Cuticle width at mid–body	2	3 ± 0.7 (1–4)	3 ± 0.7 (2–3)
Cuticle width at dorsal side of tail	3	3 ± 0.6 (2–4)	3 ± 0.2 (2–3)
Amphid from anterior	–	14[a]	–
Long cephalic setae length	4	5 ± 0.7 (4–6)	4 ± 0.3 (4–5)
Short cephalic setae length	1	2 ± 0.4 (1–3)	–
Lip region width	21	19 ± 1.5 (17–23)	19 ± 0.5 (18–20)
Stoma length	22	25 ± 1.3 (22–27)	23 ± 0.3 (22–23)
Stoma width	8	8 ± 1.0 (6–11)	9 ± 0.8 (8–10)
First tooth from anterior	16	18 ± 0.9 (16–19)	14 ± 3.6 (10–17)
Second tooth from anterior	22	21 ± 1.1 (18–22)	20 ± 0.8 (19–20)
Distance between teeth	6	3 ± 0.5 (2–4)	6 ± 2.9 (3–9)
First body setae from anterior		38 ± 10.0 (27–63)	19 ± 6.7 (14–24)
Nerve ring from anterior	–	–	102 [a]
Oesophagus length	262	269 ± 16.2 (225–288)	293 ± 7.1 (286–300)
Cardia length	14	17 ± 2.1 (13–20)	17 ± 3.9 (12–19)
Cardia width	21	22 ± 2.1(18–25)	20 ± 2.2 (18–23)
Distance from head to vulva	589	612 ± 55.3 (451–679)	645 ± 12.9 (632–658)
Anterior genital tract length	97	114 ± 50.7(75–286)	151 ± 5.9 (147–155)
Posterior genital tract length	92	112 ± 17.9 (88–151)	129 ± 19.8 (115–143)
Vagina length	14	15 ± 3.3 (10–21)	9 ± 4.0 (6–12)
Vagina as percentage of body width	33	35 ± 6.7 (24.6–46.4)	25[a]
Tail length	235	239 ± 11.5 (216–253)	216 ± 8.5(210–225)
Rectum length	32	29 ± 3.2 (23–33)	28 ± 6.0 (22–33)
Rectum / width at anus	1.2	1.2 ± 0.1 (0.9–1.4)	1.3 ± 0.2 (1.0–1.5)
Spinneret length	3	4 ± 0.5 (3–5)	4[b]

– Indicates structures that were not visible or not present.

[a] Indicates structure measured in only one specimen.

[b] Indicates value the same for all specimens.

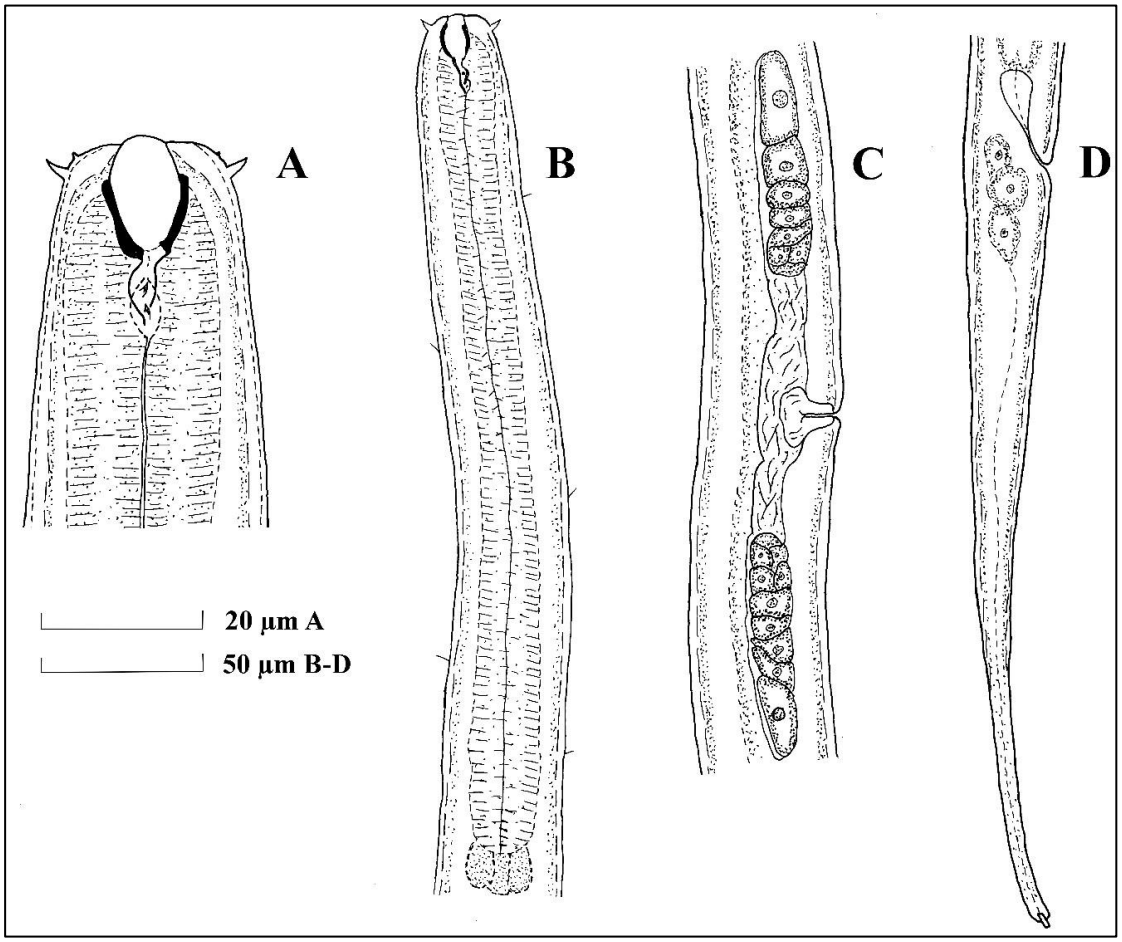


Figure 6.19: Female drawings *Eutobrilus annetteae* (Joubert & Heyns, 1979) Tsalolikhin, 1981 A: Labial region showing structure of stoma and tooth pockets; B: Anterior region showing oesophagus; C: Vulval region and genital tract; D: Tail.

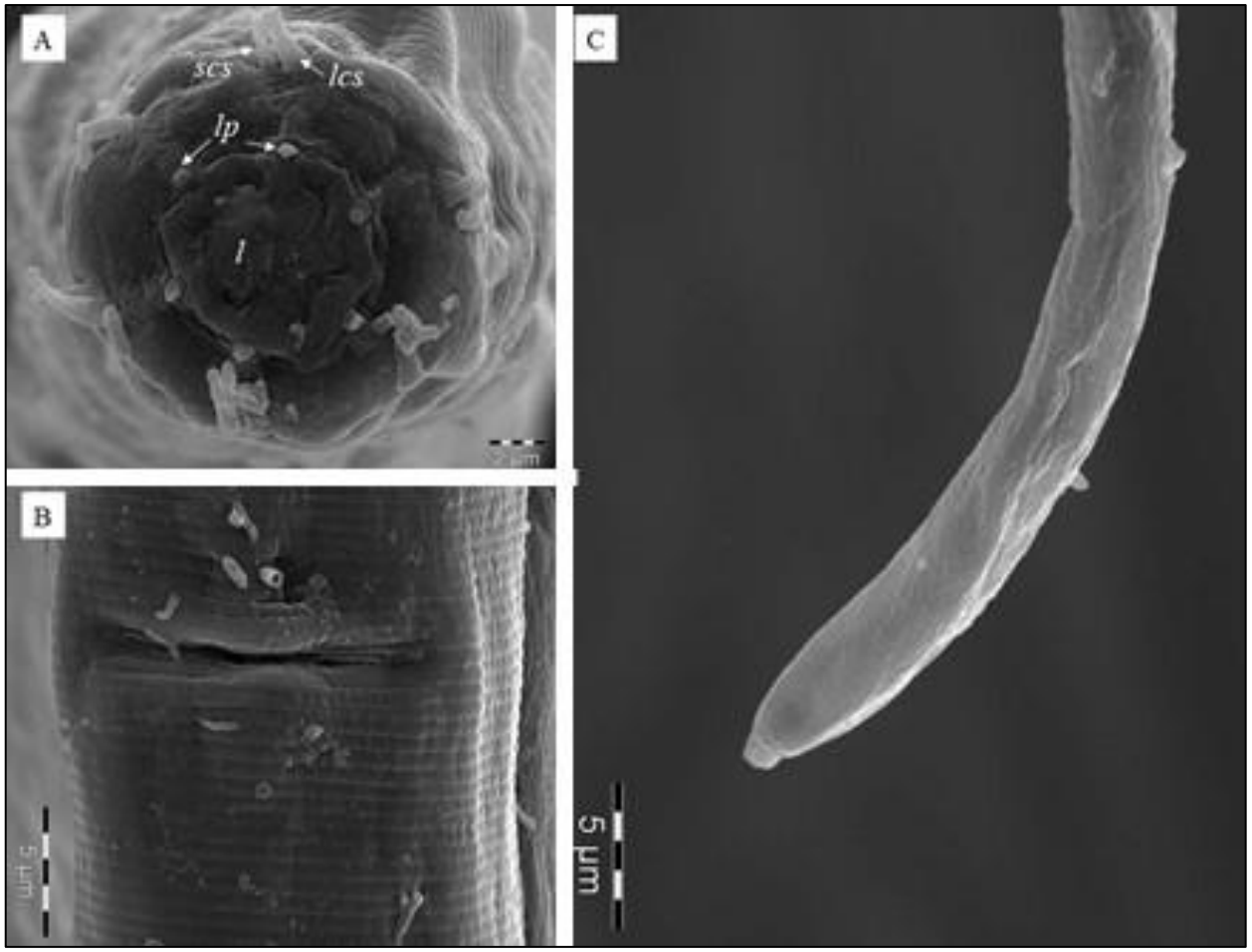


Figure 6.20: Scanning electron micrograph of *Eutobrilus annetteae* (Joubert & Heyns, 1979) Tsalolikhin, 1981, A: Anterior end showing long cephalic setae (*lcs*), short cephalic setae (*scs*) and labial papillae (*lp*) and liplets (*l*); B: Ventral side of body showing vulva (arrow); C: Tail region showing spinneret (arrow).

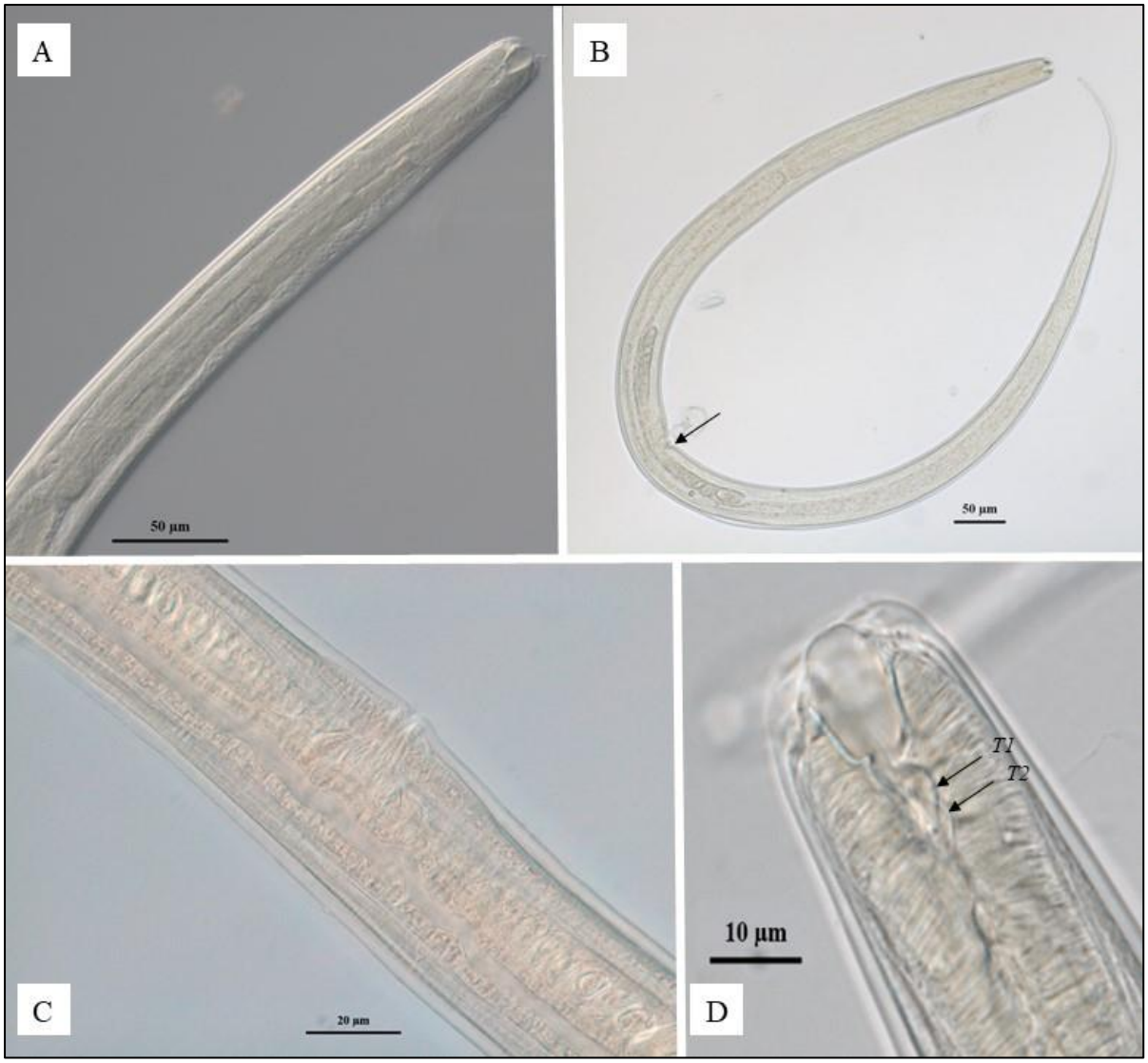


Figure 6.21: Light microscopic pictures of *Eutobrilus annetteae* (Joubert & Heyns, 1979) Tsalolikhin, 1981 female A: Anterior region indicating buccal cavity to cardia; B: Whole female specimen, arrow indicating vulva; C: Vulval region showing simple vaginal musculature; D: Labial region showing buccal cavity and two overlapping tooth pockets (*T1* and *T2*).

Discussion:

The *Eutobrilus* specimens found in the Telperion Nature Reserve were identified as *E. annetteae* based on morphometrics and morphology especially the simple morphology of the vagina, the absence of males, the cephalic setae being shorter than one third of the labial region width and two overlapping tooth pockets. Specimens found in Telperion Nature Reserve matches those from the type description as well as other described populations of *E. annetteae*. The Telperion Nature Reserve

population was compared to the type population in Joubert & Heyns (1979) as well as the populations in Botha & Heyns (1992); Eyualem-Abebe & Coomans (1995) and Yunliang & Coomans (2000). The Telperion population differs from the type population in the following aspects: slightly shorter body (1049-1547 µm *vs* 1400-1700 µm), shorter cephalic setae (4-6 µm *vs* 7-10.5 µm), narrower stoma (6.3-11 µm *vs* 14.9 µm). However, Botha & Heyns (1992) stated that this measurement of the stoma width was incorrect and measured the type material at 8.6 µm. Based on the morphometrics and morphology of other *E. annetteae* populations the Telperion Nature Reserve population falls within the range of the species, matching the morphology and morphometrics of all known populations except in the vagina length as percentage of corresponding body width (25-46 *vs* 54-64). The population from Telperion Nature Reserve is most similar to the populations of the Galápagos in Eyualem-Abebe & Coomans (1995). The morphometrics together with the South African origin and geographical distribution of this species, support the identification of the Telperion Nature Reserve *Eutobrilus* population as *E. annetteae*.

Locality and material examined: Specimens of *E. annetteae* were found at three sites i.e. river site (MP00038); wetland site (MP00013) and waterhole (MP00091) (Table 6.1) and are deposited in the NCN (ARC- PHP, Biosystematics, Pretoria) on slide numbers 50564,50565; 50574; 50577,50585.

6.4.5.2 *Neotobrilus ampiei* (Joubert & Heyns, 1979) Tsalolikhin, 1981

Figures 6.22 – 6.29

Measurements: Table 6.9

Description:

Female (n = 13): Habitus curved ventrally to various degrees, body 1868-2439 μm long. Cuticle 1-4 μm thick at mid-body, annulated. Somatic setae numerous, spread unevenly over entire body. First somatic setum located 24-41 μm from anterior end. Lip region 24-36 μm wide, cephalic setae 13-16 μm long. *En face* view of labial region indicates one circlet consisting of six labial papillae, and one circlet with six long cephalic setae and four short cephalic setae. Cephalic setae originate from the cuticle, while cephalic papillae originate subcuticular. All setae and papillae appear to be hollow tubes. Amphids stirrup shaped with slit-like apertures, located 17-19 μm from anterior end. Stoma 35-44 μm long, half the width of lip region, consisting of two parts: A distinct barrel-shaped buccal cavity separated by two separate pockets. Each pocket contains a tooth and are separated by a narrowing of the cavity. First tooth is 18-24 μm from anterior end with second tooth 13-16 μm posterior to first. Oesophagus cylindrical and muscular, 345-437 μm long. Nerve ring not observed. Cardia 22-36 μm long with three prominent cells situated at junction of cardia and oesophagus. Excretory pore not observed. Reproductive system didelpic-amphidelphic, anterior tract (195-392 μm long) almost always slightly shorter than posterior tract (228-430 μm). Vulva a transversal slit situated at 37-46 % of body. Vaginal musculature strong, vagina less than half corresponding body width. Advulval setae twice as long as somatic setae (mean length of 16.4 μm), located dorsally at one body width anterior and posterior to vulva. Uterine eggs observed in most female specimens, measuring 51-68 μm X 35-56 μm with shell. Eggshell uniquely sculptured with several prominent tubercules. *Pars dilatata oviductus* rounded, containing flaggelloid sperm cells. Ovaries reflexed, oocytes arranged in single row except at growth point. Tail 255-354 μm long ending in rounded tip with a 3-5 μm spinneret and a subterminal setum. Rectum 31-47 μm long.

Male (n = 5): Habitus curved ventrally, body 1990-2320 μm long. Morphology of stoma and oesophagus similar to that of female. Spicule 61-72 μm long, medial outgrowth from ventral limb towards dorsal limb. Gubernaculum 19-26 μm long. Reproductive system diorchic, sperm cells flaggelloid. *Ductus ejaculatoris* muscular, its length corresponds with length of supplementary row, never exceeding it. Tail 175-215 μm long. Two rows of subventral somatic setae along row of supplements, setae directed anteriorly and consisting of two parts. Supplementary row consisting of six pre-cloacal supplements, three larger and three closest to the cloaca, smaller. The supplements lose complexity nearing the cloaca. Supplement 1 to supplement 3 (starting anteriad) are complex in structure with several tubercules, whereas Supplement 4 to Supplement 6 less covered in tubercules and Supplement 6 the most simplistic. Distance between Supplement 2 and Supplement 3 is the shortest. Micro papillae present between supplements Supplement 3 and Supplement 4 (10-11 micropapillae), Supplement 4 and Supplement 5 (4-5 micropapillae) and Supplement 5 and Supplement 6 (5-6 micropapillae). According to SEM micropapillae appear as thin transversal slits.

Table 6.9: Morphometrics of *Neotobrilus ampiei* (Joubert & Heyns, 1979) Tsalolikhin, 1981 females and males found in freshwater sediment in the Telperion Nature Reserve, South Africa. All measurements in the form: mean ± standard deviation (range). - indicates structures that were not visible or not present.

Morphometric feature	♂	♀
	n = 5	**n = 13**
L	2098 ± 128.2 (1990–2320)	2095 ± 193.9 (1868–2439)
a	33.7 ± 4.4 (27.1–37.6)	28.8 ± 3.0 (23.1–33.2)
a'	30.5 ± 3.9 (24.4–34.0)	24.7 ± 2.5 (19.9–28.8)
b	5.5 ± 0.2 (5.3–5.8)	5.5 ± 0.3 (5.1–6.3)
c	10.7 ± 1.5 (9.3–13.3)	7.1 ± 0.5 (6.5–8.1)
c'	5.4 ± 0.7 (4.5–6.4)	7.3 ± 0.6 (5.7–8.3)
V	–	41 ± 2.4 (37–46)
G1	–	14 ± 2.4 (10–18)
G2	–	15 ± 2.6 (12–20)
Supplementary row as percentage of body length	15 ± 1.0 (14–17)	–
Spicule length as percentage of supplementary row	20 ± 1.1 (19–22)	–
Spicule length as percentage of body length	3 ± 0.1 (3 –3)	–
Supplementary row as percentage of *Ductus ejaculatoris*	82 ± 5.0 (76–87)	–
Cuticle width at neck	2 ± 0.3 (2–3)	2 ± 0.5 (2–3)
Cuticle width at mid–body	2 ± 0.8 (2–3)	2 ± 0.8 (1–4)
Cuticle width at dorsal side of tail	2 ± 0.5 (1–2)	2 ± 0.6 (2–4)
Amphid from anterior	–	18 ± 1.5 (17–19)
Long cephalic setae	14 ± 1.6 (13–16)	14 ± 1.2 (13–16)
Lip region width	29 ± 1.9 (27–31)	31 ± 3.3 (24–36)
Setae length / labial region width	0.5 ± 0.1 (0.4–0.6)	0.5 ± 0.1 (0.4–0.6)
Stoma length	40 ± 1.6 (38–42)	41 ± 2.3 (35–44)
Stoma width	13 ± 1.3 (12–15)	14 ± 1.8 (10–17)
First tooth from anterior	20 ± 1.5 (18–21)	21 ± 1.8 (18–24)
Second tooth from anterior	33 ± 1.8 (32–35)	36 ± 1.5 (33–37)
Distance between teeth	14 ± 2.1 (11–16)	16 ± 0.7 (13–16)
First pocket from anterior	16 ± 0.9 (15–17)	17 ± 1.4 (15–20)
Second pocket from anterior	29 ± 2.2 (26–31)	31 ± 1.5 (29–33)
Distance between pockets	4 ± 1.8 (2–7)	4 ± 0.9 (3–5)
First body setae from anterior	24 ± 3.5 (20–28)	32 ± 5.4 (24–41)
Oesophagus length	380 ± 11.8 (370–398)	380 ± 27.4 (345–437)
Cardia length	27 ± 1.9 (26–31)	29 ± 4.9 (22–36)
Cardia width	35 ± 4.4 (27–39)	41 ± 3.8 (36–49)
Body width at mid–body	63 ± 10.6 (54–76)	74 ± 9.6 (58–90)
Body width at vulva	–	74 ± 9.6 (58–90)
Body width at anus / cloaca	37 ± 2.4 (34–39)	41 ± 3.9 (35–49)
Distance from head to vulva	–	851 ± 92.1 (710–988)
Length of anterior genital tract	–	286 ± 69.9 (195–392)
Vagina length	–	19 ± 4.3 (16–29)

[a]Values the same for all specimens.

– Indicates structure not present or not measured.

Table 6.9 (continued): Morphometrics of *Neotobrilus ampiei* (Joubert & Heyns, 1979) Tsalolikhin, 1981 females and males found in freshwater sediment in the Telperion Nature Reserve, South Africa. All measurements in the form: mean ± standard deviation (range). - indicates structures that were not visible or not present.

Morphometric feature	♂	♀
	n = 5	**n = 13**
Vagina length as percentage of corresponding body width	–	30 ± 8.1 (23–46)
Length of anterior genital tract	–	286 ± 69.9 (195–392)
Length of posterior genital tract	–	315 ± 71.3 (228–430)
Egg length	–	61 ± 5.4 (51–68)
Egg length without shell	–	52 ± 4.1 (46–60)
Egg width	–	49 ± 7.9 (35–56)
Egg width without shell	–	38 ± 7.1 (27–47)
Tail length	198 ± 14.8 (175–215)	297 ± 28.1 (255–354)
Rectum length	–	42 ± 4.8 (31–47)
Rectum / width at anus	–	1.03 ± 0.1 (0.85–1.19)
Spinneret length	3 [a]	4 ± 0.5 (3–5)
Testis length	1337 ± 144.6 (1225–1500)	–
Supplementary row	320 ± 29.0 (284–359)	–
Ductus ejaculatoris	390 ± 31.2 (351–421)	–
Number of supplements	6 [a]	–
Spicule length	65 ± 3.9 (61–72)	–
Gubernaculum length	23 ± 3.2 (19–26)	–
Supplement 1– Supplement 2	72 ± 5.0 (65–78)	–
Supplement 2– Supplement 3	36 ± 5.8 (30–45)	–
Supplement 3– Supplement 4	133 ± 17.6 (105–150)	–
Supplement 4– Supplement 5	43 ± 4.8 (37–48)	–
Supplement 5– Supplement 6	37 ± 2.7 (35–42)	–

[a]Values the same for all specimens.

– Indicates structure not present or not measured.

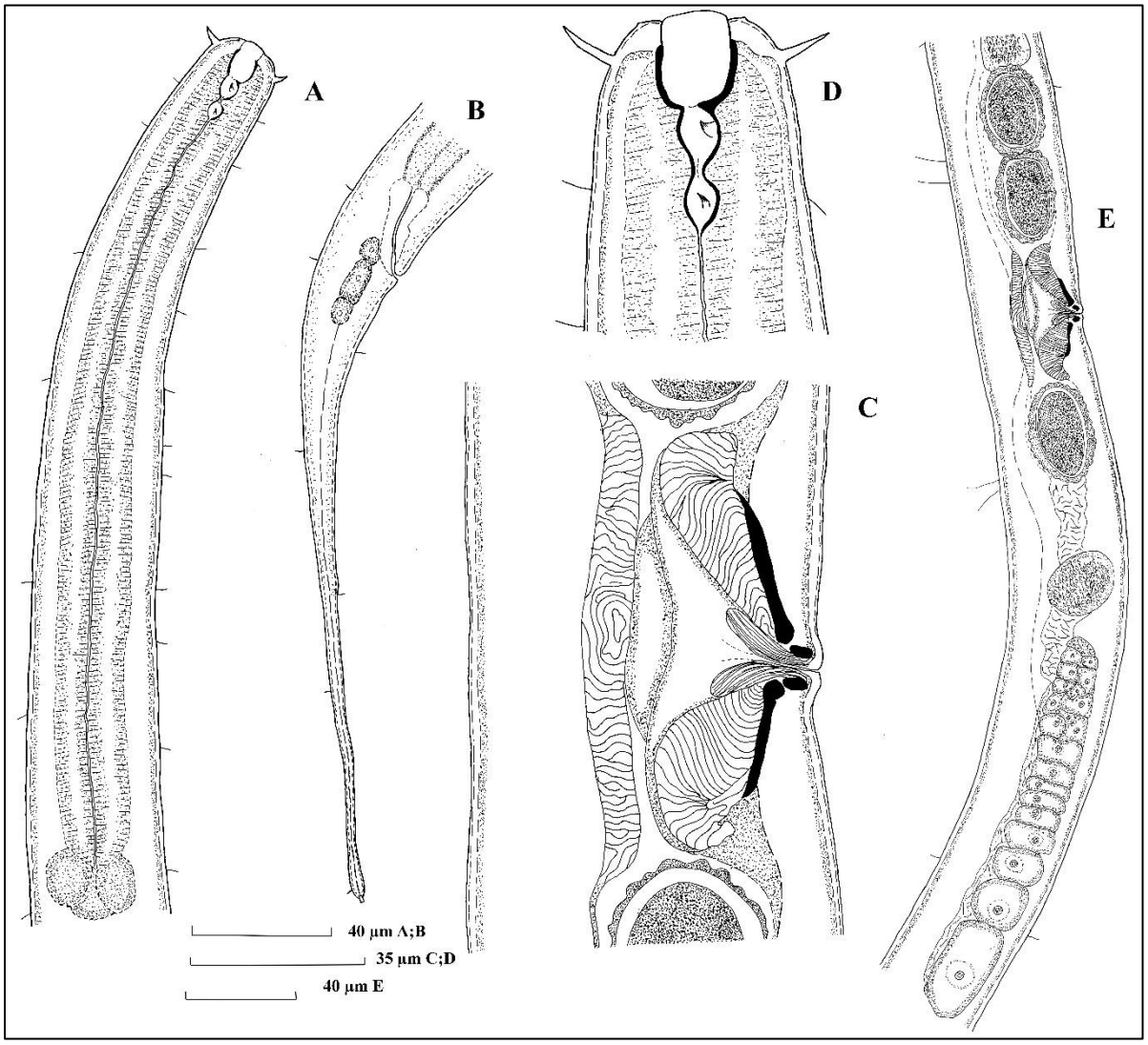


Figure 6.22: Female drawings *Neotobrilus ampiei* (Joubert & Heyns, 1979) Tsalolikhin, 1981 A: Anterior region showing oesophagus; B: Tail; C: Vulval region showing musculature of vagina; D: Anterior region showing structure of stoma and teeth pockets; E: Vulval region showing part of anterior genital tract with two eggs and full posterior tract with one egg.

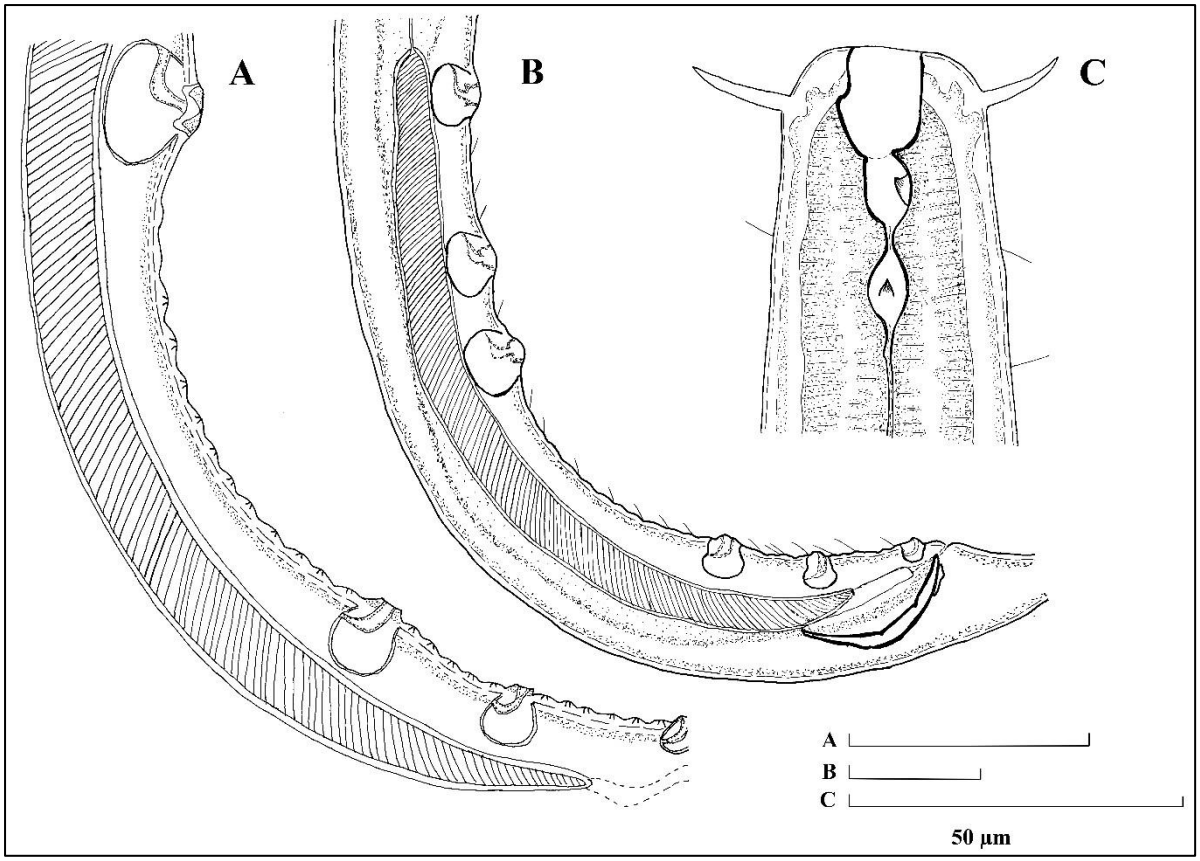


Figure 6.23: Male drawings *Neotobrilus ampiei* (Joubert & Heyns, 1979) Tsalolikhin, 1981 A: Close up of supplements 3-6 showing position of micropapillae; B: Supplementary row S1-S6 (S1 starting anteriorly:; *Ductus ejaculatoris*; spicule and gubernaculum; C: Anterior region showing stoma and arrangement of teeth pockets.

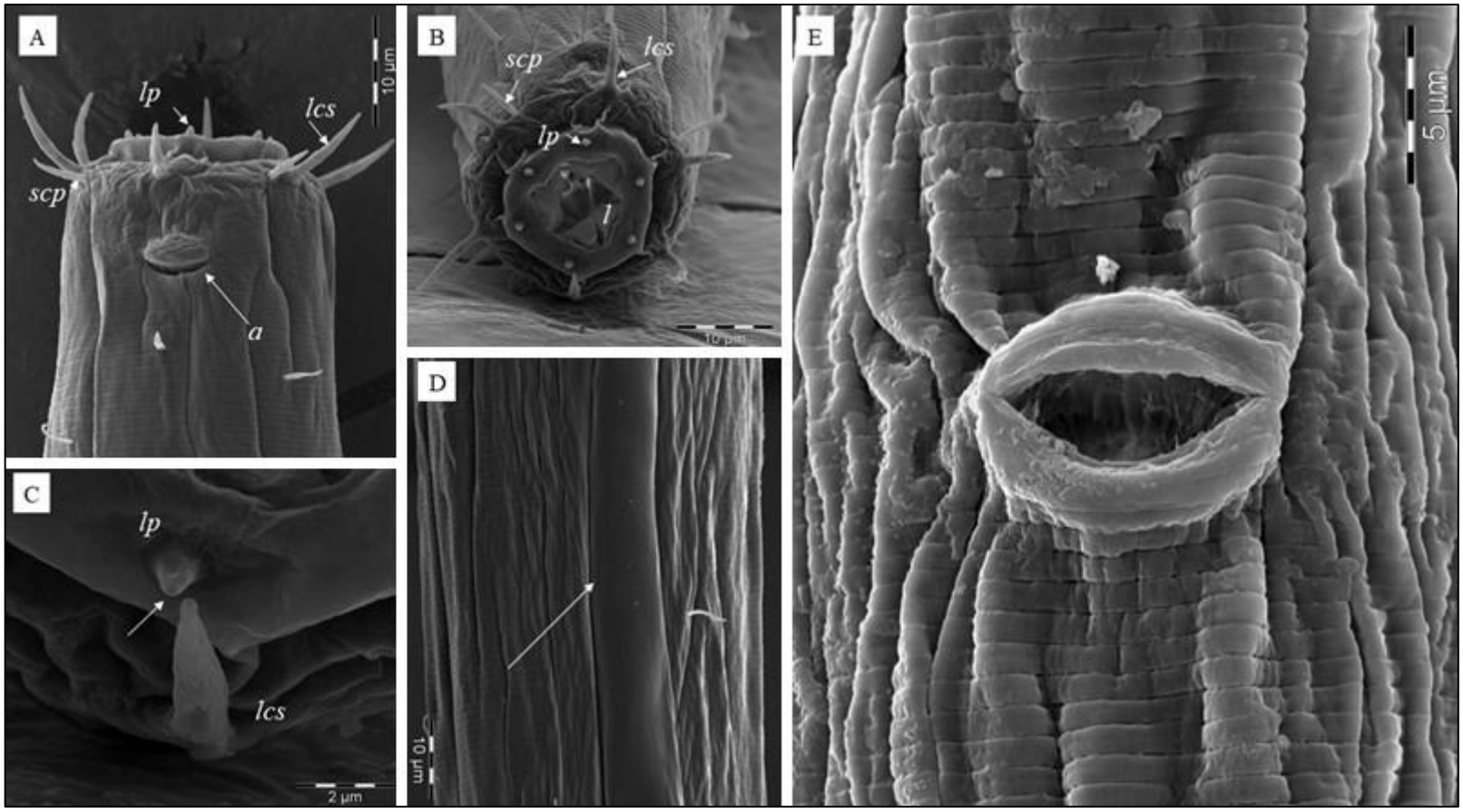


Figure 6.24: Scanning electron micrograph of *Neotobrilus ampiei* (Joubert & Heyns, 1979) Tsalolikhin, 1981; A: Lateral view of anterior end showing amphid (*a*); long cephalic setae (*lcs*), short cephalic setae (*scc*) and labial papillae (*lp*); B: Anterior end showing long cephalic setae (*lcs*), short cephalic setae (*scc*) and labial papillae (*lp*) and liplets (*l*); C: Close up of long cephalic setae (*lcs*) and labial papillae (*lp*), arrows indicating the opening of the sensory organs; D: Arrow indicating lateral cord E: Ventral side of body showing vulva.

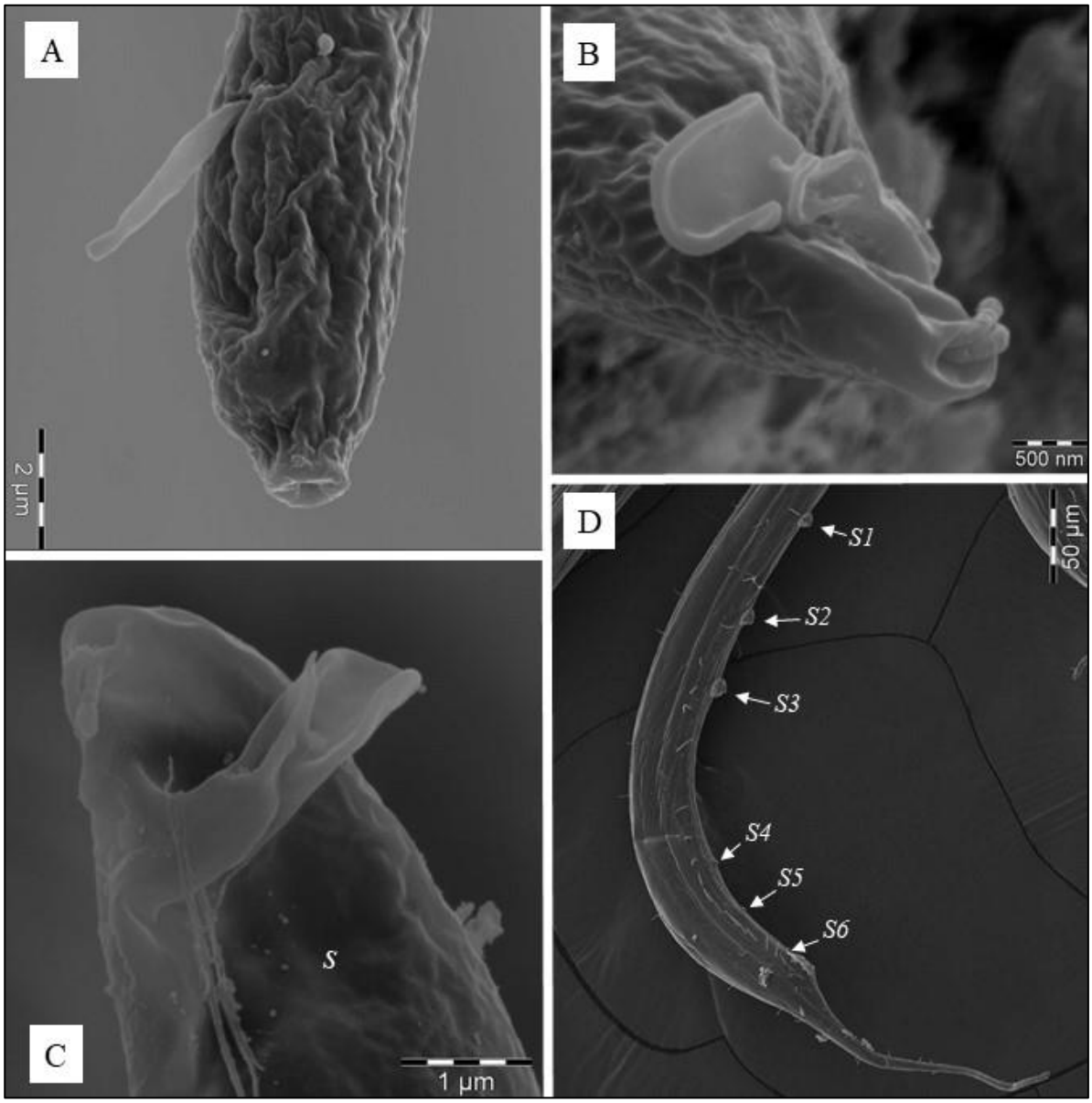


Figure 6.25: Scanning electron micrograph of *Neotobrilus ampiei* (Joubert & Heyns, 1979) Tsalolikhin, 1981 A: Female tale showing spinneret and last somatic (caudal setae); B-C: Close up of tip of spicule (*s*) showing medial outgrowth D: Male posterior part showing supplements S1-S6 and a row of ventrosublateral setae.

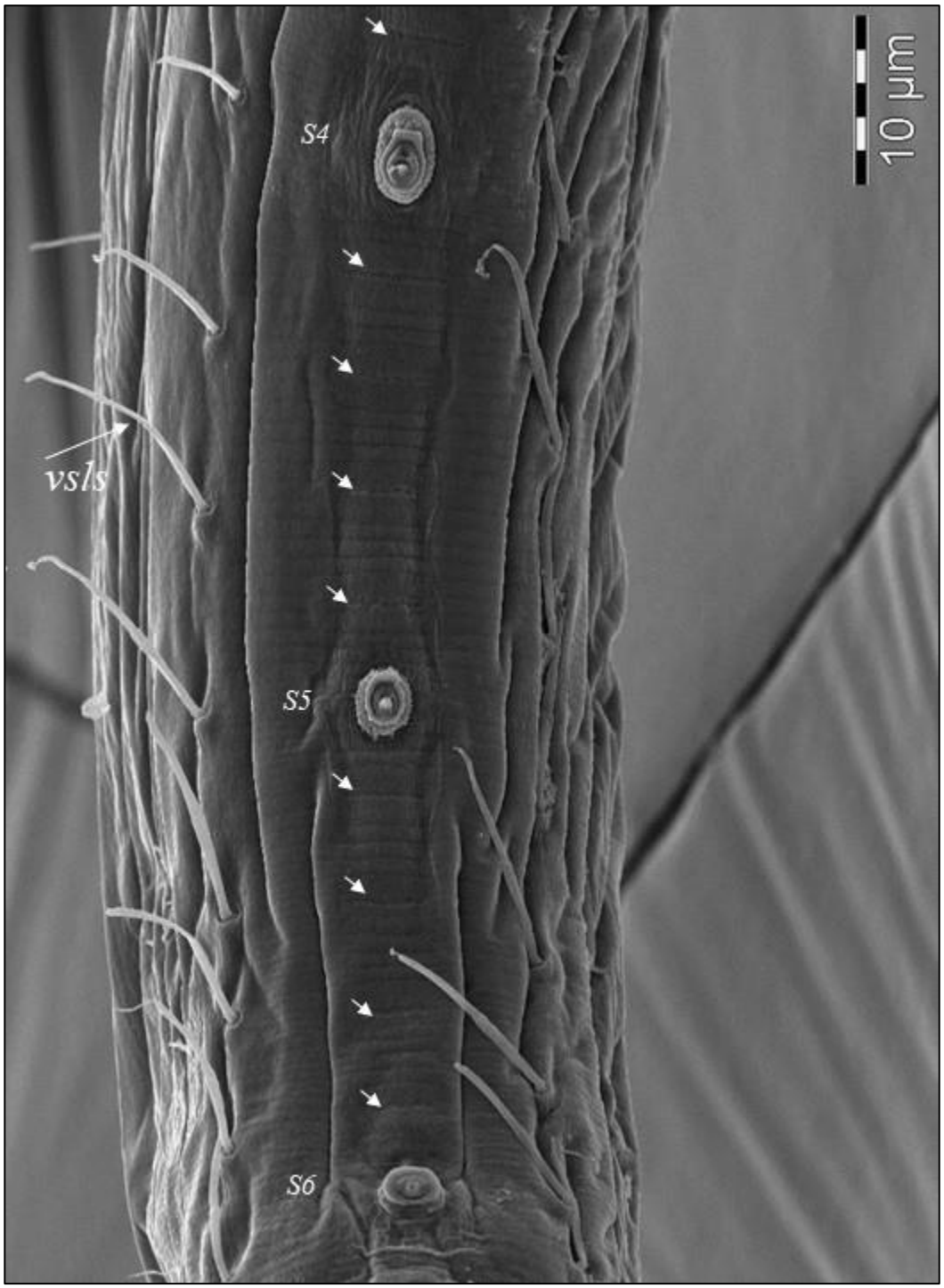


Figure 6.26: Scanning electron micrograph of *Neotobrilus ampiei* (Joubert & Heyns, 1979) Tsalolikhin, 1981 Ventral side of male showing distribution and direction of ventrosublateral setae (*vsls*); supplements S4-S6, and micropappilae (small arrows).

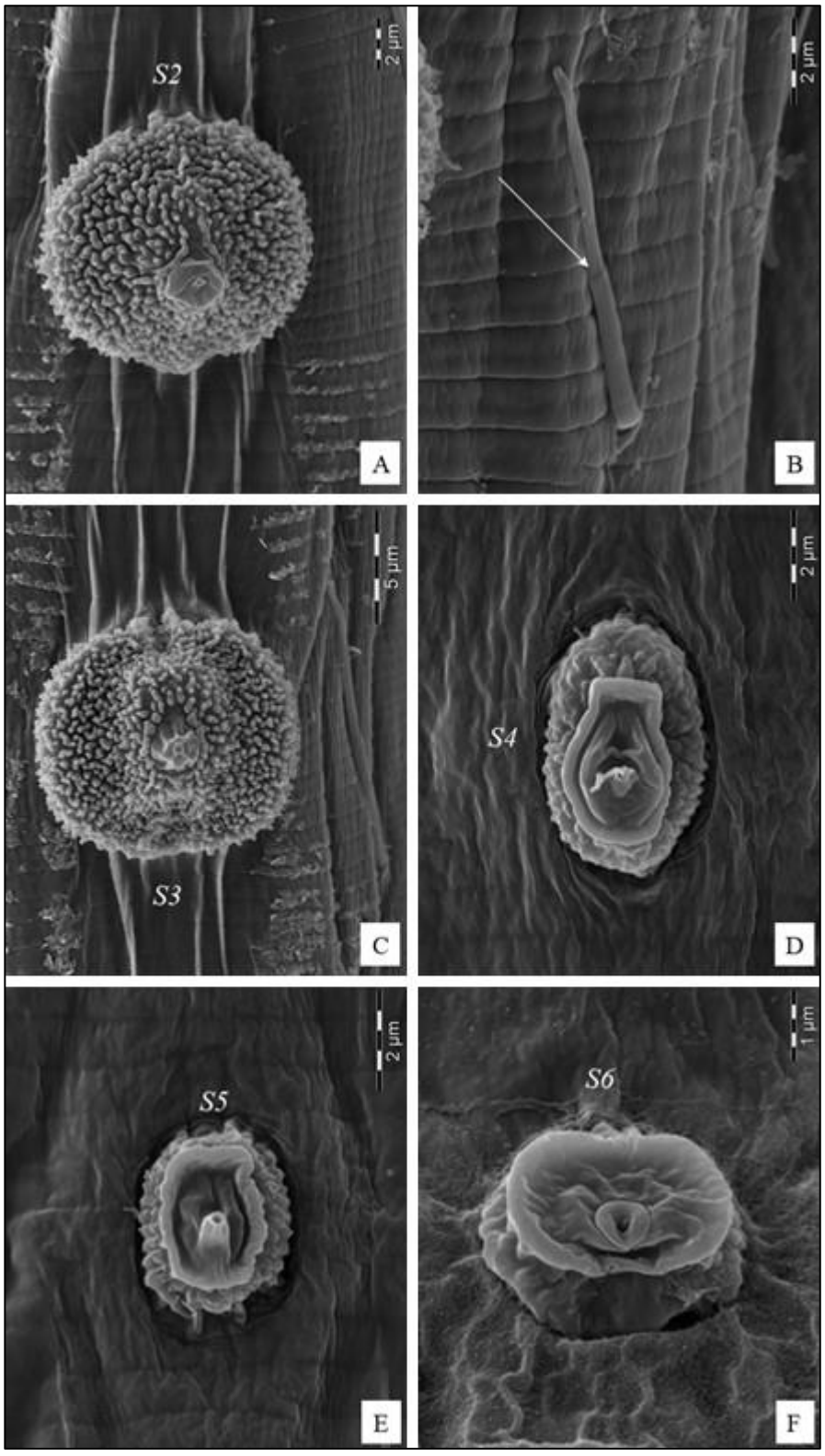


Figure 6.27: Scanning electron micrograph of *Neotobrilus ampiei* (Joubert & Heyns, 1979) Tsalolikhin, 1981 A + C: Close up of supplements S2 and S3 (S1 identical to S2 and S3); B: Close up of ventrosublateral setae, arrow showing junction where two parts meet; D-F: Close up of supplement S4-S6.

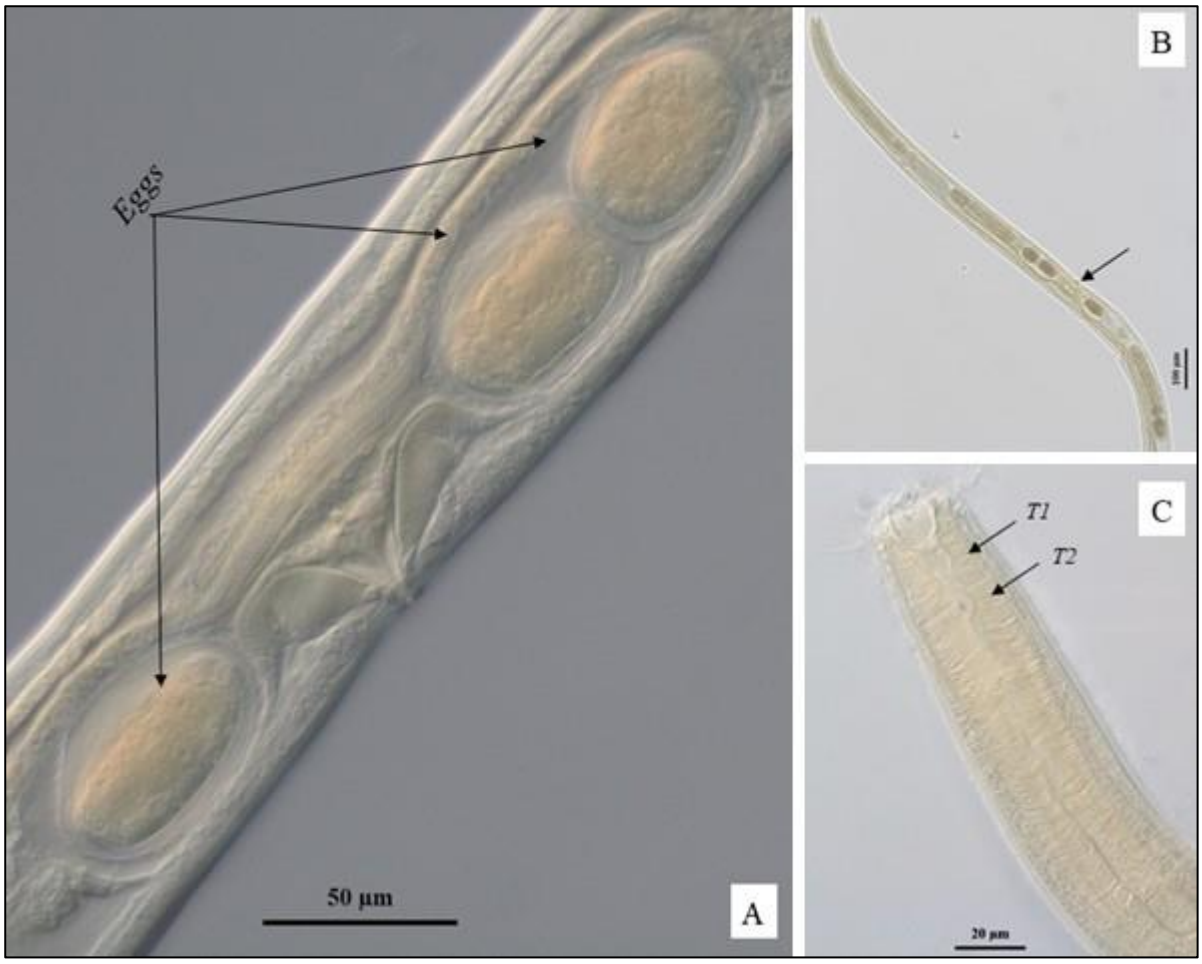


Figure 6.28: Light microscopic pictures of *Neotobrilus ampiei* (Joubert & Heyns, 1979) Tsalolikhin, 1981 female A: Vaginal region with three uterine eggs; B: Anterior end to end of posterior genital tract arrow indicating vulva; C: Labial region showing buccal cavity and two tooth pockets (*T1* and *T2*).

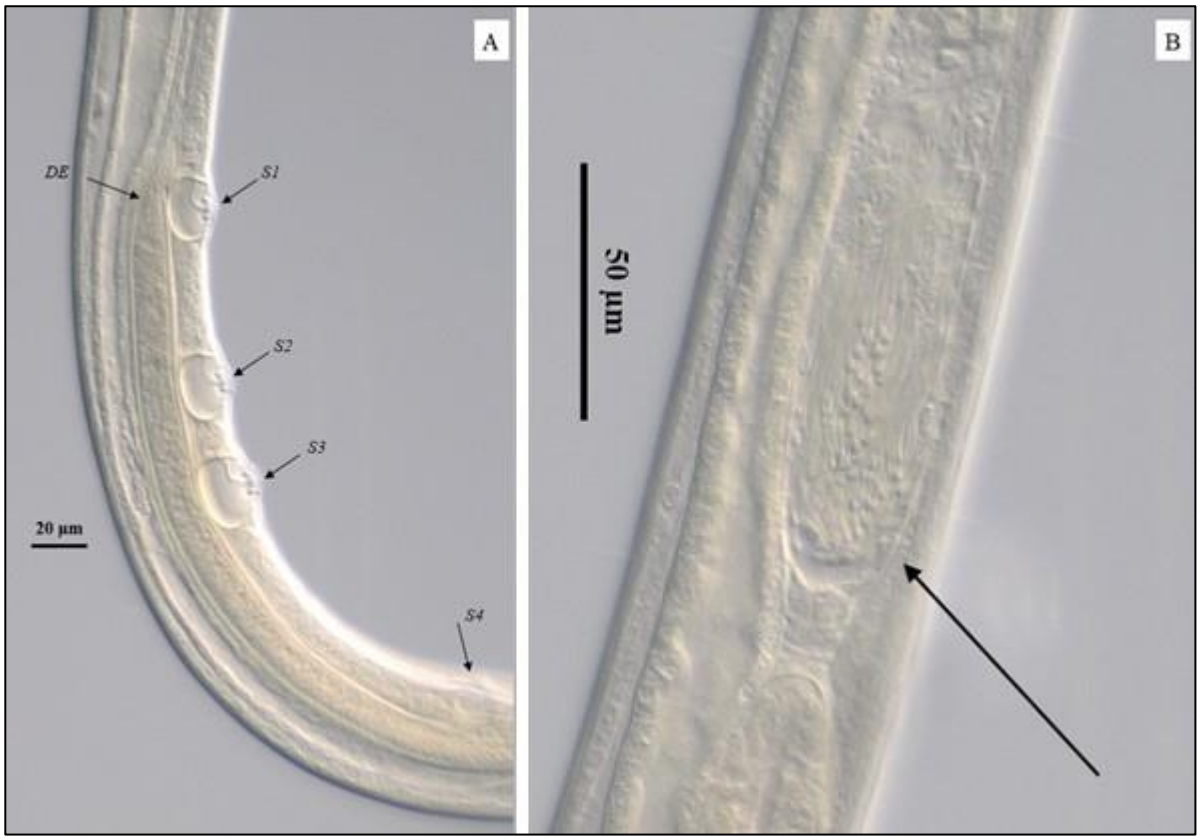


Figure 6.29: Light microscopic pictures of *Neotobrilus ampiei* (Joubert & Heyns, 1979) Tsalolikhin, 1981 Male A: Posterior region showing supplements S1-S4 and *Ductus ejaculatoris* (*DE*); B: Arrow indicating flaggelloid sperm cells in male.

Discussion:

The *Neotobrilus* specimens collected in Telperion Nature Reserve were identified following a key from Tsalolikhin & Shoshin (2009). *Neotobrilus ampiei* is distinguished from all other species in this genus by the uniquely sculptured eggshells in the females and by the closely positioned supplements 2 and 3 in males. The current female specimens exactly matches those of the original description by Joubert & Heyns (1979). However, the males differ slightly in: spicule length (61-72 µm *vs* 69-80 µm) and c-value (9.26-13.26 *vs* 8.4-9.9). *Neotobrilus ampiei* is considered endemic as the only reports are from the type locality in the Eastern Cape Province and a second population in the North West Province described by Joubert & Heyns (1979). This is only the second report of this species.

Locality and specimens examined: Specimens of *N. ampiei* were found at the Waterhole 2 (MP00031) site (Table 6.1) and are deposited in the National Collection of Nematodes (ARC- Plant Health and Protection, Biosystematics, Pretoria) on slide numbers 50591-50599.

6.5 Conclusions

Resulting from this study, three new nematode species are described from freshwater sources in the Telperion Nature Reserve, Mpumalanga, South Africa. The genus *Aphanolaimus* has already been reported from South Africa, but only *Aphanolaimus strilliae* n. sp. has been described from this country. Members of the genus *Ironus* are frequently found in South Africa and *Ironus telperionensis* n.sp. is the eighth species of the genus reported from South Africa and the fifth described from the country. *Makatinus africanus* n. sp. is the third species of this genus to be described from South Africa, with the other two species, *M. capensis* and *M. punctatus*, being reported only from their type localities. This supports the statement made by Peña-Santiago & Varela (2017) that the geographical distribution of the species in this genus is limited. *Paraphanolaimus behningi* and *C. aspinata* are reported from South Africa for the first time from freshwater sources. The SEM observations made of the species *C. africana, E. annetteae* and *N. ampiei* indicate the value in species description.. In the species *C. africana* a caudal pore, never before reported, was observed bearing two openings. The function of these pores is still unknown and needs to be investigated. In the members of Tobrilidae, SEM observations clarify the following: 1) morphology and origin of the cephalic setae: All the setae on the cephalic region of this family appear as hollow tubes confirming the sensory function of these structures, four cephalic setae are cuticular and six cephalic setae are subcuticular in origin; 2) micropapillae: although difficult to see, SEM confirmed the existence of micropapillae in the males of *N. ampiei* as seen in several of the species of *Neotobrilus*. This confirms the statement by Tsalolikhin & Shoshin (2009) that the micropapillae of most species have not been described simply because they were not seen due to the quality of the preserved specimens; 3) spicules: the spicules of *N. ampiei* from Telperion Nature Reserve revealed a never before reported structure on the tip of the spicules of which the function is unknown and needs further investigation.

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CHAPTER 7

CONCLUSIONS AND FUTURE RECOMMENDATIONS

7.1 Conclusions

The aim of the thesis was to conduct a nematode survey of the Telperion Nature Reserve and to determine the nematode diversity. Due to the undisturbed and pristine nature of the reserve two hypothesis were postulated: 1) Telperion Nature Reserve is situated in a protected part of the Grassland Biome and will reflect high nematode diversity and 2) the expected high diversity will include nematode species new to science as well as new species records for South Africa.

7.1.1 High nematode diversity of the Telperion Nature Reserve

Considering the results from Chapter 2 (Article 1) to Chapter 6 (Article 5), it can be concluded that the Telperion Nature Reserve yielded a remarkably high nematode diversity. A total of 109 nematode genera were recorded from the reserve which included 30 plant-parasitic nematode genera and 79 free-living nematode genera (both freshwater and terrestrial nematode genera, of all trophic levels). A survey of the Kruger National Park by Botha & Heyns (1990; 1993) reported 59 nematode genera from both terrestrial and freshwater habitats. Only a few nematode surveys conducted in undisturbed, natural habitats in South Africa included both free-living and plant-parasitic nematodes. Mobara (2014) conducted a survey of the Seekoeivlei Nature Reserve and reported 43 nematode genera. Durand *et al.* (2012) and Du Preez *et al.* (2015) conducted surveys of the Bakwena and Wonderfontein caves, reporting 27 and 54 nematode genera respectively. These differences in number of genera found at each protected area could be due to sample size, number of localities per area and various extraction techniques. However, the diversity found at the Telperion Nature Reserve is still comparatively high.

Globally, limited literature is available on the nematode diversity in completely natural grassland systems. Yeates (1974) and Yeates & Lee (1997) studied the diversity of nematodes in tussock grasslands in New Zealand and identified 51 and 32 genera, respectively. In various grasslands in the Slovak Republic an average of 49 genera were reported (Čerevková 2006). In four different indigenous tussock grasslands in New

Zealand, Bell *et al.* (2005) reported 70 nematode genera. At 36 grasslands in Romania an average of 33-67 genera were reported (Popovici & Ciobanu 2000). Based on the results from various surveys conducted in South Africa and other parts of the world, the total nematode diversity of the Telperion Nature Reserve is considered high as was expected from a protected area in the Grassland Biome.

The diversity of the plant-parasitic nematodes in the Telperion Nature Reserve also proved to be exceptionally high as compared to that in other protected areas. For example, 47 species of plant-parasitic nematodes (excluding the eight potential new species) were recorded in the reserve. By comparison, Van den Berg (1993; 1996) reported 19 and 23 plant–parasitic nematode species from the Wilderness and Tsitsikamma sections of the Garden Route National Park, respectively. Also, 41 and 34 plant–parasitic nematode species were reported from the Swartberg Nature Reserve (Western Cape Province) and Goegap and Witsand Nature Reserves (Northern Cape Province), respectively (Van den Berg *et al*. 2003; 2007). Marais & Swart (2013; 2014) reported 94 and 80 plant–parasitic species from 18 protected areas in the KwaZulu–Natal Province and from seven protected areas in the Eastern Cape Province, respectively. Unpublished data from six protected areas also recorded several plant-parasitic nematode species. These included the Groenkloof (14 species) and Rietvlei (19 species) Dam Nature Reserves, the Serene Valley (10 species) (South African Plant-Parasitic Nematode Survey database) and the Willem Pretorius, Erfenis Dam and Kalkfontein Dam (9 species) nature reserves (M. Marais [ARC–PHP] pers. comm., 7 March 2019). Therefore, the diversity of the plant-parasitic nematodes of Telperion proved to be higher than similar surveys dones in other protected areas of South Africa.

7.1.2 New species and new reports for South Africa

Nematode surveys have been conducted in South Africa in both cultivated and uncultivated areas. However, there are still a lack of knowledge of the nematode diversity in various habitats. This includes habitats in protected areas, especially in the Mpumalanga Province. The present study resulted in novel information on the diversity of nematodes in the Grassland Biome. *Chronogaster aspinata, Discomyctus, Drilocephalobus, Heterocephalobellus, Paraphanolaimus behningi, Pratylenchus pseudopratensis* and *Seinura* found in the terrestrial and freshwater habitats of the Telperion Nature Reserve are reported here for the first time in South Africa. *Aphelenchoides lichenicola, A. rutgersi, A. spicomucronatus* and *Panagrolaimus*

leperisini are also new reports for the country as well as new reports from grass seeds. Several new species were found during the survey of the Telperion Nature Reserve and the following are described in this thesis: *Aphanolaimus strilliae* n. sp., *Ironus telperionensis* n. sp. and *Makatinus africanus* n. sp. Several species of the genera *Helicotylenchus*, *Hemicycliophora*, *Lindseyus*, *Subanguina*, *Trichodorus*, *Tylenchorhynchus*, *Xiphinema* and *Xiphinemella* are also considered new to science and will be described in future. In conclusion, the present survey conducted in the Telperion Nature Reserve resulted in new and novel information of the nematode diversity in South Africa as well as on a global scale. Also, this sensitive Grassland Biome of South Africa proved to be rich in nematode species of all trophic levels, demonstrating the necessity of its protection.

7.2. Future recommendations

Although the diversity reported in this thesis added to our knowledge of the nematode diversity in South Africa, especially in undisturbed habitats in the Grassland Biome, it also highlighted the potential for future work:

1. In the present study, the use of classical morphological methods proved sufficient to identify nematode species. However due to the complexity of the identification of certain species especially plant-parasitic nematodes, future surveys should include both morphological and molecular identification of nematodes.
2. To describe the new species found during this survey by resampling the Telperion Nature Reserve to collect sufficient material for both morphological and molecular studies.
3. To use the results reported in this thesis, especially that of the terrestrial free-living nematodes to determine the ecology of nematodes in an undisturbed grassland ecosystem. Thereby, as nematodes are indicator organisms of soil health, these results [which includes nematode data over four consecutive seasons (winter, spring, summer and autumn)] may serve as a reference dataset for comparison to cultivated agricultural soils in future nematode ecology studies. Ultimately, this may lead to the implementation of methods to restore the microbial balance in cultivated soils, leading to increased and sustainable crop production.
4. Due to the importance of plant-parasitic nematodes in agriculture, the information gained from the present study also highlighted the importance of knowledge on the distribution of these nematodes in

the Grassland Biome. Many agricultural crops are cultivated in in this biome in South Africa and moreover, as many of the staple food crops belong to the grass family (Poaceae or Gramineae). it follows that grasses in natural grassland systems may serve as reservoir hosts of important agricultural pests. Future studies should include more surveys of the undisturbed soils in the Grassland Biome as well as the other biomes of South Africa to broaden our knowledge of the natural occurrence and distribution of plant-parasitic nematodes in South Africa.

5. One of the agriculturally most important nematode families, the Pratylenchidae, were included in the present thesis in order to fill in the gap in the current knowledge and expertise on members of this family in South Africa. Future work will be to conduct a survey of members of this family in both cultivated and uncultivated soils in South Africa to determine the distribution of this family. This work will include both morphological and molecular studies to establish a reference dataset for future identification of these nematodes.

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APPENDIX A

Instructions to authors Zootaxa (excerpt) – Magnolia Press

Preparation of manuscripts

Nomenclature must be in agreement with the *International Code of Zoological Nomenclature* (4th edition 1999), which came into force on 1 January 2000. Author(s) of species name must be provided when the scientific name of any animal species is first mentioned (the year of publication needs not be given; if you give it, then provide a full reference of this in the reference list). Authors of plant species names need not be given. Metric systems should be used. If possible, use the common font New Times Roman and use as little formatting as possible (use only **bold** and *italics* where necessary and indentions of paragraphs except the first).

The style of each author is generally respected but they must follow the following general guidelines.

Title page

The title should be concise and informative. The higher taxa containing the taxa dealt with in the paper should be indicated in parentheses: e.g. A taxonomic revision of the genus *Aus* (Order: family).

The name(s) of all authors of the paper must be given and should be typed in the upper case (e.g. ADAM SMITH, BRIAN SMITH & CAROL SMITH). The address of each author should be given in *italics* each starting a separate line. E-mail address(es) should be provided if available.

Abstract

The abstract should be concise and informative. Any new names or new combinations proposed in the paper should be mentioned. Abstracts in other languages may also be included in addition to English abstract. The abstract should be followed by a list of key words that are not present in the title. Abstract and key words are not needed in short correspondence.

Main text

The arrangement of the main text varies with different types of papers (a taxonomic revision, an analysis of characters and phylogeny, a catalogue etc.), but should usually start with an introduction and end with a list of references.

Citation

References should be cited in the text as Smith (1999), Smith & Smith (2000) or Smith *et al.* (2001) (3 or more authors), or alternatively in a parenthesis (Smith 1999; Smith & Smith 2000; Smith *et al*. 2001). All literature cited in the text must be listed in the references in the following format.

A) **Journal paper**:

Smith, A. (1999) Title of the paper. *Title of the journal in full*, volume number, page range.

B) **Book chapter**:

Smith, A. & Smith, B. (2000) Title of the Chapter. *In*: Smith, A, Smith, B. & Smith, C. (Eds), *Title of Book*. Publisher name and location, pp. x–y.

C) **Book**:

Smith, A., Smith, B. & Smith, C. (2001) *Title of Book*. Publisher name and location, xyz pp.

D) **Internet resources:**

Author (2002) Title of website, database or other resources, Publisher name and location (if indicated), number of pages (if known). Available from: http://xxx.xxx.xxx/ (Date of access).

F) **Dissertations** resulting from graduate studies and **non-serial proceedings of conferences/symposia** are to be treated as books and cited as such. Papers not cited must not be listed in the references.

Please note that:

(1) Journal titles must be written in full (not abbreviated)

(2) Journal titles and volume numbers are followed by a ","

(3) Page ranges are connected by "n dash", not hyphen "-", which is used to connect two words.

For websites, it is important to include the last date when you see that site, as it can be moved or deleted from that address in the future.

Guidelines for Reference List

Please follow the following Zootaxa reference format: (Note: Words in red are the common mistakes made by authors.)

1. Digital Object Identifier (DOI)

Zootaxa is a CrossRef member. It is required by CrossRef to include DOI links for cited papers that have DOI. If DOI is not completed, our staff will need to add these for you. Due to hundreds of papers waiting to be processed, your one will be delayed. Therefore we strongly encourage authors to add DOI links to cited papers as it will speed up the publishing process. Steps are shown as followed:

1. Go to http://iphylo.org/~rpage/zootaxa-framemaker/
2. Copy and paste your references list.
3. Tick "Double check DOIs".
4. Press "Process".
5. Copy the DOI address where the reference is highlighted in GREEN.
6. Check the DOI link and the paper shown via the link is correct one.

2. Journal Paper (Do not use Book Chapter Format)

Author's Surname/Last Name, Author's First Name Initial. (Published Year) Paper Title. *Journal Name*, Volume No (Issue No), 000–000. [in Language/in press/or other information]

Author's Surname always comes before Initials

"–" (n dash), not "-" (hyphen)

Journal Name in *Italic* & followed by a comma

Eg:

Kiss, O. (2013) Three new species of *Rhyacophila* (Trichoptera: Rhyacophilidae) from Taiwan and Nepal. *Zootaxa*, 3640 (2), 213–223. http://dx.doi.org/10.11646/zootaxa.3640.2.5

space and a bracket between Volume No and Issue No

DOI placed on the next line & the format must include http://dx.doi.org/

Page Range

3. Book Chapter

Author's Surname/Last Name, Author's First Name Initial. (Published Year) Name of the chapter. *In*: Editor's Surname/Last Name, Editor's First Name Initial. (Ed.), *Book Title. Vol. 00. Book Sub-title*. Publisher's Name, Publisher's City Location, pp. 000–000. [in Language/in press/or other information]

a comma between Initials and next Surname

a comma after Surname

No space between Initials

"*In*" in *Italic* and followed by a colon

"&" between Names and no comma

Eg:

Newton, A.F., Thayer, M.K., Ashe, J.S. & Chandler, D.S. (2000) Staphylinidae Latreille, 1802. *In*: Arnett, R.H. & Thomas, M.C. (Eds.), *American Beetles. Vol. 1. Archostemata, Myxophaga, Adephaga, Polyphaga: Staphyliniformia*. CRC Press, Boca Raton, pp. 272–418.

Must provide Page Range and follow by a period

a comma before "pp."

"–" (n dash), not "-" (hyphen)

Book Name in *Italic*

space between "pp." and Page Range and "pp." placed before the page number

4. Book

Author's Surname/Last Name, Author's First Name Initial. (Published Year) *Book Name*. Publisher's Name, Publisher's City Location, 000 pp. [in Language/in press/or other information]

Year in a bracket, not followed by a period

Book Name in *Italic*

Eg:

Baker, E.W. & Tuttle, D.M. (1994) *A Guide to Spider Mites (Tetranychidae) of the United States*. Indira Publishing House, West Bloomfield, Michigan, 347 pp.

"pp." placed after Page No

space between Total Page No and "pp."

5. Internet Resources

Author's Surname/Last Name, Author's First Name Initial. (Published Year) Paper Title. Available from: http://www.xxxxxxxxxxxx (accessed date Month Year)

Paper Title must be provided

Eg:

O'Connor, R.J., Dunn, E, Johnson, D.H., Jones, S.L., Petit, D., Pollock, K., Smith, C.R., Trapp, J.L. & Welling, E. (2000) A programmatic review of the North American Breeding Bird Survey: report of a peer review panel. U.S. Geological Survey Patuxent Wildlife Research Center, Laurel, Maryland. Available from: http://www.mp2-pwrc.usgs.gov/bbs//bbsreview/ (accessed 3 April 2003)

access date must be provided

APPENDIX B

Instructions to authors Agricultural Research Council–Plant Health and Protection Handbook and Manual Series (ARC–PHP)

The manuscript should be in the format of the series of books and manuals published by ARC–PHP for an example see one of the following publications:

- HARNEY, M., 1993. A guide to the Insects of Stored Grain in South Africa. PPRI Handbook no. 1.
- BAXTER, A.P., 1994. Elementary keys to common fungi in South Africa. PPRI Handbook no. 2.
- CAMPBELL, P., 2000 Wattle Control. PPRI Handbook no. 3.
- MILLAR, I.M., 1994. A catalogue of the aphids (Homoptera: Aphidoidea) of sub-Saharan Africa. PPRI Handbook no. 4.
- HENDERSON, L., 1995. Plant invaders of Southern Africa. PPRI Handbook no. 5.
- MEYER, M.K.P. (SMITH), 1996. Mite pests and their predators on cultivated plants in Southern Africa (vegetables and berries). PPRI Handbook no. 6, 90pp.
- UYS, V.M. & URBAN, R.P., 2006. How to collect and Preserve Insects and Arachnids. PPRI Handbook no. 7.
- KLEYNHANS, K.P.N., VAN DEN BERG, E., SWART, A., MARAIS, M. & BUCKLEY, N.H., 1996. Plant Nematodes in South Africa. PPRI Handbook no. 8.
- DIPPENAAR-SCHOEMANN, A.S. & JOCQUÉ, R., 1997. African spiders, and identification manual. PPRI Handbook no. 9, 392pp.
- ROUX, C. & BOTHA, W.J., 1997. An Introduction to the Pythiaceae in South Africa. PPRI Handbook no. 10.
- KLEIN, H., 1999. Biocontrol Agents Against Alien Invasive Plants in the Fynbos. PPRI Handbook no. 10.
- VAN TONDER, H.J. & PRINSLOO, G.L., 2000. Insect pests of stored products in southern Africa: a pocket guide. Sponsored by GOSA. PPRI Handbook no. 11.
- CAMPBELL, P.L., 2000. Rehabilitation recommendations after alien plant control. PPRI Handbook no. 11B.
- HENDERSON, L., 2000. Alien Weeds and invasive plants. PPRI Handbook no. 12.

- DIPPENAAR-SCHOEMAN, A.S., 2002. The baboon and trapdoor spiders of Southern Africa: an identification manual. PPRI Handbook no. 13, 130pp.
- JOHANNSMEIER, M.F., 2001. Beekeeping in South Africa. PPRI Handbook no. 14.
- UYS, V.M., 2002. A guide to the termite genera of Southern Africa. PPRI Handbook no. 15.
- HENDERSON, L. & CILLIERS, C.J., 2002. Invasive aquatic plants. PPRI Handbook no. 16.
- SWART, A. & MARAIS, M., 2017. The Kleynhans Manual. Collecting and Preserving Nematodes. PPRI Handbook no. 16.
- JOHANNSMEIER, M.F., 2005. Beeplants of South-Western Cape. PPRI Handbook no. 17.
- DIPPENAAR-SCHOEMAN, A.S. & VAN DEN BERG, A.M., 2010. Spiders of the Kalahari. PPRI Handbook no. 18, 114pp.
- DIPPENAAR-SCHOEMAN, A.S., 2014. Spiders of the Grassland Biome. PPRI Handbook no. 19.

Referencing style:

In text:

Single author: (Ramavhoya 2005) **or** Ramavhoya (2005)

Two authors: (Tripathi & Dubey 2004) **or** Tripathi & Dubey (2004)

Three or more authors: (Macarisin *et al*. 2007) **or** Macarisin *et al*. (2007)

Reference list:

Book:

Van Den Berg M A, De VilliersEA& Joubert P H 2001. Pests and beneficial arthropods of tropical and non-citrus subtropical crops in South Africa. Dynamic Ad, Nelspruit.

Chapter in book:

Grove T, De Villiers E A. & Schoeman P S 2015. Litchi. In: *Insects of cultivated plants and natural pastures in southern Africa*, 554–555 (Eds G L Prinsloo & V M Uys). Entomologal Society of Southern Africa, Pretoria

Journal paper:

Al-Samarrai G, Singh H & Syarhabil M 2012. Evaluating eco-friendly botanicals (natural plant extracts) as alternatives to synthetic fungicides. *Annals of Agricultural and Environmental Medicine* **19**: 673–675.

Dissertations:

Ramavhoya M R 2005. Chemical and biological properties of *Euphorbia ingens* E. Mey. MSc dissertation. North West University, Potchefstroom, South Africa

APPENDIX C

Instructions to authors Journal of Nematology (excerpt) – Exeley, Inc.

Preparation of manuscripts

Abstract

The abstract page includes the abstract and the list of key words and begins with the indented subheading Abstract: Note that the colon is also italicized, like all colons in all JON subheadings. The abstract serves as the summary, written in past tense, one paragraph of no more than 1,300 spaces, and must be on a separate page, along with the key words. It should be self explanatory and intelligible in itself and include the rationale for the study, objectives and topics covered, a brief description of methods, results, and conclusions. Do not include authorities for Linnaean binomials or trinomials, footnotes, statistical probability levels, or literature references.

Key words: The list of key words begins with the indented subheading Key words: and includes those key words appearing in the title. Include words or short phrases that would be useful in index retrieval systems. Choose key words thoughtfully, as they are the sole basis of the JON annual subject index. List key words alphabetically. Most key words should be in the singular form. Include both the binomial and common name of nematodes and hosts, common names of pesticides, experimental techniques (e.g., electron microscopy, modeling, electrophoresis), specific subjects (e.g., soil temperature, suppression), and general subject areas.

Main text

General Principles

International code: Manuscripts proposing new species must conform to requirements of the International Code of Zoological Nomenclature (http://www.nhm.ac.uk/hosted-sites/iczn/code/). Taxonomic authority: With the first usage of the name of each taxonomic category, cite the taxonomic authority and year of authorship for that name. Examples are as follows: *Meloidogyne incognita* Kofoid and White, 1919; *Globodera pallida* Stone, 1973. In the case of changed combinations, the original author should also be given, e.g., *Pristionchus maupasi* (Potts, 1910) Paramonov, 1952. References for authorities should be included in the

Literature Cited. The first usage of a newly proposed species name should be acknowledged as in the following example: Meloidogyne incognita n. sp. Additional newly proposed taxonomic categories should be similarly acknowledged as in the following example: Heteroderidae n. fam.

General organization for taxonomic descriptions: The overall organization of the article is recommended as follows:

Introduction

Materials and Metohds

Descriptions

Discussion

Literature cited

Citation

Authors have an ethical responsibility to fairly and correctly attribute previous work by citation. In most cases, it is preferable to cite the primary literature instead of a review. Authors should make every effort to cite only articles that are readily available and are particularly encouraged to cite work published in the JON.

References in Text

In the body of the manuscript, publications are cited using the name-year system, e.g., (Bird, 1996). In some instances, such as at the start of a sentence, or if the intent is to particularly stress the cited author's contribution, only the date is in parentheses: e.g., "Bird (1996) showed that" However, authors are encouraged to limit their use of this form of citation. To cite a publication with two authors, list both: e.g., (Bird and Kaloshian, 2003); with three or more authors use et al.: e.g., (Bird et al., 2003). Note the comma used after the author name(s) and the comma and period after et al. When multiple citations are listed in parentheses, they are listed in chronological order from oldest to most recent and separated by semicolons, for example, (Zuckerman, 1980; Bird, 1996). If two or more cited papers are from the same year, list the papers from that year alphabetically: e.g., (Bird, 2003; Bird and Kaloshian, 2003; Bird and Opperman, 2003; Bird et al., 2003). For two or more

articles by the same author(s) in the same year use letters: e.g., (Houser, 1992a, 1992b). The same applies if two multiple-author citations are from the same year: e.g., (Bird et al., 2003a, 2003b). Note that in these cases, the years are separated by a comma rather than a semicolon. Some manuscripts are published by consortia and do not list individual authors. In those cases, the name of the consortium should be considered to be the author's name, e.g., (C. elegans Sequencing Consortium, 1998). On the rare occasions where the author of a legitimate published work is truly undisclosed, use "Anonymous" as the author's name. Thus, a complex example that incorporates the aforementioned rules might be: (Zuckerman, 1980; Houser, 1992a, 1992b; Bird, 1996, C. elegans Sequencing Consortium, 1998; Bird, 2003; Bird and Kaloshian, 2003; Bird and Opperman, 2003; Bird et al., 2003a, 2003b).

Reference List

The major subheading LITERATURE CITED is capitalized and centered, and begins on a separate page. List references alphabetically by authors' surnames. Alphabetical order is determined by the first author's surname (family name) and then, if necessary, by surnames of succeeding coauthors. When the author names are completely identical in two or more references, these references are sequenced by publication date (earliest to latest) and, if necessary, by the page numbers of articles published in the same journal. In rare instances where different authors share the same surname, it may be necessary to also consider the alphabetical order of the author initials. When citing multiple articles by the same author, list articles with one author before those with several authors. When author(s) are the same for articles published in the same year, use letters: e.g., 1987a, 1987b. Each reference cited in the text (including figure legends, etc.) must be in the Literature Cited; the converse also must apply. Each citation should be checked against the original publication. Authors must pay precise attention to spelling, spacing, capitalization, indentation, and format in their literature citations.

Citing a journal article

Lohar, D. P., Schaff, J. E., Laskey, J. G., Kieber, J. J., Bilyeu, K. D., and Bird, D. McK. 2004. Cytokinins play opposite roles in lateral root formation, and nematode and rhizobial symbioses. The Plant Journal 38:203-214.

In this example, note the following points:

1) All authors are listed (never use et al.) in the order in which they appeared in the original publication, with the surname, followed by all initials. Note the comma after the surname, periods and comma after initials, and space between initials.

2) The year given is the year of publication listed by the journal. In some cases this may differ from the year in which the article was actually published. In case of an article in press, give the best estimate of the year that the journal will list publication. Authors should be prepared to supply the JON editorial board with documented proof that a manuscript claimed as "in press" truly has been accepted.

3) The title should be given in sentence case. If the title contains a colon, the word after the colon should begin with a capital letter, even if not done so in the original reference. Do not use a capital letter after a dash.

4) Journal names must be spelled out in full. There is no period after the name of the journal.

5) The volume number is followed by a colon, and then the inclusive page numbers. Note the lack of spacing. Generally the issue number within a volume is not given, but in rare cases in which all issues of a journal begin with page 1, it may be required: e.g., Journal Name 34(4):78-93. For "in press" articles, include as much information as is known (such as the volume number). Page numbers can be added at the proof stage as they become known.

Citing a book

Thorne, G. 1961. Principals of nematology. New York: McGraw Hill.

In this example, note the following points:

1) The format for author (or editor) name(s), date, and title are the same for journal articles (above).

2) List the place of publication, then the publisher. Do not use "Inc." or "Ltd." for a publisher, and for publishers with multiple locations, list only one city.

3) The number of total pages is not listed.

Citing a book chapter.

Herman, R. K., and Horvitz, H. R. 1980. Ge 1 netic analysis of Caenorhabditis elegans. Pp.227-261 in B. M. Zuckerman, ed. Nematodes as biological models, vol. 2. New York: Academic Press.

In this example, note the following points:

1) The page range of the chapter is included, after the term “Pp.” and is followed by the word

“in” in italics.

2) The initials of the editor(s) come before the surname(s); note the spaces between initials.

3) Information about the volume (abbreviated “vol.”) or edition (“ed.”) number comes after the name of the book.

Citing electronic sources.

In most instances, journals that are published only as electronic journals will have a bona fide citation and can be cited like a normal publication. Articles that do not have a true citation probably should not be cited. The Editor handling the manuscript (or EiC) can make format recommendations for cases that don’t readily fit standardized JON rules. The following citation is to an electronic-only journal:

> Scholl, E. H., Thorne, J. L., McCarter, J. P., and Bird, D. McK. 2003. Horizontally transferred genes in plant-parasitic nematodes: A high-throughput genomic approach. Genome Biology 4:R39.1-R39.12.

Similarly, many electronic resources, such as an on-line database, have been published in journals, and thus can be cited in the standard manner. For example, the widely used BLAST tools can be cited as:

> Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. 1990. Basic local alignment search tool. Journal of Molecular Biology 215:403-410.

Another example of an on-line publication is WormBook, which is edited by a community rather than a single person and has no city for the publisher. A Wormbook citation may be formatted as:

> Eisenmann, D. M. 2005. Wnt signaling. (June 25, 2005). The C. elegans Research Community, ed. WormBook. doi/10.1895/wormbook.1.7.1, http://www.wormbook.org.

Information about the web sites actually queried (including the URL and date), or versions of computer programs or databases used, should rightfully be included in the Materials and Methods section.

Citing other sources.

Authors should cite only those articles that a 1 re readily available, and should minimize the citation of theses, bulletins, company brochures and the like. Here are some examples of formatting for those types of articles:

Anonymous. 1945. Article title. Washington, DC: EPA Publications.

Jones, B. T. 1980. Article title. In P. L. Bond, ed. Title of larger work. Serial Publication 23:23-34.

Jones, B. T. 1987. Dissertation title. M.S./Ph.D. dissertation (or thesis), University Name, Place.

McDowell, R. I., Jr., Dewey III, F. R., and Resh, Jr., H.-T. 1985. Title. Publication and no., Name of Experiment Station, University (if needed), Place.

Teller, A. P., and Green, B. S. Title. Journal Name 28:561 (Abstr.).

APPENDIX D

Instructions to Authors (excerpt) Koedoe - African Online Scientific Information Systems (Pty) Ltd

Checklists

Checklists aim to provide a platform for publication of long species lists and other similar data that provide particularly important baseline information for research and monitoring. Checklists should be as completely documented as short communications and should include reference to relevant literature and a description of the data collection and other procedures employed.

Word limit	3000 words (excluding the unstructured abstract and references)
Unstructured abstract	300 words [250 words main part (Background, Objectives, Method, Results and Conclusion) and 50 words 'Conservation Implications']
Abstract requirement	a labelled heading, Conservation implications
References	25 or less
Tables/Figures	no more than 7 Tables/Figure
Ethical statement	should be included in the manuscript
Compulsory supplementary file	ethical clearance letter/certificate

Checklist full structure

Title: The article's full title should contain a maximum of 95 characters (including spaces).

Abstract:

The abstract should be no longer than 300 words (250 words main part and 50 words 'Conservation Implications') and must be written in the past tense. The abstract should give a concise account of the Introduction, Patient presentation, Management and outcome and significance of the matter. The unstructured abstract for an Original Research article should consist of five paragraphs unlabelled Background, Objectives,

Method, Results and Conclusion. Then there should follow a labelled paragraph, namely Conservation implications.

Introduction:

This section must contain a clear statement of the aims of the work or of the hypotheses being tested. A brief account of the relevant background that supports the rationale of the study and checklist should also be given. The length of the introduction should not exceed 750 words.

Methods and materials:

This section should contain explicit, concise descriptions of all new methods or procedures employed, for example:

Results:

This section provides a synthesis or integration of the obtained literature grouped or categorised according to some organising or analysis principle. The data are contained in this section, but without consideration of their significance. Results are typically presented in figures or tables, summarising the lists or findings, with no duplication of information presented in the text.

these definitions should be considered when assessing internal validity.

Discussion:

This section normally contains the following: Discussion on issues relating to the completeness of the list or how representative it is considered to be of the taxa (that is, the system of classification of **organisms**, e.g. a **phylum**, **order**, **family**, **genus** or **species**) and area covered, as well as any other relevant uncertainties. Discussion on or comparison of previous lists and/or other similar areas. Discussion on species or records of high importance; key issues. Discussion on threats or concerns (where possible). Discussion on taxonomic updates or new species since last list of area or survey. Discussion on the potential value or uses of the list and associated information.

Conclusion:

This should state clearly the main conclusions of the study and give a clear explanation of their importance and relevance, with a recommendation for future research (implications for practice). Provide a brief conclusion that restates the objectives, the research design and the results with their meaning.

Acknowledgements:

Those who contributed to the work but do not meet our authorship criteria should be listed in the Acknowledgments with a description of the contribution. Authors are responsible for ensuring that anyone named in the Acknowledgments agrees to be named.

Also provide the following, each under their own heading:

Competing interests: This section should list specific competing interests associated with any of the authors. If authors declare that no competing interests exist, the article will include a statement to this effect: *The authors declare that they have no financial or personal relationship(s) that may have inappropriately influenced them in writing this article.* Read our policy on competing interests.

Author contributions: All authors must meet the criteria for authorship as outlined in the authorship policy and author contribution statement policies.

Funding: Provide information on funding if relevant

Disclaimer: a statement that the views expressed in the submitted article are his or her own and not an official position of the institution or funder.

References:

Authors should provide direct references to original research sources whenever possible. References should not be used by authors, editors, or peer reviewers to promote self-interests. Refer to the journal referencing style downloadable on our *Formatting Requirements* page.

Harvard Reference Style Guide

Introduction

Notes: Please 'copy' the title of a book, an article or whatever (as far as the spelling of words such as 'behaviour' or 'behavioural' are concerned (and this also goes for direct quotations) exactly as in the original:

- When referring to any work that is NOT a journal, such as a book, article, or Web page, capitalise only the first letter of the first word of a title and subtitle, the first word after a colon or a dash in the title, and proper nouns. Do not capitalise the first letter of the second word in a hyphenated compound word.
- Capitalise all major words in journal titles, full names for journal titles, not abbreviations.
- If within the same paragraph, reference is made to **the same author(s) for a second and further time(s)**, the year of publication is omitted in the second and further references – as long as it does not lead to confusion
- Always cite page numbers within in-text citation – even if paraphrasing - example: Hallinan (2000:66)

Multiple publications; same author:

- Same author; **different years**
  Normal conventions (author, year, title, etc).
- Same author; **same year**
  More than one reference by an author in the same year: these are distinguished in **order of publication** using a lower-case alphabetical suffix after the year of publication (e.g. 1988a, 1988b, 1988c, etc). The same suffix is used to distinguish that reference for the in-text citations.

Order of Listing

The List of References is ordered **alphabetically** by primary authors' surnames.

- Multiple authors.
  - Use the sequence of authors' surnames exactly as given in the publication. The primary author, i.e., major contributor, is listed first by the publisher.
- Same author:
  - different years: list the author's references chronologically, starting with the earliest date.
  - same year: use an alphabetical suffix (e.g. 1983a, 1983b).

If you are going to use the abbreviation for USA state names then please make sure the abbreviation is correct, for example:

- Doss, G., 2003, *IS project management handbook,* Prentice Hall, Upper Saddle River, NJ. (No full stops between the abbreviations).

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African Online Scientific Information Systems (Pty)Ltd t/a AOSIS Reg No: 2002/002017/07
Updated 13 April 2017 by AOSIS

Books

Books	In-Text Example	Reference List Example
Single Author	(Doss 2003)	Doss, G., 2003, *IS project management handbook*, Aspen Publishers, New York. Karskens, G., 1997, *The rocks: Life in early Sydney*, Melbourne University Press, Carlton.
Two Author	(Laudon & Laudon 2003)	Laudon, K.C. & Laudon, J.P., 2003, *Essentials of management information systems: Managing the digital firm*, Prentice Hall, Upper Saddle River.
Three authors	**In-text: initially** (Coveney, Ganster & King 2003) **In-text: thereafter** (Coveney et al. 2003)	Coveney, M., Ganster, S. & King, D., 2003, *The strategy gap: Leveraging technology to execute winning strategies*, Wiley, Hoboken.
Corporate author	**In-text: initially** (Department of Foreign Affairs and Trade 2002) **In-text: thereafter** (DFAT 2002)	Department of Foreign Affairs and Trade, 2002, *Connecting with Asia's tech future: ICT export opportunities*, Economic Analytical Unit, Commonwealth Government, Canberra.
Book Editor	(ed. Shaw 2003) Shaw (ed. 2003:87) indicates that '... Current essays edited by Shaw (2003) suggest ...	Shaw, M.J. (ed.), 2003, *E-business management: Integration of Web technologies with business models*, Kluwer Academic, London. Schuman, N.A., 2001, 'Psalm 91: Tekst, context, en een diversiteit aan herlezingen', in P. Post, G. Rouwhorst, T. Sheer, R. Steensma & L. Tongeren (reds.), *Jaarboek voor liturgieonderzoek deel*, vol. 17, pp. 237–256, Instituut voor Liturgie-wetenschap, Groningen. Spieckermann, H., 2003, 'Hymnen im Psalter – Ihre Funktion undn ihre Verfasser', in E. Zenger (Hrsg.), *Ritual und poesie*, pp. 90–136, Herder, Freiburg.

Book collection or One volume of multi-volume work	(eds. Hudson & Bolton 1997) Hudson and Bolton (eds. 1997:32) stated that '...'.	Hudson, W. & Bolton, G. (eds.), 1997, *Creating Australia: Changing Australian history*, Allen & Unwin, Sydney.
Chapter: single author	(Howard 1998)	Howard, S., 1998, 'Verbal Protocol Analysis', in B. Henderson-Sellers, A. Simons & H. Younessi (eds.), *The open process specification*, pp. 272–274, Addison Wesley, Sydney.
Chapter: three authors	In-text: initially (Johnston, Mak & Kurnia 2001) In-text: thereafter (Johnston et al. 2001)	Johnston R.B., Mak H.C. & Kurnia S., 2001, 'The contribution of Internet Electronic Commerce to advanced supply chain reform - a case study', in S. Barnes & B. Hunt (eds.), *E-Commerce and V-Business*, pp. 232–249, Butterworth-Heinemann, Oxford.
Four or more authors but equal to six. **See reference on how to cite more than 6 authors in this reference guide below**	A comprehensive study conducted in 1998 indicated that business in Australia is growing exponentially (Jones et al. 1999). *Or* Jones et al. (1999:34) suggested in their comprehensive study that '...'. **Note: If there is another reference starting with Jones, for example, Jones, Larsen, Green and Matthews, the names of all the authors should be given in both cases to avoid confusion.**	Jones, P., Smith, A., Hudson, T., Etherton, J., Connelly, W. & Gardener, J., 1999, *Business management for the new era*, Wyland Publishing, Adelaide. **Note: Use et al. in all in-text entries. Include all of the authors in the reference list.**
Article cited in a book		Oppenheim, P.L., 1981, 'Power politics', *Journal of Power Engineering* 1(3), 19–26, quoted in K. Strong, 1985, *Advances in power engineering*, Springer-Verlag, Berlin, p. 70.
Second, further or revised editions		Dyson, G.G.H., 1977, *The mechanics of athletics*, 7th edn., Homes and Meier, New York. Cohen, J., 1977, *Statistical power analysis for the behavioural ciences*, rev. edn., Academic Press, New York.
Translated works		Bakhtin, M., 1984, Rabelais and his world, transl. H. Iswolsky, Indiana University Press,

		Bloomington. Izedinova, S.V., 1977, *A few months with the Boers: The war reminiscences of a Russian nursing sister,* transl. C Moody, Perskor, Johannesburg.
Multiple works – same author	University research (Brown 1982, 1988) has indicated that … *or* Recent reports (Napier 1993a, 1993b) indicate that … **Note: When using two studies by the same author in different years, paraphrasing is essential. Place in chronological order—oldest first.** Ideas by Napier (1993b) were implemented … **Note: You may use direct quotes when using the sources separately.** **Add a, b, c, etc. to differentiate between works in the same year by using the alphabetical order of the title.**	Brown, P., 1982, *Corals in the Capricorn group*, Central Queensland University, Rockhampton. Brown, P., 1988, *The effects of anchors on corals*, Central Queensland University, Rockhampton. Napier, A., 1993a, *Fatal storm*, Allen & Unwin, Sydney. Napier, A., 1993b, *Survival at sea*, Allen & Unwin, Sydney.
Works by different authors—same family name—same year	A. Carter (1999) proposed that class size seriously limited creativity in the lower school. Further investigation proved there were notable weaknesses in this claim (Carter, T 1999). **Note: As a general rule, it is advisable to paraphrase in this instance.**	Carter, A., 1999, *Issues in Australian education*, Cherokee Publications, Brisbane. Carter, T., 1999, *Creativity in the classroom*, Watkins & O'Hara Publishers, Darwin.
Second or later edition with an author	Group dynamics has been identified as … (Johnston 1993). *Or* A recent theory (Johnston 1993:5) on group dynamics states that '…'.	Johnston, K., 1993, *Surviving the first year experience, 2nd edn.*, Macmillan, Melbourne.
Several sources are cited at once	Bradford (1992), Curtis (1983), and Graham (1997) all agree … *Or* Recent studies (Bradford 1992; Curtis 1983; Graham 1997) agree that …	Bradford, C., 1992, *Genre in perspective: A whole language approach*, Bookshelf, Gosford. Curtis, A., 1983, *Practical math for business*, Houghton Mifflin, Boston. Graham, A., 1997, *Managing*

	Note: Paraphrasing is essential if you are going to cite the essence of what the authors all agree upon. Alphabetise according to the name of the first author in each source. Separate entries by using semicolons.	*more postgraduate research students*, Oxford Centre for Staff Development, Oxford. Note: Each author will appear as a separate entry in the reference list.
No date can be established	Bridging courses are studied by students who have not previously … (Lansdown n.d.). *Or* Lansdown (n.d.:13) found that '…'	Lansdown, M., n.d., *Bridging courses*, Central Queensland University, Rockhampton.
The date can be established but only approximately	In a draft policy release, the Queensland Education Department (c. 1995) suggests… or 'Disciplining a child should not invoke …' (Queensland Education Department c.1995:xxii).	Queensland Education Department, c. 1995. *Draft policy on school discipline*, Author, Gladstone.

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Journals

Journals	In-Text Example	Reference List Example
Single author	(Hammer 1990:104–112)	Hammer, M., 1990, 'Reengineering Work: Don't Automate, Obliterate', *Harvard Business Review*, July–August, 104–112. Kozulin, A., 1993, 'Literature as a psychological tool', *Educational Psychologist* 28(3), 253–265.
Two authors	(Lamb & Kling 2003:197)	Lamb, R. & Kling, R., 2003, 'Reconceptualizing users as social actors in information systems research', *MIS Quarterly* 27(2), 197.
No volume or issue number	Sprague and Shameen (1999) indicate that alternatives to continual economic growth... *Or* It has been suggested that '[g]rowth at all cost is no longer a viable option' (Sprague & Shameen 1999: 50).	Sprague, J. & Shameen, A., 1999, 'Boosting growth, courting disasters?', *Asiaweek*, 31 July, 50–51.
Journal articles and book chapters from books of readings		Kuebler, S.A., 2004, 'OSHA's enforcement strategy', Occupational Health & Safety 73(12), 12–3, in I. Eddington (ed.), *MGT 8015 Corporate occupational health and safety: Selected readings*, 2005, University of Southern Queensland, Toowoomba, Reading 4.1, pp. 71–72. Hancock, L., 2002, 'Australian federalism, politics and health', in H. Gardner & S. Barraclough (eds.), Health policy in Australia, 2nd edn.,

		Oxford University Press, South Melbourne, in I. Eddington (ed.), *MGT 8015 Corporate occupational health and safety: Selected readings*, 2005, University of Southern Queensland, Toowoomba, Reading 1.2, pp. 28–35.
Non-English journal article		Give the original title, as well as an English translation in brackets. Ising, M., 2000,'Intensitätsabhängigkeit evozierter Potenzial im EEG: Sind impulsive Personen Augmenter oder Reducer?' [Intensity dependence in eventrelated EEG potentials: Are impulsive individuals augmenters or reducers?], *Zeitschrift für differentielle und diagnostische Psychologie* 21, 208–217.
Back dating articles		The volume number should be provided in Arabic numeral, without the preceding abbreviation 'v', 'vol.' or 'jrg.'. Full page numbers must be given. If the date or place of publication is missing, the abbreviation 'n.d.' is used instead of referring to the date of publication and 's.l.' instead of referring to the place of publication. **The date is placed in square brackets if the date of publication is known to the user but is not explicitly mentioned in the book or journal.** Pont, A.D., [1978], 'Die herderlijke brief van die sinode van 1837', *HTS*

		Teologiese Studies/Theological Studies 34(4), 91–105.
Supplement journal		Fisk, B.N., 2001, 'Do you not remember? Scripture, story and exegesis in the rewritten Bible of Pseudo-Philo', *Journal for the Study of the Pseudepigrapha*, suppl. ser. 37.

Proceedings

Proceedings	In-Text Example	Reference List Example
Published Conferences, seminars and meetings	(Eidenberger et al. 2002)	Eidenberger, H., Breiteneder, C. & Hitz, M., 2002, 'A framework for visual information retrieval', in S-K. Chang, Z. Chen & S-Y. Lee (eds.), *Recent advances in visual information systems: 5th International conference, VISUAL 2002 proceedings*, Hsin Chu, Taiwan, March 11–13, 2002, pp. 105–116.

Unpublished material

Unpublished material	In-Text Example	Reference List Example
Unpublished conference paper	(Fitzsimmons 2005)	Fitzsimmons, D., 2005, 'Who chooses who belongs: Tactics and strategies and migrant literature', paper presented at the AULLA & FILLM conference, James Cook University, Cairns, 15–19th July.
Theses and Reports	(Rouse 2002)	Rouse, A.C., 2002, 'Information technology outsourcing revisited: success factors and risks', PhD thesis, Dept. of Information Systems, University of Melbourne.

Encyclopedias and Dictionaries

Encyclopedias and Dictionaries	In-Text Example	Reference List Example
Encyclopedias and Dictionaries	(Karlof 2002)	Karlof, B., 2002, 'Benchmarking', in H. Bidgoli (ed.), *Encyclopedia of information systems*, Academic Press, New York, vol. 1, pp. 65–80.

Newspaper

Newspaper	In-Text Example	Reference List Example
Print unattributed	(Sydney Morning Herald 7 March 1994:8)	'UNSW gains top ranking from quality team', Sydney Morning Herald, 30 February, 1994, p. 21.
Print attributed	(Barker 2004)	Barker, G., 2004, '$54m Deal To Heat Up Broadband War', *The Age*, Business, 24 February, p. 2. Donaghy, B., 1994, 'National meeting set to review tertiary admissions', Campus News, 03–09 March, p. 3.
Online	(Varghese 2004)	Varghese, S., 2004, 'The

		Linux desktop is here', *The Age*, viewed 01 March 2004, from http://www.theage.com.au/articles/2004/02/13/1076548215848.html.

Magazine

Magazine	In-Text Example	Reference List Example
Single author	(Knight 2004)	Knight, W., 2004, 'How to second guess the next hack attack', *New Scientist*, 24 January, p. 19. Note: Always evaluate information found in magazines for 'scholarliness'– including bias, validity, trustworthiness of the authors etc. Magazines are not generally considered scholarly pieces of work for research.

Software

Software		
Named author	(Rawson-Tetley 2005)	Rawson-Tetley, R., *Animal Shelter Manager*, computer software, viewed 11 January 2005 from http://sheltermanager.sourceforge.net.
No named author	(OpenOffice.org 2005)	*OpenOffice.org*, computer software, viewed 11 January 2005, from http://www.openoffice.org.
Corporate publisher	(MATLAB 2003)	*MATLAB* version 6.5.1, 2003, computer software, The MathWorks Inc., Natick, Massachusetts. 1. Title of software - in italics: *MATLAB* 2. Version of

		software: version 6.5.1, 3. Year of publication: 2003, 4. Identifier: computer software, 5. Publisher: The MathWorks Inc., 6. Place of publication: Natick, Massachusetts.

World Wide Web

World Wide Web		
Document on the WWW (author or sponsor given but not dated)	According to Greenpeace (n.d.), genetically modified foods are ... *or* Greenpeace (n.d.:1 of 2) recommends that 'fewer genetically ...'.	Greenpeace n.d., *The future is GE free*, viewed 28 September 2005, from http://www.greenpeace.org.au/ge/farming/canola.html. **Note: The title of a webpage is treated like the title of a book. It is written in italics in the reference list.**
Identifiable, personal author	(Arch & Letourneau 2002)	Arch, A. & Letourneau, C., 2002, 'Auxiliary Benefits of Accessible Web Design', in *W3C Web Accessibility initiative*, viewed 26 February 2004, from http://www.w3.org/WAI/bcase/benefits.html.
E-book	(Eck 2002)	Eck, D.J., 2002, *Introduction To Programming Using Java*, 3rd edn., OOPWeb.com, viewed 26 February 2004, from http://www.oopweb.com/Java/Documents/IntroToProgrammingUsingJava/VolumeFrames.html.
E-journal: single author	(Lenoir 2003)	Lenoir, L., 2003, 'Response of the

		foraging behaviour of red wood ants (Formica rufa group) to exclusion from trees', *Agricultural and Forest Entomology* 5(3), 183–189, viewed 10 September 2003, from http://www.blackwell-synergy.com/links/doi/10.1046/j.1461-9563.2003.00176.x/full/.
E-journal: three authors	(Mueller et al. 2003)	Mueller, J.K., Heckathorn, S.A. & Fernando, D., 2003, 'Identification of a chloroplast dehydrin in leaves of mature plants', *International Journal of Plant Sciences* 164(4), 535–542, viewed on 10 September 2003, from http://www.journals.uchicago.edu/IJPS/journal/no.s/v164n4/164053/164053.html.
E-journal: Abstract and more than 6 authors	(Wolter et al. 2003)	Wolter, B.F., Ellis, M., Corrigan, B.P., DeDecker, J.M., Curtis, S.E., Parr, E.N. et al., 2003, 'Effect of restricted postweaning growth resulting from reduced floor and feeder-trough space on pig growth performance to slaughter weight in a wean-to-finish production system', *Journal of Animal Science*, (abstract), vol. 81, viewed 11 September 2003, from http://buffy.lib.unimelb.edu.au:2148/jas/abs/2003/a0340836.htm..

Other References

Other References		
CD-ROM	(World Atlas & Almanac 1995)	*World Atlas & Almanac*, 1995, CD-ROM, Mindscape Inc., Novato, California, USA.
Video & Audio	(*Grumpy meets the orchestra* 1992)	*Grumpy meets the*

Recordings: named		*orchestra*, 1992, video recording, Australian Broadcasting Corporation, Sydney, Featuring the Sydney Symphony Orchestra.
Video & Audio Recordings: named performer	(Sangare 1997)	Sangare, O., 1997, 'Dugu Kamalemba', in *The divas from Mali*, audio CD, Network Medien GmbH, D-60316 Frankfurt. Track #10. 1. Author (performer): Sangare, O., 2. Date (year): 1997, 3. Title (piece) - in single quotes: 'Dugu Kamalemba', 4. Title (collection) - in italics: in *The divas from Mali*, 5. Identifier: audio CD, 6. Publisher: Network Medien GmbH, 7. Place: D-60316 Frankfurt. 8. Qualifier: Track #10.
Personal Communication: individual	• When interviewed on 15 May 2001, Mr R. Forbes stated... OR • Mr R. Forbes confirmed his statement by fax on 15 May 2003... OR • The statement claimed that Martians had landed in Moonee Ponds (R. Forbes [Australian UFO Society] pers. comm., 15 May 2003). Not included in the Reference list.	
Personal Communication: email	(Milnthorpe 2002)	Milnthorpe, R.Q., 2002, email, 26 November, milnthorpe@scraggemback.com.au 1. Author: Milnthorpe, RQ 2. Date (year): 2002, 3. Identifier: email, 4. Date: 26 November, 5. Address: milnthorpe@scrag

		gemback.com.au
Personal Communication: lectures	• 'There is no need to panic', stated Mr R. Forbes at his public lecture on 18 May 2003 ... OR • The Chair (Seminar on the Moonee Ponds Phenomena 11 June 2003) drew attention to the unexplained spontaneous combustion of Mr Ron Forbes ... Not included in the Reference list.	
Maps: print	(Viking O'Neil 1991:32–33)	Viking O'Neil, 1991, *Australian Road Atlas*, 10th edn., Penguin Books Australia, Melbourne, pp. 32–33. 1. Author: Viking O'Neil 2. Date: 1991, 3. Title - in italics: *Australian Road Atlas*, 4. Edition: 10th edn., 5. Publisher: Penguin Books Australia, 6. City: Melbourne, 7. Pages: pp. 32–33.
Maps: online	(maps.com 1999)	maps.com, 1999, *Bhutan*, viewed 11 September 2003, from http://www.maps.com/cgi-bin/search/hyperseek.cgi?search=CAT&Category=Asia%3ABhutanP&Qualifier= 1. Author: maps.com, 2. Date: 1999, 3. Title - in italics: *Bhutan*, 4. Viewed: viewed 11 September 2003, 5. URL: from http://www.maps.com/cgi-bin/search/hyperseek.cgi?search=CAT&Category=Asia%3ABhutanP&Qualifier=

**** Guidelines for presenting lists and appendices ****

- Lists may be included in the paper provided they are not too long – that is, when they do not make the paper longer than a generally accepted publication length (about 10 pages).
- Details will necessarily differ according to different taxonomic groups, but family names should always be provided.
- Ensure tables are carefully constructed to be clear and unambiguous and always containing:
  - full naming conventions
  - synonyms (or, where multiple synonyms, provide a reference to these)
  - citations for literature sources used for names (if you have more than 25 references that are stipulated for the main manuscript, please place the exceeding references in the online lists as well; e.g. extra references, personal communications, or other sources from which the species lists or data were derived from).
- Clear indication is needed of who provided the identifications: the author(s), or specialists in an institution such as a herbarium or a museum (include person and institution name).
- A description of the general localities and more specific features of the study area(s) such as GPS points should be provided. However, in the case of threatened biota, locality details should be excluded (GPS localities, maps etc.), but reference to the relevant authority housing such records may be provided.
- Information on date of record should be provided (first date of record for the species in the area, as well as most recent record).
- Maps and figures can be included to provide further details, if appropriate.
- If herbarium or museum specimens are referred to, please provide relevant details.
- If data and other information are captured in specific data repositories, please provide details.
- All symbols and other notations should be well described in the footnote of the checklist as this will ensure the data or lists are still understandable and usable in the future. Use standardised symbols, for example (=) for synonyms.

- In lists annotated with additional information, such as species conservation status (e.g. red data list status), aspects of their biology will be valuable. If this becomes extensive, more than a single list may be provided: one with the taxonomic details and the other with the ecological details.

APPENDIX E

Copyright Zootaxa – Letter from the Chief Editor: Zhi-Qiang Zhang

Chantelle Girgan

From: Zhi-Qiang Zhang <ZhangZ@landcareresearch.co.nz>
Sent: Monday, 28 January 2019 10:16 PM
To: Chantelle Girgan
Subject: RE: Zootaxa Copyrigth

Dear Chantelle:

I herein grant your permission to reproduce the content from Zootaxa* on two conditions.
In addition to citing the paper, you will need to indicate that the reproduction of copyright content is permitted. You need to add "© Magnolia Press" in the reproduced content and mention "reproduced with permission from copyright holder".

*Requested materials:
Girgan, C., Swart, A., Marais, M. & Fourie, H. 2018. *Aphelenchoides* spp. (Nematoda: Aphelenchida) and *Panagrolaimus leperisini* (Nematoda: Rhabditida) found associated with grass seeds in the Telperion Nature Reserve, South Africa. *Zootaxa* 4370(2): 137-155.

Please keep this letter for legal purpose.

Zhi-Qiang Zhang
Chief editor/publisher of Magnolia Press

From: Chantelle Girgan [mailto:JansenC@arc.agric.za]
Sent: Tuesday, 29 January 2019 12:13 AM
To: Zhi-Qiang Zhang <ZhangZ@landcareresearch.co.nz>
Subject: Zootaxa Copyrigth

Dear Dr Zhi-Qiang Zhang

How are you? I am hoping you will be able to help me with some information. I published an article in Zootaxa last year (see reference below) which formed part of my PhD thesis. I am submitted the thesis soon and need to include this article as it was published in my thesis. However, the university requires a letter from Zootaxa or the publisher that states that I have permission to do so and that there will be no copyright problems.

Please can you write my such a letter or indicate to me who I can contact in this matter.

Thank you in advance.

Kind regards
Chantelle Girgan

Girgan, C., Swart, A., Marais, M. & Fourie, H. 2018. *Aphelenchoides* spp. (Nematoda: Aphelenchida) and *Panagrolaimus leperisini* (Nematoda: Rhabditida) found associated with grass seeds in the Telperion Nature Reserve, South Africa. *Zootaxa* 4370(2): 137-155.

APPENDIX F

Link to raw data of Chapters 2, 5 and 6

https://drive.google.com/drive/folders/1dKOGEbFhzPKaM-mi-f5PLyPOIe4I8DbO?usp=sharing

Appendix G:

Permission to collect samples



E OPPENHEIMER & SON

SOUTH AFRICA

9th November 2017

Mariette Marais
Agricultural Research Council

Dear Dr Marais,

We give full permission to you and your team to conduct Nematode biodiversity research and to sample the soil and plant material from which nematodes are extracted on Telperion Nature Reserve.

The owners give full permission that the nematodes collected be deposited in the National Collection of Nematodes housed at biosystematics of ARC-PPRI and the information recorded on the species published in scientific journals.

We trust that the National Collection of Nematodes will adhere to the policies regarding history collection.

Should you have any queries, please feel free to contact me.

Yours sincerely,

Dr Duncan MacFadyen

Manager: Research and Conservation

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